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Kristen J. Nadeau *Editors*

Insulin Resistance

Childhood Precursors of Adult Disease

Second Edition

Humana Press

Contemporary Endocrinology

Series Editor

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Series Editor Foreword

The concept of insulin resistance was placed on a firm scientific ground by Rosalyn Yalow and Solomon Berson in their Nobel prize-winning work, which described the development of radioimmunoassay (the first radioimmunoassay was for insulin) [1]. The authors demonstrated that circulating insulin levels in individuals with type 2 diabetes were not low or absent, as was assumed at the time, but are often increased.

With one-third of the US population developing prediabetes and two-thirds either overweight or obese, insulin resistance is arguably the most common disease-related condition in the United States and probably worldwide. Although pathophysiology of insulin resistance is relatively well understood, its specific causes in individual patients remain unknown (with some rare exceptions, e.g., in individuals with syndromes of extreme insulin resistance due to the mutations of insulin receptor gene or autoantibodies to the insulin receptor). This is true even for such common conditions as obesity or type 2 diabetes mellitus. Further, a diagnosis of insulin resistance is virtually impossible to make with precision in a clinical setting and is difficult in a research setting.

When much about insulin resistance remains to be learned, it is useful to summarize the current state of the art. Because the roots of insulin resistance are often established in childhood, the current volume, edited by Drs. Philip S. Zeitler and Kristen J. Nadeau, is an invaluable resource for anyone interested in pathophysiology, diagnosis, and management of insulin resistance. The chapters, written by world-class authorities, cover the entire spectrum of knowledge about insulin resistance including molecular mechanisms of insulin action, genetics, diagnosis, role of environment, puberty, pregnancy, and a variety of disease states. Exceptionally well written, referenced, and edited, the book is a welcome addition to the knowledge armamentarium of both pediatric and adult endocrinologists, as well as clinical investigators and basic scientists who continue to work tirelessly in order to shed more light on this common but still mysterious condition.

New York, NY, USA — Leonid Poretsky, MD

Reference

1. Kahn CR, Roth J. Berson, Yalow, and the JCI: the agony and the ecstasy. J Clin Invest. 2004;114(8):1051–4.

Preface to the Second Edition

Since the publication of the first edition of this book, there has been substantial progress in the understanding of insulin resistance and β-cell function, as well as the contribution of these two factors to the pathophysiology of type 2 diabetes and other disorders associated with obesity. In particular, a deeper understanding of the unique pathophysiology of insulin resistance in childhood and the impact of insulin resistance across the lifespan is emerging. This understanding includes a greater appreciation of the impact of insulin action on development of body composition in the fetus, child, and adolescent; the unique impact of puberty and sex steroids; the role of insulin resistance in the fetus and early life in the programming of glucose metabolism and pancreatic function; the effect of insulin resistance on development of target organ abnormalities in the liver, kidney, and heart; and the now-established role of insulin resistance in the young as a risk for disease in adulthood. In the process, there has been increased recognition that, beyond risk for the future, disorders related to insulin resistance are also having serious metabolic and cardiorenal impacts resulting in life-altering diseases at a time when individuals should be reaching their prime years for education, work, and family. As a reflection that insulin resistance in the young is having both short-term and long-term effects, we have changed the name of this book to *Insulin Resistance: Childhood Precursors of Adult Disease*.

In this second edition, we have again brought together experts in the field to provide a comprehensive overview and update on a wide variety of topics related to insulin resistance in youth. In some cases, these contributions are updated and timely chapters from the first edition. In other cases, we have added new chapters to address areas not previously covered. The first part of the edition addresses the clinical presentation and assessment of insulin resistance in youth, as well as a new chapter on insulin resistance in chronic childhood disease, an increasing contributor to the clinical picture of insulin resistance in children. Part II reviews the pathophysiology of insulin resistance in youth, including mechanism of insulin resistance and impact on metabolism, contributions of the in utero and early childhood environments, the growing understanding of the relationship between puberty and insulin resistance, and current perspectives on the role of body composition and ectopic fat. It closes with reviews of the impact of insulin resistance on the heart, liver, and kidneys. Part III addresses unique clinical settings for insulin resistance in youth, including type 1 diabetes, intrauterine growth retardation, and adolescent polycystic ovary syndrome. Finally, Part IV focuses on treatment,

with a discussion of whether insulin resistance itself should be considered a treatment target, as well as impact of exercise, weight loss medications, and bariatric surgery.

Insulin resistance continues to grow as a serious public health concern underlying the leading causes of morbidity and mortality in most of the world. This problem is increasingly affecting youth and threatens their long-term well-being and productivity, as well as boding crippling health-care costs. While we have made strides in understanding the root causes of insulin resistance, much remains to be explored in terms of molecular, biochemical, and physiological aspects of the disorder, as well as the human face reflected in the epidemiology, and how this understanding can be translated into intervention. This second edition provides perspective on insulin resistance as it affects individuals across the lifespan. As with the first edition, we hope that bringing together these updated contributions will continue to spur interest in the topic on the part of clinicians and researchers, as well as public health experts and decisionmakers.

Aurora, CO, USA

Philip S. Zeitler
Kristen J. Nadeau

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Part I

Definition and Epidemiology

Clinical Manifestations of Insulin Resistance in Youth

1

Melinda E. Chen and Tamara S. Hannon

Introduction

The obesity epidemic has led to a significant proportion of youth with comorbid conditions related to reduced insulin sensitivity, or "insulin resistance." Insulin resistance manifests as a reduction in the metabolic effects of insulin on liver, muscle, and adipose tissue—the body tissues that store energy. Because insulin directly or indirectly affects nearly every tissue in the body, physical signs of insulin resistance or associated conditions may be evident. These include skin findings, such as acanthosis nigricans, and systemic conditions such as polycystic ovary syndrome (PCOS) and the cluster of metabolic abnormalities referred to as the metabolic syndrome. Prediabetes and type 2 diabetes are well-recognized diagnoses associated with insulin resistance in adults and are becoming more prevalent in children. The ongoing increase in obesity and insulin resistance makes a thorough understanding of this topic more salient to both pediatric endocrine and general pediatric practice. This chapter will provide an introduction to the understanding, the recognition of, and the care for insulin resistance in children and youth. Focusing on the core concept of insulin sensitivity and its contribution to related clinical conditions, fundamental aspects of development and assessment of insulin resistance will be briefly discussed. Additionally, recognition of various syndromes and health conditions associated with insulin resistance will be reviewed. Some are mild and a direct result of exogenously driven conditions, such as obesity-related insulin resistance. However, more severe forms of insulin resistance, such as the inherited lipodystrophies, can be the result of an endogenous genetic defect in insulin action. The current understanding of various medical and lifestyle interventions and reasonable expectations for their success are also examined.

Because this chapter is intended to be an introductory review, the topics covered here will be necessarily brief, and a great deal of complexity and interrelationships between the conditions covered will be left to later chapters. The overarching concept that should be clear throughout the following pages is that youth with insulin resistance should be assessed, evaluated, and treated commensurate with the risks and outcomes known for their age group, which is not necessarily comparable to those found in adults.

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Metabolic Effects of Insulin

Insulin has metabolic effects in virtually every tissue of the body. Insulin acts in balance with glucagon and other hormones to maintain glucose homeostasis. Specifically, it acts as the anabolic end of the balance by encouraging cellular uptake of glucose and glycogen synthesis. Simultaneously, it decreases glycogenolysis and gluconeogenesis and limits provision of substrates (amino acids, fatty acids, and ketone bodies) for these processes, leading to a net effect of utilization of immediately available glucose [1, 2].

Once insulin binds to its target receptor, the exact pathway to the metabolic action of insulin depends on the tissue itself. In the liver, insulin inhibits glycogen phosphorylase, directly modulating glycogenolysis [1]. Insulin also decreases gluconeogenesis by decreasing free fatty acid and amino acid provision to the liver, as well as modulating glucagon secretion that would otherwise promote gluconeogenesis [2, 3]. In addition, insulin directly inhibits ketone production in the liver [1].

In skeletal muscle and, to a lesser degree, adipose tissue, insulin stimulates increased localization of cytoplasmic glucose transporter 4/GLUT-4 receptors to the cell membrane, resulting in greater intracellular glucose transport [4, 5]. Insulin binding also triggers a coordinated series of responses that favor the use of immediately available glucose over use of amino acids [2]. This is achieved by increasing utilization of amino acids for protein synthesis, inhibiting breakdown [2], and more indirectly through the inhibition of gluconeogenesis [2, 3].

Insulin additionally inhibits lipolysis in adipose tissue, thereby indirectly increasing glucose utilization as a fuel source in other tissues. Simultaneously, differential tissue effects of insulin favor the diversion of circulating lipids to adipose for storage, rather than for immediate use in muscle [6]. Decreased utilization of fatty acids minimizes available substrate for ketone production, which would be used as an alternative source of fuel in the absence of glucose. The decreased substrate exaggerates the direct inhibition of liver ketone production [1].

Lastly, insulin exaggerates its own effects by negating hormonal stimulation toward opposite goals. It does this by directly inhibiting glucagon secretion, while hyperglycemia can induce somatostatin, an additional inhibitor of glucagon secretion [3].

Insulin Resistance

Insulin resistance is a fundamental underlying mechanism in the development of type 2 diabetes. Whereas normal responsivity to insulin is integral for the multitude of tissue responses that contribute to glucose homeostasis, resistance to insulin action is characterized by diminishing metabolic response for a given degree of insulin secretion. Total body insulin resistance largely reflects reduced insulin action at the skeletal muscle tissue, resulting in lowered intracellular glucose uptake [5, 7], but tissue-specific effects are nevertheless important to understand overall effects of insulin resistance. Hepatic insulin resistance, for example, results in reduced inhibition of glycogen phosphorylase, increased hepatic glucose output, and reduced inhibition of ketone production [1, 5, 8]. Insulin resistance in adipose tissue results in increases in circulating lipids because of reduced insulin-mediated expression of genes vital for lipogenesis and, importantly, loss of suppression of hormone-sensitive lipase and lipoprotein lipase. Absent suppression of these enzymes controlling triglyceride mobilization leads to inappropriate fatty acid secretion from the adipocyte [8], contributing to the dyslipidemia often seen in association with insulin resistance.

In childhood, normoglycemia is predominantly maintained in the face of insulin resistance, where decreased tissue sensitivity is compensated for by higher insulin secretion to maintain normal glucose concentrations [9]. However, continued stress to the pancreatic β(beta)-cell, decreased total body insulin action, and increased demand for higher insulin concentrations can lead to progressive β(beta)-cell dysfunction and failure [9]. Further exacerbation of insulin resistance and β(beta)-cell dysfunction

leads to an ever-greater mismatch between insulin needs and insulin secretion, ultimately resulting in type 2 diabetes [9, 10].

Insulin resistance is directly measured by hyperinsulinemic-euglycemic clamp studies, which are unreasonable to do in the clinical setting due to several practical barriers [11]. Fasting insulin, though easily obtainable in the clinical setting, is a poor correlate of clamp study results [10, 11]. Because of these issues, and because there are no widely accepted standards by which insulin resistance can be defined, assessment of insulin resistance in the clinical setting measuring fasting insulin is generally not recommended [10].

Contributory Factors to Developing Insulin Resistance

Risk factors for developing insulin resistance can be grossly divided into nonmodifiable or modifiable categories. Non-modifiable risk factors, though unavoidable, do not consistently affect an individual over time and may result in fluctuating risk for dysglycemia throughout the lifespan.

Non-modifiable Factors

Puberty

Puberty is associated with rapid growth and development during which anabolic hormones, including insulin, are required in higher concentrations. The physiologic insulin resistance associated with the onset of puberty is thereby necessary to promote optimal growth and is naturally a self-limited process [12, 13]. Nonetheless, it is during this time that the risk for developing conditions associated with insulin resistance is heightened [12]. Typically, insulin sensitivity is decreased 30–50% even in lean and healthy children and is compensated for by a doubling of insulin secretion [12, 14]. In healthy children, this phenomenon is seen as early as the onset of puberty (Tanner 2), and insulin sensitivity returns to near prepuberty levels by the time puberty has completed [13].

Genetics

The heritable nature of susceptibility to insulin resistance is frequently seen in clinical practice, with a family history of type 2 diabetes considered a risk factor for developing type 2 diabetes in the individual [15]. Nevertheless, the development and progression of insulin resistance is multifactorial, and widespread genetic screening is not currently recommended. The list of genes associated with type 2 diabetes via increased propensity for insulin resistance or β(beta)-cell dysfunction is ever expanding, but the clinical utility of genetic studies is limited by expense and practicality, as well as overall usefulness. The many known polymorphisms explain only a small proportion of interindividual phenotypic variance [16], and broad assessments of the use of biomarkers have demonstrated only modest improvements in the ability to predict development of type 2 diabetes [15].

Epigenetics

More recently, research into the epigenetics of obesity has revealed potential implications for insulin resistance. Genome association studies demonstrating methylation alterations as a consequence of adiposity have identified influences on genes involved in lipid transport or dyslipidemia [17], as well as genes previously implicated in insulin resistance and type 2 diabetes risk or other metabolic defects [17]. Though causation of insulin resistance is unknown, changes such as hypomethylation of particular regions predispose the individual to later development of type 2 diabetes, rather than appearing after the development of insulin resistance or diabetes [16]. That the pattern of hypomethylation can be independent of the DNA sequence itself is suggestive of an entirely separate layer of control over metabolic processes affecting risk for insulin resistance [16]. Some of these methylation differences are present even in young adulthood, and it is postulated that resultant differences in expression, even if initially minor, may have substantial impact on risk for developing type 2 diabetes over the long term. In addition, specific epigenetic studies of high-risk ethnic populations have demonstrated an association of specific loci with

high methylation scores and future development of type 2 diabetes relative to age- and gender-matched controls without diabetes, independent of more well-known risk factors.

Methylation is postulated to occur early in human development, including during fetal life. Infants who are born small for gestational age [18] are well known to be at risk for insulin resistance and metabolic sequelae in later life [19]. Animal models have provided early evidence of possible mechanisms for these sequelae, including methylation of genes that could result in decreased insulin sensitivity and altered response to lipid delivery to muscle cells, leading to metabolic inflexibility that furthers insulin resistance at the level of the muscle cell [20].

Ethnicity

Certain minority populations are well recognized to have a higher burden of type 2 diabetes, and this seems to be a reflection of their susceptibility to develop insulin resistance or to demonstrate inadequate compensatory insulin secretion. Physiologic differences have been revealed with ethnic minorities demonstrating greater insulin secretion compared to their non-Hispanic white peers, even at equal degrees of insulin sensitivity, causing subsequently higher β(beta)-cell stress [21]. In both healthy youth and youth with diabetes, for example, African Americans have been shown to have lower visceral adipose tissue and higher insulin secretion at a given degree of insulin sensitivity, compared to non-Hispanic white counterparts [22, 23]. Studies on racial differences in insulin sensitivity show that while different ethnic groups have similar patterns of adaptation to insulin resistance, African Americans have somewhat lower insulin sensitivity than Latinos. This would require more exaggerated hyperinsulinemia to maintain euglycemia, possibly creating greater demand on β(beta)-cells and encouraging decompensation with clinically evident dysglycemia [23–25]. Latinos, though they may have higher insulin sensitivity than African Americans as a whole, tend to have more dramatic declines in measured insulin sensitivity as they become more obese [25]. However, the more fundamental differences between ethnic groups contributing to differences in insulin sensitivity and β(beta)-cell response are as yet unknown [25]. These inequities are aggravated with higher treatment failure rates among African-American and Hispanic youth with diabetes in comparison to non-Hispanic white youth in large treatment-based studies such as the TODAY study (Treatment Options for type 2 Diabetes in Adolescents and Youth) [26].

The predilection for obesity and insulin resistance seems to be especially true when ethnic minorities are placed into an obesogenic environment, demonstrating a genetic vulnerability toward metabolic decompensation when exposed to the appropriate environmental stress on β(beta)-cell function. Native Americans, for example, have the highest prevalence of type 2 diabetes in the SEARCH for Diabetes in Youth study, followed by African Americans, Hispanics, and Asians [27]. These differences are likely to be multifactorial, incorporating influences from genetics and epigenetics, as well as environmental stressors and cultural differences in diet and activity. There is some evidence for differential epigenetic changes in certain ethnic groups [17], while others have demonstrated some physiologic basis for these differences at the level of the β(beta)-cell.

Modifiable Factors

Though characteristics such familial traits, genetics, ethnicity, and the onset of puberty are uniformly unavoidable, other contributory factors toward insulin resistance can be successfully modified, though many patients struggle to do so.

Obesity

Obesity, one of the most well-known contributors to insulin resistance, is a prevalent issue nationwide [28], with approximately 32% of children 2–19 years considered overweight or obese and 17% considered obese. This places approximately a third of the nation's children at risk of complications associated with insulin resistance. Though alarming, this is only the beginning of a problem that increases exponentially by adulthood, with about 70% of the adult US population

>20 years old considered overweight or obese, about 38% of the population considered obese, and an estimated 12% of the population estimated to have diabetes [29].

Consequently, the combination of obesity and puberty composes a "double hit" to adolescents in the development of pathologic insulin resistance. Physiologic insulin resistance in puberty is already characterized by decreased glucose oxidation and decreased insulin-mediated suppression of free fatty acid oxidation. Increased need for fat oxidation rising from obesity during puberty may exacerbate competition with glucose oxidation and amplify glycemic manifestations of insulin resistance [12]. Meanwhile, obesity alone is associated with a measured insulin response about half that seen in normal weight youth, after adjusting for individual insulin sensitivity [30]. This indicates poorer β(beta)-cell function in the face of insulin resistance occurring even among obese adolescent youth with normal glucose tolerance. Consequently, the insulin resistance of puberty in addition to excessive substrate for fatty acid oxidation may create enough demand to overwhelm β(beta)-cells resulting in prediabetes or diabetes, particularly in those patients who are predisposed to have underlying β(beta)-cell dysfunction.

The fundamental role of obesity in the development of insulin resistance becomes especially important when the epidemiology of the condition is considered. Unfortunately, those race/ethnic groups that already show a preexisting vulnerability toward insulin resistance often have higher rates of obesity, with almost 40% of Hispanic youth being overweight or obese and about 35% of African-American youth being overweight or obese [28]. This trend continues in the more severe categories, with 22% and 20% of Hispanic and African-American youth, respectively, categorized as obese, compared to 14% of non-Hispanic white youth [28]. This distribution of obesity essentially places the greatest physiological stress on those individuals who are intrinsically most likely to decompensate from that stress and places a disproportionate burden of morbidity from type 2 diabetes and metabolic syndrome within these populations.

Diet and Lifestyle

One important factor in constant interaction with ethnic or genetic predisposition toward insulin resistance is the surrounding environment [31]. The influence of an "obesogenic environment" common to a Westernized lifestyle, corresponding to higher fat, salt, sugar, refined grains, and high-calorie foods, and lacking in physical activity has been reported especially within countries with higher income levels [32, 33]. While the effects of an unhealthy diet can impact anyone, this influence is seen most strikingly in groups that shift toward higher rates of obesity upon encountering a truly obesogenic lifestyle for the first time. A number of studies, for example, have shown that adolescent and adult immigrants to the United States from lower-income countries have an increasing prevalence of obesity with increased years since immigration and, over the long term, the prevalence begins to approach rates similar to the country to which they have immigrated [33, 34]. This trend, on large national surveys, is at least present among Hispanics and Asians, two ethnic groups that are particularly susceptible to developing insulin resistance. Furthermore, these populations are less likely to discuss diet and exercise options with their clinicians, potentially leading to an education gap that can increase their participation in obesogenic habits [34].

Even in children, a similar association seems to hold true, with children of acculturated parents (i.e., having lived in a higher-income country for a longer period of time and implicitly having adapted more of the surrounding obesogenic behaviors and diet) much more likely to be overweight or obese than children in the originating country, children of new immigrants, or even children native to the high-income country [33, 35]. When examining dietary shifts, changes toward increased consumption of processed foods and decreases in high-fiber, high-nutrient foods are often reported particularly in younger generations, though it is by no means exclusive [32]. This shift is likely the result of multiple influences [32], including food availability (forced shift away from traditional foods), relative income (lower income possibly creating a

reliance on cheaper and lower quality foods), education level, generation (younger generations being reported as more likely to change dietary habits), and others. These generalizations between time and risk for obesity, however, are often drawn from studies that fail to draw a distinction between acculturation (assimilation and exchange of cultural behaviors) and enculturation (a lesser degree of assimilation despite living within a different culture). Among studies that used scales to measure degree of acculturation rather than time from immigration in relationship with the development of obesity, there is generally a positive association between high degrees of acculturation and higher risk for obesity. Authors have theorized that migration forces a more rapid nutritional transition toward increased consumption of high-fat foods that are lower in nutrition, a transition that would normally occur over years along with the economic development of their country of origin [36].

Physical Activity

The other end of the equation in caloric balance affecting insulin resistance is the degree of physical activity. Longitudinally, a shift toward more sedentary activities is part of the obesogenic lifestyle. A lack of calorie expenditure leading to overall caloric excess can lead to increased weight gain, increasing fatty acid oxidation as competition for glucose oxidation as outlined above. However, even moderate physical activity increases muscle cell uptake of glucose by insulin-independent mechanisms. Exercise modulates translocation of the same GLUT4 transporters that are typically controlled by insulin secretion [37], increasing glucose uptake in an additive manner to insulin-mediated mechanisms and contributing to more efficient glucose homeostasis overall [38].

Conditions Associated with Insulin Resistance

Though the development of type 2 diabetes is the most obvious and direct consequence of insulin resistance and β(beta)-cell stress that proceeds unchecked, there are myriad other associations that healthcare providers should be aware of and screen for as indicated. Like insulin resistance, many of these conditions are more commonly seen in adulthood, but can certainly affect the pediatric population. Many of these disease associations and syndromes are not entirely surprising, given the multiorgan effects of insulin even beyond glucose homeostatic mechanisms. Resistance to insulin-mediated control of circulating fatty acids may predispose to worsening dyslipidemia, a well-known risk factor for cardiovascular disease. Insulin, in addition, increases androgen synthesis and simultaneously decreases liver production of sex hormone-binding globulin, thus providing a mechanism for the reproductive disruption seen in PCOS [39, 40]. Consequently, insulin resistance itself is associated with many well-known diseases and syndromes that incorporate other organ and system effects of insulin.

Metabolic Syndrome

In adults, the metabolic syndrome is comprised of a collection of cardiovascular risk factors associated with insulin resistance, including abdominal obesity (elevated waist circumference), hypertension, dyslipidemia, and dysglycemia. Approximately one-third of the adult population is estimated to have metabolic syndrome according to 2003–2012 National Health and Nutrition Examination Survey (NHANES) data [41]. Of note, criteria for metabolic syndrome in the adult population are known to underdiagnose the condition in certain ethnic populations, and thus the reported prevalence of the syndrome in African Americans may in fact be an underrepresentation [42].

With the increase in pediatric obesity, greater attention has been given to criteria that would be analogous in the pediatric population. As always in pediatrics, the growth and ever-changing physiology of children create a unique challenge in the recognition of important health conditions, and a single standard definition does not yet exist. Furthermore, the absence of long-term

epidemiologic data linking any definition of metabolic syndrome to cardiovascular risk makes the designation of an evidence-based definition of metabolic syndrome impossible. However, based on extrapolation from adults, the International Diabetes Federation defines metabolic syndrome in children 10 to <16 years old as the presence of obesity >90th percentile by waist circumference, replacing the adult criterion for waist circumference alone, and the presence of at least two other criteria defined similarly to adults (hypertriglyceridemia, low high-density lipoprotein [HDL], hypertension, and dysglycemia or type 2 diabetes) [43]. Meanwhile, children 16 and older should be diagnosed according to adult criteria, and children <10 do not have particular criteria for diagnosis of metabolic syndrome, but should be screened for risk factors if abdominal obesity >90th percentile by waist circumference is present and if their family history of metabolic syndrome or cardiovascular risk is strong. Based on adolescent-adapted criteria, the overall prevalence of metabolic syndrome in patients 12–19 years old is thought to be about 10%, with an overall higher prevalence in males, Hispanics, and non-Hispanic whites [42]. In recent years, the prevalence of metabolic syndrome has been seen to decrease slightly in youth [42], while the prevalence has stabilized but not decreased among adults [41].

Like insulin resistance, the presence of metabolic syndrome has been shown to differ by ethnic group [21], and the prevalent components of metabolic syndrome also differ by ethnic background. African Americans, for example, have a somewhat lower prevalence of the diagnosis overall and are less likely than non-Hispanic whites to have dyslipidemia, but are more likely to demonstrate hypertension [21].

In the pediatric population, as in adults, the association between obesity and metabolic syndrome is strong, with higher prevalence in more severe degrees of obesity. In a population of obese children, almost 40% of moderately obese subjects and almost half of severely obese subjects met criteria for metabolic syndrome, while overweight or normal weight subjects are unlikely to meet criteria [44]. Severely obese white subjects have an especially high rate of metabolic syndrome even with an intermediate degree of insulin resistance. Other studies have shown the dyslipidemia associated with insulin resistance (low HDL, high triglycerides) is frequently found in adolescents [21].

In addition, the prevalence of metabolic syndrome increases with increasing insulin resistance, even after adjusting for race or obesity [44]. When followed longitudinally without intervention, most youth persisted in having metabolic syndrome and even progressed at 2-year follow-up, with lower body mass index (BMI) being associated with a lower likelihood of persisting. This alarming persistence of cardiovascular risk factors early in life warrants close attention, follow-up, and intervention.

Prediabetes and Type 2 Diabetes

Insulin resistance is a major contributing factor to the development of prediabetes and type 2 diabetes. Within the general adolescent population, the prevalence of prediabetes (either impaired fasting glucose [IFG] or impaired glucose tolerance [IGT]) has been reported to be approximately 16.1% [45]. This is compared with 27.5% of US adults with prediabetes [46]. As with the prediabetic state, adults represent a far greater proportion of the burden of type 2 diabetes within the United States with a population prevalence of about 12.3 per 100 compared to 0.5 per 1000 in individuals <20 years of age [47]. Though adults have also seen greater increases in the prevalence of diabetes within the population, the recent 30–35% increase in type 2 diabetes in youth is nevertheless alarming [27, 48].

Polycystic Ovary Syndrome (PCOS)

Other hormonal alterations in association with insulin resistance together form the polycystic ovary syndrome (PCOS) in adolescent females—a collection of symptoms that classically encompasses chronic anovulation, polycystic ovaries,

and hyperandrogenism [49]. Signs of insulin resistance are commonly present, and there is increased risk for the development of glucose intolerance and type 2 diabetes [50]. As with metabolic syndrome, PCOS was first defined in adults but is increasingly recognized within the pediatric population. And like metabolic syndrome, diagnostic criteria for PCOS are less clear in youth, owing to practical differences in clinical evaluation and assessment. Some elements of PCOS, such as irregular menses and anovulation, can be seen as components of normal puberty, making diagnosis more difficult [49, 51]. The core pathophysiological elements of hyperandrogenism, insulin resistance, and anovulation remain the same. However, thresholds for declaring these abnormalities differ by criteria, and currently obesity and measures of insulin resistance are not included in most proposed diagnostic criteria for adolescents.

Hyperandrogenism can be assessed as clinical symptoms or biochemical evidence. Cutoffs for laboratory tests when evaluating for biochemical evidence of hyperandrogenism differ by developmental stage and must be evaluated within the clinical context [51]. Currently, biochemical evaluation lacks standardization between assays and, especially in younger females, sensitivity to detect lower levels. Though some cutoff values have been proposed, these depend on the use of reliable assays and laboratories [49]. The assessment of hirsutism in a female who is not yet fully pubescent is difficult and highly subjective. The use of a Ferriman–Gallwey score has many limitations even in adults, among them subjectivity and ethnic variation, and data on use in adolescents is even more scarce. Acne vulgaris is common during puberty, though moderate or severe acne early in puberty is less common and should prompt further evaluation [49].

Though the presence of oligomenorrhea is recommended as a diagnostic feature in some criteria, the clinical assessment of menstrual irregularity in adolescents is confounded by the fact that anovulatory cycles may persist for years after menarche even in normal females. However, the overall pattern is to have progressively more regular cycles with time, causing some experts to recommend considering menstrual intervals of <20 days or >45 days 2 or more years after menarche, or consecutive menstrual intervals >90 days at any point, as evidence of persistent amenorrhea [49]. The presence of ovaries meeting the adult definition of "polycystic" is actually quite common in normal adolescent females, so is an unreliable predictor for PCOS. Consequently, most criteria do not require visualization of polycystic ovaries themselves for diagnosis in adolescents [52].

PCOS is well recognized to be associated with insulin resistance. Hyperinsulinemia is associated with increased androgen production and decreased sex hormone-binding globulin (SHBG) production. This creates functional hyperandrogenism that impairs normal GnRH regulation and creates or exacerbates the menstrual irregularities characteristic of PCOS [49, 52, 53]. Thus, the presence of insulin resistance should raise greater concern for PCOS, and the reverse is also true. However, currently, no adolescent criteria require obesity, insulin resistance, or hyperinsulinemia [49]. These and other commonly seen features in PCOS overlap with several features of metabolic syndrome, and the prevalence of metabolic syndrome is thought to be 37–47% within the adolescent PCOS population—higher than in adults with PCOS and much higher than in the general population [53]. Adolescents with PCOS have a similar fasting glucose and higher concurrent fasting insulin even compared to obese adolescents and a roughly 50% reduction in peripheral insulin sensitivity and subsequent higher insulin secretion during a hyperglycemic clamp [52]. Adolescents may have exaggerated compensatory insulin secretion in response to the insulin resistance of PCOS, in contrast to a dysfunctional secretory response, suggesting that adult PCOS could represent an extension of adolescent-onset PCOS. In this postulated scenario, a longer time of disease exposure results in gradual dysfunction and failure of β(beta)-cells to compensate for increased insulin resistance over the long term. In addition, recognition of these differences in adults and adolescents in PCOS could indicate that differential treatment by age and clinical stage may be warranted [52].

Current treatment of PCOS in the pediatric population, while materially similar to adults, should be approached differently in the clinic. Intervention has been shown to benefit hirsutism, quality of life, and cardiovascular risk profile at least in the short term [49]. While adolescents may consider issues of fertility to be a less immediate concern, lifestyle modifications, if successfully implemented, can still be beneficial to quality of life. Oral contraceptive pills, especially antiandrogenic forms such as drospirenone- or cyproterone-containing formulations, can help to combat hyperandrogenism and restore regular menses, but may worsen insulin resistance or other cardiovascular risk factors such as dyslipidemia [51]. In addition, considering the postulated fundamental role of insulin resistance in driving other features of PCOS, insulin sensitizers such as metformin have been shown to increase glucose tolerance and decrease androgen concentrations, in some studies promoting improved lipid profiles and restoring regular menses when in combination with lifestyle interventions [54, 55].

Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in pediatric patients [56]; estimates of the prevalence of NAFLD are about 30–40% in obese youth [56]. The clinical spectrum can range from mild steatosis to cirrhosis. Hispanic individuals have the highest risk, and Asian and non-Hispanic white individuals have a higher risk of hepatic steatosis than African Americans. Studies consistently show higher prevalence of NAFLD among those with insulin resistance [57] and a higher likelihood of persistence of glucose dysregulation among patients with NAFLD [58]. Others have suggested that because fatty liver can be seen even before frank type 2 diabetes has developed, and because steatotic livers tend to be insulin resistant and produce excess glucose, NAFLD may be a contributor toward the progression of insulin resistance to type 2 diabetes [59]. However, few studies have truly addressed causality in the association between insulin resistance and NAFLD.

About a fourth of children in the Nonalcoholic Steatohepatitis Clinical Research network had prediabetes, and 6.5% had type 2 diabetes, rates far higher than within the general pediatric population. Children with evidence of dysglycemia tend to be older and more obese, with a higher waist circumference. In addition, females with NAFLD are more likely to have prediabetes and type 2 diabetes than males with NAFLD [57], despite a clear predominance of males within the overall population with NAFLD.

The frequent coexistence of type 2 diabetes and NAFLD in adults is even more clinically relevant because type 2 diabetes is associated with nonalcoholic steatohepatitis (NASH), the more progressive form that is more often associated with end-stage liver disease. As in adults, biochemical or clinical evidence of insulin resistance is associated with NASH, and individuals with type 2 diabetes and NAFLD have approximately double the risk of NASH compared to those with normal glucose tolerance.

Lipodystrophies: Inherited and Acquired

Four broad categories of genetic lipodystrophies exist, each with multiple genetic causes or multiple subtypes: (1) congenital generalized lipodystrophy, (2) acquired generalized lipodystrophy, (3) familial partial lipodystrophy, and (4) acquired partial lipodystrophy—though lipodystrophy can also be a feature of other syndromes. In particular, lipodystrophy secondary to highly active antiretroviral therapy (HAART) is not genetically inherited but is now the most common form of lipodystrophy, present in about half of patients receiving HAART.

Lipodystrophy is rare in children, with an overall prevalence of less than one in a million individuals. The disorder consists of the absence of adipose, either generalized or partial. Consequently, individuals can appear unusually muscular; but despite this clinical feature, insulin

resistance is frequently found in association with lipodystrophies. When adipocytes are not present, lipid molecules normally stored within the adipocyte are stored in other organs, including the muscle, myocardium, pancreas, and liver, causing metabolic instability [60]. Metabolic derangements can occur when the limited ability to store triglycerides causes increased fatty acid circulation. Increased substrate availability promotes utilization by skeletal muscle, competing with glucose utilization, thus resulting in clinical manifestations of insulin resistance [61]. Specifically, diabetes, hypertriglyceridemia, and acanthosis nigricans are frequently reported in association with lipodystrophy, and PCOS is common in women with lipodystrophy [62] Women with partial lipodystrophy may have more severe manifestations of metabolic complications in general [63]. The insulin resistance can be worsened by reduced levels of leptin in patients with severe loss of adipose tissue, promoting increased appetite that can make insulin resistance harder to treat clinically [64].

The prevalence of diabetes mellitus in lipodystrophy patients varies by type from 35% to 70%. Patients with acquired generalized lipodystrophy have the highest rate of diabetes mellitus, though patients with congenital generalized lipodystrophy have the youngest age of onset. In these patients, diabetes can present as young as school age. However, the absence of ketosis and presence of acanthosis nigricans and elevated serum insulin suggests dysglycemia secondary to insulin resistance rather than an autoimmune process [62].

The etiology of lipodystrophies involves mutations directly related to lipid storage or adipocyte differentiation and function. As adipocytes are an important target of insulin action and vital for decreasing fatty acid circulation and competition for glucose oxidation, any fundamental disruption in this process would directly decrease tissue responsiveness to insulin. Of the known genetic mutations resulting in autosomal recessive congenital generalized lipodystrophy, many—including AGPAT2, BSCL2, CAV1, and PTRF—are related to appropriate adipocyte development and function [64]. In (mostly) autosomal dominantly inherited familial partial lipodystrophy, the most commonly mutated gene is LMNA, encoding lamin A and lamin C, which are thought to result in abnormal nuclear interaction with transcription factors involved in adipogenesis [65], or premature apoptosis in adipocytes. Mutations in PPARG, a regulator of adipogenesis and target for thiazolidinediones [66], are present in many cases of lipodystrophy, while PLIN1 mutations affect a protein that is necessary for normal lipid storage [60].

Acquired lipodystrophy seen with HAART therapy may be related to protease inhibitor inhibition of zinc metalloprotease, which is involved in processing of lamin A, thus possibly resulting in lipodystrophy in a manner similar to LMNA mutations [67].

Management of lipodystrophies primarily centers on management of metabolic abnormalities. As with other patients with insulin resistance, diet and exercise are important. A recommended diet for lipodystrophy patients contains a somewhat lower proportion of carbohydrate compared to other patients, at about 50–60% of daily calories [64]. In addition, many patients require metformin, or a combination of metformin and insulin; insulin needs may be substantial due to the severe insulin resistance. Though metreleptin, or recombinant human leptin, has not been effective in treatment of insulin resistance in obese subjects, who presumably have high endogenous levels of leptin, the administration of metreleptin seems to be beneficial for lipodystrophy patients who have low baseline levels of leptin [68]. In very limited data available on metreleptin use in children, it reduces serum insulin and hemoglobin A1c when used in combination with lifestyle changes [62]. Metreleptin continues to be experimental for partial lipodystrophy.

Interventional Options

In both adults and children with insulin resistance, lifestyle modifications including diet and exercise have long been mainstays of treatment. Many intervention studies are multifaceted, aim-

ing to alter both, and seem to show overall improvement in insulin resistance when there is weight loss and supervised physical activity [69, 70]. Studies that examine exercise or diet modifications alone are few, and results have been variable. Aerobic exercise specifically has been associated with improvement in insulin resistance in some studies [71], whereas resistance exercise has been shown to improve insulin resistance in male adolescents but not females [72, 73]. Studies assessing exercise independent of weight loss or attempted calorie restriction show transiently improved insulin sensitivity. While physical activity alone does not completely reverse the metabolic consequences of obesity alone, studies specifically examining physical activity suggest independent contributions from obesity and physical inactivity toward cardiometabolic risk [74]. Some have noted correlations between insulin sensitivity and changes in body composition, suggesting that improvements can come from changes in adiposity and muscle mass, even without weight change. Other studies show inverse associations between the time spent on moderate and vigorous physical activity and fasting insulin in adolescents, a relationship that remains relevant regardless of the time spent sedentary [75]. Perhaps most encouraging for the sedentary obese individual, greater improvements come from the transition from being sedentary to being moderately active, though additional benefits accrue from greater degrees of exercise [73].

Though significant periods of exercise may be difficult to achieve as a consistent lifestyle change, adult data suggest that even small improvements in daily physical activity can lead to positive gains. Studies examining various degrees of interrupted sedentary activity show that at least moderate activity is required to induce a change in ATP production that would likely lead to weight change, but even light activity could result in modulated glucose uptake and improved postprandial glycemic indices within days of intervention, possibly through both activity- and insulin-mediated pathways [37]. Adults with type 2 diabetes who replaced several hours a day of sitting with standing or light walking have been shown to have significantly lower glycemia over a 24-hour period compared with adults who did not replace periods of sitting with activity. Over the course of 4–5 days, insulin sensitivity also improved in groups that replaced periods of sitting with exercise. These changes appear to occur through mediation of contraction-induced glucose uptake rather than insulin-mediated glucose uptake. Thus, improvement in insulin sensitivity can be seen even over a short period of dedicated increase in non-strenuous activity [76, 77].

Reversal of obesity may also reverse its deleterious effects in the insulin-resistant individual. In cohort studies that demonstrate successful weight control through intensive lifestyle intervention, an improvement in insulin sensitivity can be seen with a BMI-SDS (standardized BMI) reduction as small as 0.25–0.5 mg/m^2—a difference that was most apparent among pubertal and extremely obese children [69]. Clinically, this is manifested as improvement in rates of successful medical treatment with the addition of lifestyle intervention in youth with type 2 diabetes, though the overall treatment success rates were still discouraging [78].

More holistic lifestyle intervention programs have been shown to prevent the progression of prediabetes to diabetes in adults, but similar programs have not been as frequently studied in the pediatric population. In an intensive diet, exercise, and behavioral modification program for children with clinically measurable insulin resistance, children showed greater improvements in insulin sensitivity compared to those receiving counseling alone. In addition, a greater proportion of children in the intensive program converted to normal glucose tolerance as shown by oral glucose tolerance test (OGTT) on follow-up [79].

While insulin sensitizers such as metformin have been studied in adults and shown to decrease risk for type 2 diabetes, equivalent studies in children are scarce. Several studies have shown modest benefit of metformin in reducing BMI in obese children in the short term only [18, 80–83]. These studies have been limited by small size, and differences in measures of

insulin resistance have been modest [80]. At this time, metformin is only approved for use in youth with type 2 diabetes. Metformin is an accepted part of treatment of PCOS, but few studies have closely examined the effect of the medication alone on long-term risk for progression in the obese, insulin-resistant child.

Conclusions

The phenomenon of insulin resistance is widespread among both adults and youth. Insulin resistance can exist without further clinical symptoms or in the context of several syndromes that can further negatively impact an individual's health; most importantly, insulin resistance can be present even with normal glucose tolerance. The risk for insulin resistance is greatly increased in the setting of obesity, and youth have unique risk factors, such as puberty, that create added stress to the β(beta)-cell.

Syndromes that were previously associated with adulthood—such as fatty liver, metabolic syndrome, and PCOS—are now frequently recognized in association with insulin resistance in youth. As in adults, these syndromes have a great deal of overlap with each other and convey additional cardiovascular risk and potential long-term health consequences. The early onset of these syndromes is alarming, as the lengthened time of exposure likely places adolescents at higher risk of developing end-organ damage and long-term consequences, which will then cause personal health and healthcare system burden on a massive scale.

Many, if not all, interventions recommended as effective treatment for insulin resistance, prediabetes, or type 2 diabetes require diligence on the part of both patient and provider. Poor adherence observed in clinical practice is a testament to the difficulty of building new and healthier habits. However, patients and providers may be encouraged that improvement in insulin resistance and general health does not always require herculean transformation and that small victories and simpler interventions are not wasted. Small changes to BMI or moderate interruptions to an otherwise sedentary lifestyle can create improvements to cardiovascular risk factors that, though modest, could have impressive impact on the population scale. Therefore, prompt attention and intervention to obesity and insulin resistance to prevent adverse long-term health consequences are both vital and urgent, in order to provide the greatest benefit to patients.

References

1. Keller U, Gerber PP, Stauffacher W. Fatty acid-independent inhibition of hepatic ketone body production by insulin in humans. Am J Physiol. 1988;254(6 Pt 1):E694–9.
2. Jefferson LS. Role of insulin in the regulation of protein synthesis. Diabetes. 1980;29(6):487–96.
3. Philippe J. Insulin regulation of the glucagon gene is mediated by an insulin-responsive DNA element. Proc Natl Acad Sci U S A. 1991;88(16):7224–7.
4. Kahn BB. Glucose transport: pivotal step in insulin action. Diabetes. 1996;45(11):1644–54.
5. DeFronzo RA. The triumvirate: cell, muscle, liver: a collusion responsible for NIDDM. Diabetes. 1988;37(6):667–87.
6. Fielding BA, Frayn KN. Lipoprotein lipase and the disposition of dietary fatty acids. Br J Nutr. 1998;80(6):495–502.
7. Carnagarin R, Dharmarajan AM, Dass CR. Molecular aspects of glucose homeostasis in skeletal muscle–a focus on the molecular mechanisms of insulin resistance. Mol Cell Endocrinol. 2015;417:52–62.
8. Meshkani R, Adeli K. Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. Clin Biochem. 2009;42(13–14):1331–46.
9. Hannon TS, Rao G, Arslanian SA. Childhood obesity and type 2 diabetes mellitus. Pediatrics. 2005;116(2):473–80.
10. Levy-Marchal C, Arslanian S, Cutfield W, Sinaiko A, Druet C, Marcovecchio ML, et al. Insulin resistance in children: consensus, perspective, and future directions. J Clin Endocrinol Metab. 2010;95(12):5189–98.
11. Brown RJ, Yanovski JA. Estimation of insulin sensitivity in children: methods, measures and controversies. Pediatr Diabetes. 2014;15(3):151–61.
12. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. Pediatr Res. 2006;60(6):759–63.
13. Moran A, Jacobs DR, Steinberger J, Hong CP, Prineas R, Luepker R, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes. 1999;48(10):2039–44.
14. Caprio S, Plewe G, Diamond MP, Simonson DC, Boulware SD, Sherwin RS, et al. Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. J Pediatr. 1989;114(6):963–7.

15. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med. 2008;359(21):2220–32.
16. Toperoff G, Aran D, Kark JD, Rosenberg M, Dubnikov T, Nissan B, et al. Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. Hum Mol Genet. 2012;21(2):371–83.
17. Chambers JC, Loh M, Lehne B, Drong A, Kriebel J, Motta V, Wahl S, Elliot HR, Rota F, Scott WR, Zhang W, Tan ST, Campanella G, Chadeau-Hyam M, Yengo L, Richmond RC, Adamowicz-Brice M, Afzal U, Bozaoglu K, Kooner JS. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. Lancet Diabetes Endocrinol. 2015;3(7):526–34.
18. Wilson DM, Abrams SH, Aye T, Lee PD, Lenders C, Lustig RH, et al. Metformin extended release treatment of adolescent obesity: a 48-week randomized, double-blind, placebo-controlled trial with 48-week follow-up. Arch Pediatr Adolesc Med. 2010;164(2):116–23.
19. Vaag A, Jensen CB, Poulsen P, Brons C, Pilgaard K, Grunnet L, et al. Metabolic aspects of insulin resistance in individuals born small for gestational age. Horm Res. 2006;65(Suppl 3):137–43.
20. Holness MJ, Sugden MC. Epigenetic regulation of metabolism in children born small for gestational age. Curr Opin Clin Nutr Metab Care. 2006;9(4):482–8.
21. Weiss R, Kaufman FR. Metabolic complications of childhood obesity: identifying and mitigating the risk. Diabetes Care. 2008;31(Suppl 2):S310–6.
22. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J. Hyperinsulinemia in African-American children: decreased insulin clearance and increased insulin secretion and its relationship to insulin sensitivity. Diabetes. 2002;51(10):3014–9.
23. Bacha F, Gungor N, Lee S, Arslanian SA. Type 2 diabetes in youth: are there racial differences in beta-cell responsiveness relative to insulin sensitivity? Pediatr Diabetes. 2012;13(3):259–65.
24. Weiss R, Dziura JD, Burgert TS, Taksali SE, Tamborlane WV, Caprio S. Ethnic differences in beta cell adaptation to insulin resistance in obese children and adolescents. Diabetologia. 2006;49(3):571–9.
25. Hasson RE, Adam TC, Davis JN, Weigensberg MJ, Ventura EE, Lane CJ, et al. Ethnic differences in insulin action in obese African-American and Latino adolescents. J Clin Endocrinol Metab. 2010;95(8):4048–51.
26. TODAY Study Group, Zeitler P, Hirst K, Pyle L, Linder B, Copeland K, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med. 2012;366(24):2247–56.
27. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA. 2014;311(17):1778–86.
28. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. JAMA. 2014;311(8):806–14.
29. National Center for Health Statistics. Health, United States, 2015: with special feature on racial and ethnic health dsiparities. Hyattsville: National Center for Health Statistics; 2016.
30. Sjaarda L, Lee S, Tfayli H, Bacha F, Bertolet M, Arslanian S. Measuring beta-cell function relative to insulin sensitivity in youth: does the hyperglycemic clamp suffice? Diabetes Care. 2013;36(6):1607–12.
31. Wagner KA, Armah SM, Smith LG, Pike J, Tu W, Campbell WW, et al. Associations between diet behaviors and measures of glycemia, in clinical setting, in obese adolescents. Child Obes. 2016;12(5):341–7.
32. Gilbert PA, Khokhar S. Changing dietary habits of ethnic groups in Europe and implications for health. Nutr Rev. 2008;66(4):203–15.
33. Kaufman-Shriqui V, Fraser D, Friger M, Bilenko N, Vardi H, Abu-Saad K, et al. Factors associated with childhood overweight and obesity among acculturated and new immigrants. Ethn Dis. 2013;23(3):329–35.
34. Goel MS, McCarthy EP, Phillips RS, Wee CC. Obesity among US immigrant subgroups by duration of residence. JAMA. 2004;292(23):2860–7.
35. Gualdi-Russo E, Zaccagni L, Manzon VS, Masotti S, Rinaldo N, Khyatti M. Obesity and physical activity in children of immigrants. Eur J Pub Health. 2014;24(Suppl 1):40–6.
36. Delavari M, Sonderlund AL, Swinburn B, Mellor D, Renzaho A. Acculturation and obesity among migrant populations in high income countries–a systematic review. BMC Public Health. 2013;13:458.
37. Bergouignan A, Latouche C, Heywood S, Grace MS, Reddy-Luthmoodoo M, Natoli AK, et al. Frequent interruptions of sedentary time modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: ancillary analysis from randomized clinical trials. Sci Rep. 2016;6:32044.
38. McGarrah RW, Slentz CA, Kraus WE. The effect of vigorous-versus moderate-intensity aerobic exercise on insulin action. Curr Cardiol Rep. 2016;18(12):117.
39. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72(1):83–9.
40. Engmann L, Jin S, Sun F, Legro RS, Polotsky AJ, Hansen KR, et al. Racial and ethnic differences in the polycystic ovary syndrome (PCOS) metabolic phenotype. Am J Obstet Gynecol. 2017;216(5):493.e1–493.e13.
41. Aguilar M, Bhuket T, Torres S, Liu B, Wong RJ. Prevalence of the metabolic syndrome in the United States, 2003–2012. JAMA. 2015;313(19):1973–4.
42. Lee AM, Gurka MJ, DeBoer MD. Trends in metabolic syndrome severity and lifestyle factors among adolescents. Pediatrics. 2016;137(3):e20153177.

43. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059–61.
44. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004;350(23):2362–74.
45. Li C, Ford ES, Zhao G, Mokdad AH. Prevalence of pre-diabetes and its association with clustering of cardiometabolic risk factors and hyperinsulinemia among U.S. adolescents: National Health and Nutrition Examination Survey 2005–2006. Diabetes Care. 2009;32(2):342–7.
46. American Diabetes Association. Statistics about diabetes. Available from: http://www.diabetes.org/diabetes-basics/statistics/.
47. Centers for Disease Control and Prevention. National Diabetes Statistics Report: estimates of diabetes and its burden in the United States. Atlanta: U.S. Department of Health and Human Services; 2014.
48. Hamman RF, Bell RA, Dabelea D, D'Agostino RB Jr, Dolan L, Imperatore G, et al. The SEARCH for Diabetes in Youth study: rationale, findings, and future directions. Diabetes Care. 2014;37(12):3336–44.
49. Witchel SF, Oberfield S, Rosenfield RL, Codner E, Bonny A, Ibanez L, et al. The diagnosis of polycystic ovary syndrome during adolescence. Horm Res Paediatr. 2015;83(6):376–89.
50. Carreau AM, Baillargeon JP. PCOS in adolescence and type 2 diabetes. Curr Diab Rep. 2015;15(1):564.
51. Williams RM, Ong KK, Dunger DB. Polycystic ovarian syndrome during puberty and adolescence. Mol Cell Endocrinol. 2013;373(1–2):61–7.
52. Lewy VD, Danadian K, Witchel SF, Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. J Pediatr. 2001;138(1):38–44.
53. Tfayli H, Arslanian S. Menstrual health and the metabolic syndrome in adolescents. Ann N Y Acad Sci. 2008;1135:85–94.
54. Glueck CJ, Aregawi D, Winiarska M, Agloria M, Luo G, Sieve L, et al. Metformin-diet ameliorates coronary heart disease risk factors and facilitates resumption of regular menses in adolescents with polycystic ovary syndrome. J Pediatr Endocrinol Metab. 2006;19(6):831–42.
55. Arslanian SA, Lewy V, Danadian K, Saad R. Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. J Clin Endocrinol Metab. 2002;87(4):1555–9.
56. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children. J Pediatr Gastroenterol Nutr. 2017;64(2):319–34.
57. Newton KP, Hou J, Crimmins NA, Lavine JE, Barlow SE, Xanthakos SA, et al. Prevalence of prediabetes and type 2 diabetes in children with nonalcoholic fatty liver disease. JAMA Pediatr. 2016;170(10):e161971.
58. Kim G, Giannini C, Pierpont B, Feldstein AE, Santoro N, Kursawe R, et al. Longitudinal effects of MRI-measured hepatic steatosis on biomarkers of glucose homeostasis and hepatic apoptosis in obese youth. Diabetes Care. 2013;36(1):130–6.
59. Cali AM, De Oliveira AM, Kim H, Chen S, Reyes-Mugica M, Escalera S, et al. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? Hepatology. 2009;49(6):1896–903.
60. Nolis T. Exploring the pathophysiology behind the more common genetic and acquired lipodystrophies. J Hum Genet. 2014;59(1):16–23.
61. Abate N. Adipocyte maturation arrest: a determinant of systemic insulin resistance to glucose disposal. J Clin Endocrinol Metab. 2012;97(3):760–3.
62. Gupta N, Asi N, Farah W, Almasri J, Moreno Barrionuevo P, Alsawas M, et al. Clinical features and management of non-HIV related lipodystrophy in children: a systematic review. J Clin Endocrinol Metab. 2016;102(2):363–74. https://doi.org/10.1210/jc.2016-2271.
63. Garg A. Gender differences in the prevalence of metabolic complications in familial partial lipodystrophy (Dunnigan variety). J Clin Endocrinol Metab. 2000;85(5):1776–82.
64. Hussain I, Garg A. Lipodystrophy syndromes. Endocrinol Metab Clin N Am. 2016;45(4):783–97.
65. Gambineri A, Semple RK, Forlani G, Genghini S, Grassi I, Hyden CS, et al. Monogenic polycystic ovary syndrome due to a mutation in the lamin A/C gene is sensitive to thiazolidinediones but not to metformin. Eur J Endocrinol. 2008;159(3):347–53.
66. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000;26(1):76–80.
67. Hudon SE, Coffinier C, Michaelis S, Fong LG, Young SG, Hrycyna CA. HIV-protease inhibitors block the enzymatic activity of purified Ste24p. Biochem Biophys Res Commun. 2008;374(2):365–8.
68. Diker-Cohen T, Cochran E, Gorden P, Brown RJ. Partial and generalized lipodystrophy: comparison of baseline characteristics and response to metreleptin. J Clin Endocrinol Metab. 2015;100(5):1802–10.
69. Reinehr T, Lass N, Toschke C, Rothermel J, Lanzinger S, Holl RW. Which amount of BMI-SDS reduction is necessary to improve cardiovascular risk factors in overweight children? J Clin Endocrinol Metab. 2016;101(8):3171–9.
70. Garnett SP, Gow M, Ho M, Baur LA, Noakes M, Woodhead HJ, et al. Improved insulin sensitivity and body composition, irrespective of macronutrient intake, after a 12 month intervention in adolescents

with pre-diabetes; RESIST a randomised control trial. BMC Pediatr. 2014;14:289.
71. Marson EC, Delevatti RS, Prado AK, Netto N, Kruel LF. Effects of aerobic, resistance, and combined exercise training on insulin resistance markers in overweight or obese children and adolescents: a systematic review and meta-analysis. Prev Med. 2016;93:211–8.
72. Lee S, Deldin AR, White D, Kim Y, Libman I, Rivera-Vega M, et al. Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. Am J Physiol Endocrinol Metab. 2013;305(10):E1222–9.
73. Lee S, Kim Y. Effects of exercise alone on insulin sensitivity and glucose tolerance in obese youth. Diabetes Metab J. 2013;37(4):225–32.
74. Loprinzi PD, Tudor-Locke C. Weight-activity associations with cardiometabolic risk factors among U.S. youth. Physiol Behav. 2015;149:165–8.
75. Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A, et al. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. JAMA. 2012;307(7):704–12.
76. Duvivier BM, Schaper NC, Hesselink MK, van Kan L, Stienen N, Winkens B, et al. Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. Diabetologia. 2016;60(3):490–8.
77. Dunstan DW, Kingwell BA, Larsen R, Healy GN, Cerin E, Hamilton MT, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. Diabetes Care. 2012;35(5):976–83.
78. TODAY Study Group. Effects of metformin, metformin plus rosiglitazone, and metformin plus lifestyle on insulin sensitivity and beta-cell function in TODAY. Diabetes Care. 2013;36(6):1749–57.
79. Savoye M, Caprio S, Dziura J, Camp A, Germain G, Summers C, et al. Reversal of early abnormalities in glucose metabolism in obese youth: results of an intensive lifestyle randomized controlled trial. Diabetes Care. 2014;37(2):317–24.
80. Kendall D, Vail A, Amin R, Barrett T, Dimitri P, Ivison F, et al. Metformin in obese children and adolescents: the MOCA trial. J Clin Endocrinol Metab. 2013;98(1):322–9.
81. Burgert TS, Duran EJ, Goldberg-Gell R, Dziura J, Yeckel CW, Katz S, et al. Short-term metabolic and cardiovascular effects of metformin in markedly obese adolescents with normal glucose tolerance. Pediatr Diabetes. 2008;9(6):567–76.
82. Yanovski JA, Krakoff J, Salaita CG, McDuffie JR, Kozlosky M, Sebring NG, et al. Effects of metformin on body weight and body composition in obese insulin-resistant children: a randomized clinical trial. Diabetes. 2011;60(2):477–85.
83. Clarson CL, Mahmud FH, Baker JE, Clark HE, McKay WM, Schauteet VD, et al. Metformin in combination with structured lifestyle intervention improved body mass index in obese adolescents, but did not improve insulin resistance. Endocrine. 2009;36(1):141–6.

Techniques to Assess Insulin Action in Youth

2

Sara Fleet Michaliszyn and Silva Arslanian

Assessing Insulin Sensitivity

Obesity, metabolic syndrome, prediabetes, and type 2 diabetes all have insulin resistance as a common pathophysiologic contributor. Therefore, the assessment of insulin sensitivity provides both researchers and clinicians with information to objectively evaluate the efficacy of interventions to reverse the escalating rates of these disorders. There are two very important considerations when assessing glucose metabolism: (1) in vivo insulin action or the sensitivity of body tissues to insulin and (2) the pancreatic β(beta)-cell response to glucose. Though investigations in the field of insulin resistance have been ongoing in adults for several decades, the study of insulin resistance in pediatrics has emerged more recently. This chapter will discuss not only the various methodologies for assessing insulin sensitivity but also simple surrogate estimates that can be valuable in large-scale epidemiological studies.

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Techniques

Insulin–Glucose Clamp Technique

The insulin–glucose clamp technique [1] is universally accepted as the gold standard in determining insulin-stimulated whole-body glucose disposal, insulin sensitivity, and pancreatic insulin secretion. This technique is advantageous because (1) both glucose and insulin levels are controlled, or "clamped," at the desired experimental level; (2) both hepatic and peripheral tissue insulin sensitivity can be measured when conjoined with isotope infusion; (3) when combined with indirect calorimetry, substrate oxidation can be calculated with assessment of glucose oxidation and storage; and (4) the test is highly reproducible, with a coefficient of repeatability of 1.0 and low intraindividual coefficient of variation [2–4]. However, only a few pediatric centers in the United States utilize this technique for research purposes, because it is time consuming, labor intensive, and expensive and requires significant participant burden and highly skilled one-to-one research nursing and a "clamper" familiar with the technique. The two variations of the clamp technique are the hyperinsulinemic–euglycemic clamp and the hyperglycemic clamp.

Hyperinsulinemic–Euglycemic Clamp

The hyperinsulinemic–euglycemic clamp is the standard test for determining peripheral insulin

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sensitivity, particularly muscle, which consumes approximately 70% of infused glucose under hyperinsulinemic conditions [5]. The goal of the hyperinsulinemic clamp technique is to acutely raise and maintain the plasma insulin concentration at the desired experimental level through a constant rate infusion of insulin, while holding the plasma glucose concentration constant at euglycemia or at another desired concentration based on experimental design; in other words, both insulin and glucose levels are "clamped" during the procedure. The clamp technique requires the following materials:

1. Vascular access to an antecubital catheter for:
   (a) Infusion of insulin and glucose
   (b) An "arterialized" heated-hand intravenous line for frequent sampling of blood to measure glucose concentration derived from the arterial equivalent of venous data [6]
2. Bedside glucose analyzer, typically a Yellow Springs Instrument (YSI), for the accurate measurement of plasma glucose obtained every 2.5–5 minutes
3. Twenty percent dextrose for intravenous infusion
4. Regular crystalline human insulin infusion, 5–120 mU/m^2·min^{-1} depending on the desired experimental steady-state plasma insulin concentration to be achieved

Typically, 40 mU/m^2·min^{-1} raises the plasma insulin concentration by approximately 100 mU/ml above baseline. The constant rate infusion of insulin gradually increases plasma insulin concentrations, reaching a plateau at around 30 minutes [7]. Consequently, glucose disposal in skeletal muscle and adipose tissue increases while hepatic glucose production is suppressed. The level of steady-state hyperinsulinemia achieved during the clamp may reflect a supraphysiological state and, therefore, may not accurately reflect insulin action and glucose dynamics under "normal" physiological conditions that a dynamic test, such as an oral glucose tolerance test (OGTT), can.

Arterialized plasma glucose concentration is maintained at the desired level by a variable rate infusion of 20% dextrose. Within 2–10 minutes of initiating the insulin infusion, glucose infusion is begun empirically at 1–2 mg/kg/min. In individuals with diabetes and hyperglycemia, the plasma glucose concentration is allowed to decline to euglycemic levels after starting the insulin infusion and before beginning the glucose infusion. Thereafter, the glucose infusion rate is adjusted to maintain a steady plasma glucose concentration. To achieve this, the plasma glucose concentration is measured using a bedside glucose analyzer every 5 minutes (more frequently if glucose metabolism is fast), and the variable rate glucose infusion is adjusted appropriately. In other words, if the actual glucose concentration is lower than desired, the glucose infusion rate is increased and vice versa.

For the novice who is performing a clamp experiment for the first time, altering the glucose infusion rate based on a given plasma glucose reading may seem impossible. However, algorithms have been developed by several groups to facilitate setting the glucose infusion rate appropriate to the measured plasma glucose level and its rate of change [8]. The required calculations for most methods are quite simple and can be performed on a hand-held calculator. Within a 1- to 2-week period, anyone working with someone experienced in the use of the clamp technique can quickly become more proficient than the best computer algorithm. It must be stressed that the pumps used for insulin and dextrose infusion should be accurate and they should be recalibrated frequently to ensure their accuracy.

Steady-state calculations are routinely performed over the last 30 minutes of a 3-hour clamp. The measurements obtained during a hyperinsulinemia–euglycemic clamp are (1) whole-body insulin-stimulated glucose metabolism (M) and/or glucose disposal (Rd), (2) insulin sensitivity, and (3) the metabolic clearance rate (MCR) of insulin (Table 2.1) [1, 9–22]. Under steady-state plasma glucose conditions, the amount of glucose infused is equal to the amount of glucose metabolized (mg/kg/min) by the total body, provided that endogenous (hepatic) glucose production is

Table 2.1 Methods and parameters used for assessing insulin sensitivity in pediatrics

Parameters obtained	Calculation
Hyperinsulinemic–euglycemic clamp [1, 9, 10]	
Glucose disposal (M) (mg/kg/min)	Exogenous glucose infusion + Endogenous glucose production
Insulin sensitivity (mg/kg/min per μ[mu]U/ml)	Glucose disposal/steady – state $\bar{x}$ insulin concentration during last 30 minutes of the clamp
MCR (ml/kg/min)	Insulin infusion/steady − state $\bar{x}$ insulin concentration during last 30 minutes of the clamp − baseline insulin
Hyperglycemic clamp [9, 10]	
First phase insulin (μ[mu]U/ml) [9, 10]	$\bar{x}$ insulin at 2.5, 5, 7.5, 10, and 12.5 minutes after bolus dextrose infusion
Second phase insulin secretion (μ[mu]U/ml) [9, 10]	$\bar{x}$ insulin at 15, 30, 45, 60, 75, 90, 105, and 120 minutes
Glucose disposal (M) (mg/kg/min)	Exogenous glucose − Urinary glucose loss
Insulin sensitivity (mg/kg/min per μ[mu]U/ml)	Glucose disposal/ $\bar{x}$ insulin concentration over the last 60 minutes
Surrogates derived from fasting data [11–14]	
Inverse of insulin	1/fasting insulin
Glucose-to-insulin ratio	Fasting glucose/fasting insulin
The homeostasis model assessment (HOMA-IR)	(Fasting insulin × fasting glucose)/22.5 (glucose in mmol/l, insulin in μ[mu]U/ml)
HOMA for insulin sensitivity (HOMA-IS)	1/HOMA-IR
HOMA2 model	http://www.dtu.ox.ac.uk
Quantitative insulin sensitivity check index (QUICKI)	1/log fasting insulin + log fasting glucose
Surrogates derived from OGTT	
Insulinogenic index [16]	Insulin 30 min − Insulin 0 min/glucose 30 min − Glucose 0 min
C-peptide index [16]	C-peptide 30 min − C-peptide 0 min/glucose 30 min − Glucose 0 min
Area under the curve (AUC) [17]	Trapezoidal method
Whole-body insulin sensitivity index (WBISI) or matsuda index [15]	$10{,}000/\sqrt{(\text{fasting glucose} \times \text{fasting insulin}) \times (\bar{x}\ \text{glucose} \times \bar{x}\ \text{insulin})}$
Insulin sensitivity index − Cederholm [19]	75,000 + (fasting glucose − 2 h glucose) × 1.15 × 180 × 0.19 × body weight 120 × log ($\bar{x}$ insulin × $\bar{x}$ glucose)
Insulin sensitivity index − Stumvoll [21]	0.22 − 0.0032 × BMI − 0.0000645 × 2 h insulin − 0.0037 × 1.5 h glucose
Insulin sensitivity index − Gutt [22]	$\dfrac{75{,}000 + (\text{fasting glucose} - 2\,\text{h glucose}) \times 0.19 \times \text{body weight}}{120 \times \log([\text{fasting insulin} + 2\,\text{h insulin}]/2) \times [\text{fasting glucose} + 2\,\text{h glucose}]/2}$
Insulin sensitivity index − vignon [20]	$\dfrac{(0.137 \times \text{insulin sensitivity index basal}) + \text{insulin sensitivity index at 120 min}}{2}$
Mathematical modeling [18]	B(beta)-cell glucose sensitivity Rate sensitivity Potentiation factor Total insulin output

completely suppressed. [*6,6-$^2H_2$*]glucose stable isotope can be used in combination with insulin clamp studies to quantitate hepatic glucose production [9]. Multiple isotopes can also be infused simultaneously to assess protein and fat metabolism in conjunction with indirect calorimetry (Fig. 2.1) [9]. If endogenous glucose production is not suppressed, it is added to the exogenous glucose infusion rate to provide a measure of total-body glucose uptake [23]. The insulin clamp technique thus allows the assessment of the individual contributions of both hepatic and peripheral tissues to the observed insulin resistance. Peripheral tissue insulin sensitivity is calculated by dividing M and/or Rd by the steady-state plasma insulin concentration (I) (expressed in mg/kg/min per μ[mu]U/ml) [1]. When comparing two groups of individuals with different clamp steady-state insulin concentrations, expressing M is not sufficient; rather M has to be adjusted or expressed for the steady-state plasma insulin concentration, that is, expressed as insulin sensitivity [10]. An index of hepatic insulin sensitivity can be calculated as the inverse of the product of hepatic glucose production and the plasma insulin concentration [24, 25]. The MCR of insulin (ml/kg/min) can be computed by dividing the insulin infusion rate by the increase in circulating insulin concentration during the clamp [1, 10, 26]. In most investigations, the duration of the clamp varies from 2 to 4 hours, and plasma samples are obtained for insulin measurement at 10–15-minute intervals throughout the clamp with at least three to four determinations during the baseline 30-minute period and the last 30 minutes of the clamp steady state.

Hyperglycemic Clamp

The hyperglycemic clamp determines the response of the β(beta)-cell to glucose and quantifies the amount of glucose metabolized during a controlled hyperglycemic condition [1, 27–29]. During a hyperglycemic clamp, the plasma glucose concentration is rapidly raised by a bolus infusion of 25–50% dextrose over a 2-minute period and maintained at a given degree of hyperglycemia for the duration of the experiment using a variable rate infusion of 20% dextrose. In most hyperglycemic clamp experiments, the plasma glucose concentration is raised to and maintained at the desired experimental concentration, often around 225 mg/dl ± 5% for 2 hours (Fig. 2.2a) [28]. During the first 15 minutes of the hyperglycemic clamp, very little of the infused glucose is metabolized; most goes to fill the glucose space. We have been successful in using the following formula to attain a desired hyperglycemia of 225 mg/dl:

$$225 - \left(\text{mean of three baseline fasting plasma glucose concentration}\left[\text{mg/dl}\right]\right) \times \text{weight}\left(\text{kg}\right) \times \text{glucose distribution factor}\left(\text{dl/kg}\right)$$

for calculating the glucose priming bolus (expressed in mg) to raise the plasma glucose concentration to 225 mg/dl. We have used a glucose distribution factor (expressed in dl/kg) of 1.5 for children of normal weight and 1.1 for overweight and obese children. Occasionally, this may overshoot or undershoot the desired glucose plateau. However, since the plasma glucose concentration

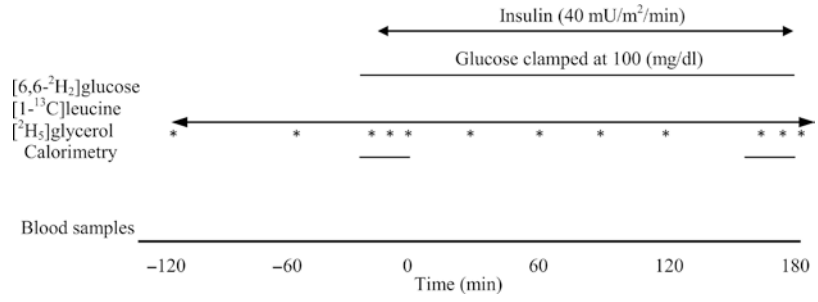

Fig. 2.1 Hyperinsulinemic–euglycemic clamp protocol. (Adapted from Arslanian [9])

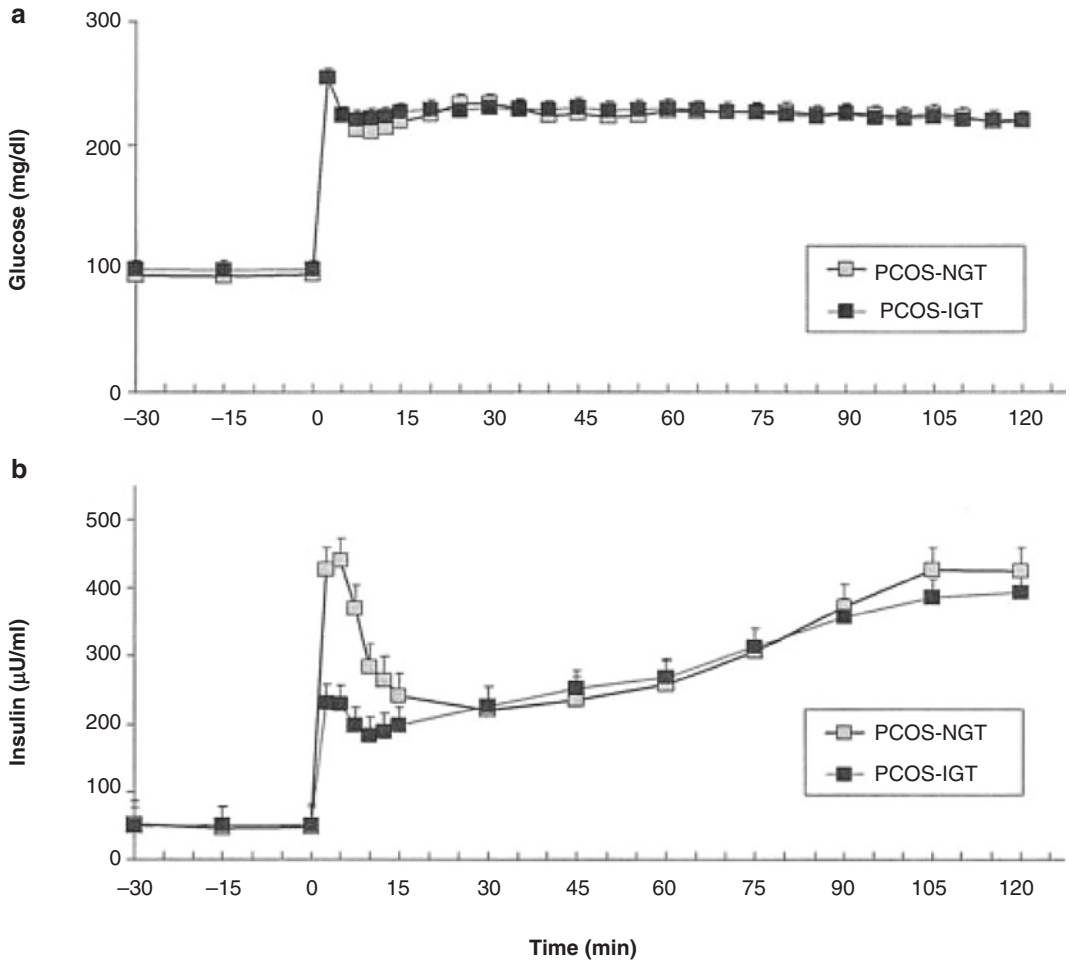

Fig. 2.2 Labels for PCOS NGT are polycystic ovary syndrome with normal glucose tolerance and PCOS-IGT are polycystic ovary syndrome with impaired glucose tolerance. Glucose (upper panel) and insulin concentrations (lower panel) during a hyperglycemic clamp in adolescents with polycystic ovary syndrome (PCOS) with NGT versus IGT. *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance. (Reproduced with permission from Arslanian et al. [28])

is measured at 0.5 minutes after the bolus infusion and every 2.5 minutes thereafter, rapid adjustments in the variable rate infusion of dextrose can be made to achieve the desired hyperglycemia.

During the first 15 minutes of the hyperglycemic clamp, plasma samples are obtained every 2.5 minutes for determination of glucose, insulin, and C-peptide (Fig. 2.3). Thereafter (between 15 and 120 minutes of the clamp), samples are obtained every 5 minutes for glucose and every 15 minutes for insulin and C-peptide determination. Measurement of C-peptide is important, especially if individuals differ in their insulin clearance rates.

The measurements obtained during a hyperglycemic clamp include (1) the plasma insulin and C-peptide response to glucose and (2) whole-body glucose metabolism (Table 2.1). The plasma insulin response to glucose is biphasic, with a rapid first phase in the first 10 minutes after the glucose bolus, followed by a gradual increase in the second phase (Fig. 2.2b) [28]. Traditionally, the first-phase insulin or C-peptide concentration is calculated as the mean of five determinations at times 2.5, 5.0, 7.5, 10.0, and 12.5 minutes of the clamp and the second phase as the mean of eight determinations from 15 to 120 minutes [27, 28, 30]. It is important to note

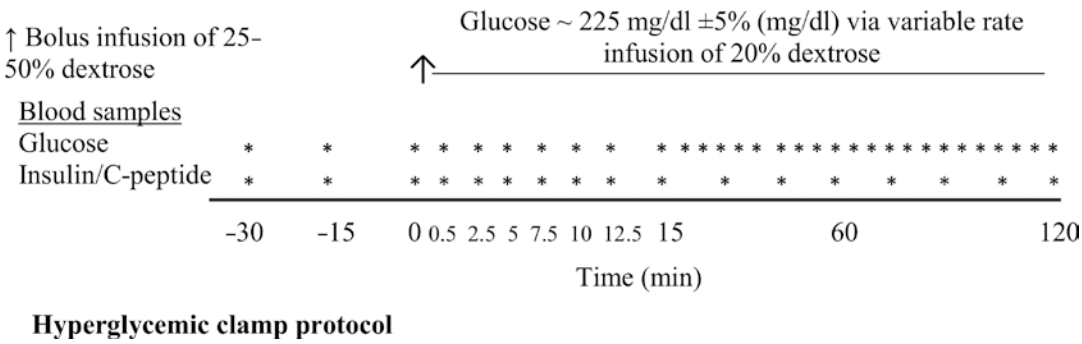

Fig. 2.3 Hyperglycemic clamp protocol

that one can calculate the post-hepatic systemic delivery of insulin if the MCR of insulin is known [31]. However, because the portal insulin concentration cannot be measured in man, the true rate of insulin secretion cannot be determined [29]. Alternate estimates have been proposed using mathematical modeling of C-peptide [32] and C-peptide deconvolution methods in adults [33, 34] and youths [35] to overcome these complexities. Mathematical modeling of insulin secretion during the hyperglycemic clamp provides three components: (1) a basal post-absorptive secretion rate, (2) a dynamic secretion component (glucose sensitivity of first-phase insulin secretion), and (3) a static secretion component (glucose sensitivity of second-phase secretion) [32, 35–38]. Such modeling has been applied in a limited number of studies in pediatrics demonstrating a significant stepwise decline in glucose sensitivity of first-phase secretion from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) [35, 37, 38] to type 2 diabetes [35, 38].

During the last 60 minutes of the hyperglycemic clamp, the rate of glucose metabolism (M) (expressed in mg/kg/min) is calculated from the rate of exogenous glucose infusion minus urinary glucose loss, as renal threshold is exceeded secondary to hyperglycemia. Hepatic glucose production is completely suppressed in healthy individuals under conditions of hyperglycemia. However, this may not be the case in patients with diabetes associated with severe insulin resistance; the concurrent use of isotopes will allow for the determination of hepatic glucose production in such individuals. In addition to obtaining M, an index of insulin sensitivity can be calculated by dividing M by the plasma insulin concentration over the last 60 minutes of the clamp. This calculation assumes a linear relationship between the plasma insulin concentration and M, which is a simplification of a more complex relationship. In our studies, insulin sensitivity index, calculated during the last 60 minutes of the hyperglycemic clamp, and insulin sensitivity from the euglycemic clamp correlated strongly ($r = 0.90$, $p < 0.001$) [39]; however, others have reported lower correlations, ranging from 0.45 to 0.65 [40].

Use of Isotopes and Indirect Calorimetry with the Insulin–Glucose Clamp Technique

Substrate turnover and the effects of insulin on different metabolic pathways can be determined during insulin clamp experiments with the concurrent use of isotopes. We have used multiple stable isotopes simultaneously with clamp experiments to study glucose, protein, and fat metabolism at baseline and during hyperinsulinemia [26, 41] (see Wolfe [42] and Kim et al. [43], for a review of isotope tracers in biomedicine).

Indirect calorimetry is often used in conjunction with the hyperinsulinemic–euglycemic clamp to provide information on intracellular rates of glucose and fat oxidation during different insulin concentrations and rates of whole-body glucose uptake (see Schutz [44] for a review). Prior to calculating substrate utilization, rates of protein oxidation must be known and routinely estimated from urinary nitrogen excretion. The use of urinary nitrogen assumes that this measurement accurately reflects protein oxi-

dation and is unaltered by the clamp technique. The use of indirect calorimetry combined with clamp experiments has enabled the investigation of glucose-fatty acid competition in explaining pubertal insulin resistance [23, 45] and racial differences in resting fat oxidation [46].

The Frequently Sampled Intravenous Glucose Tolerance Test with Minimal Model Analysis

Bergman and colleagues introduced the minimal model method in an attempt to provide a simpler technique for in vivo assessment of metabolic function of islet cells and insulin-sensitive tissues [47–49]. After an overnight fast, a bolus of 50% glucose solution (0.3 g/kg body weight) is infused over a 0–1 minute period. Basal samples are collected for 15 minutes at −15, −10, −5, and − 1 minutes, and post-injection samples are obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes [31], although the sampling times have been altered in different publications [50, 51] A computer mathematical minimal model (MINMOD) provides an indirect measure of insulin sensitivity based on glucose utilization and insulin kinetics obtained during the frequently sampled intravenous glucose tolerance test (FSIGTT) [48]. Two modifications were introduced to ensure an endogenous insulin response of sufficient magnitude to enhance the ability of the computer to estimate the model parameters. The first modification was the tolbutamide-enhanced FSIGTT [51]. This modification involves the intravenous injection of tolbutamide (300 mg in normal weight individuals, 500 mg in overweight or obese subjects) at 20 minutes, with additional blood samples obtained at 23, 24, and 27 minutes. The insulin-modified FSIGTT was an attempt to use the FSIGTT model in patients with diabetes [52, 53]. Insulin (0.03 units/kg) is administered as an intravenous (IV) bolus at 20 minutes of the FSIGTT with additional sampling similar to the tolbutamide protocol. Insulin sensitivity estimates from both protocols correlate with each other in the same individual; however, they are quantitatively discrepant [50, 52].

Several other modifications of the minimal model include radioactive or stable isotope labeling of the IVGTT [54, 55], which has also been employed in pediatrics [56, 57], and decreasing the total number of samples from $n = 30$ to $n = 12$ [58]. It is important to note that it is scientifically more accepted to use the full sample ($n = 30$) protocol in studies with a smaller sample size or where small differences in insulin sensitivity need to be detected, whereas the small sample protocol ($n = 12$) is appropriate for large-scale epidemiological studies [59].

The parameters derived from the MINMOD FSIGTT are (1) insulin sensitivity index (S_I), which is the increase in fractional glucose disappearance per unit insulin increase, that is, the ability of insulin to enhance glucose uptake and to inhibit hepatic glucose production; (2) insulin-independent fractional glucose disappearance (S_G), which is the increase in fractional disappearance of glucose per unit increase of glucose at basal insulin; and (3) β(beta)-cell function with the most commonly used index being acute insulin release (AIR_G), an index of first-phase insulin secretion (Table 2.1). Mathematical modeling of β(beta)-cell function yields two parameters describing the sensitivity to glucose of first- and second-phase insulin secretion, respectively ($\phi[phi]_1$, $\phi[phi]_2$), and (4) fractional insulin clearance [31].

An advantage of the MINMOD FSIGTT is that it is easier than the glucose clamp method because it is less labor intensive, steady-state concentrations are not required, and there are no intravenous infusions that require constant adjustment. However, despite the extensive use of FSIGTT in adults, repeat experiments demonstrate that the interday coefficients of variation were 20.2 ± 3.2% (range 6–44%) for S_I, 25.1 ± 8.8% for S_G, and 20.1 ± 3.5% for first-phase insulin secretion [59]. Though S_I estimates from FSIGTT correlate with insulin sensitivity measured during the hyperinsulinemic–euglycemic clamp, S_I from the tolbutamide FSIGTT is estimated to be 13 ± 6% lower and 44 ± 4% lower during the insulin FSIGTT compared with clamp S_I [50].

There are several inconsistencies and wide variations worth mentioning in using the FSIGTT in pediatrics. In 1990, the FSIGTT protocol in pediatrics was shortened to 90 minutes in children, with a tolbutamide dose of 5 mg/kg [60].

Though S_I derived from the 90-minute FSIGTT and the actual FSIGTT correlated, the fractional standard deviation for S_I from the former was significantly greater than that from the full study. Furthermore, 5% of participants had insufficient insulin response during the FSIGTT. The authors mentioned that there were "several shortcomings in the measurement of insulin sensitivity by the minimal model" [60]. To address these shortcomings, several methodological changes in pediatrics have included variability in sampling schedules and dosing concentrations of both glucose and tolbutamide. For example, sampling schedules have varied from 11 to 13 [61–64] to 18 to 19 [65–67] to 22 to 24 [68, 69]. The variability in glucose bolus has spanned from 0.3 g/kg [63, 67, 69] to 11.4 g/m^2 [65] to 100 gm without accounting for body weight [64], tolbutamide from 5 mg/kg [60, 67] to 125 mg/m^2 [65, 66] to 300 mg/1.73 m^2 [62–64], and insulin from 0.02 to 0.03 U/kg [61, 70, 71]. Almost all of the aforementioned studies using IFGTT report injecting the glucose bolus over the course of 0.5–1 minutes. This is concerning as it remains unclear if infusing large volumes of 25% dextrose solutions is feasible in obese children weighing more than 100 kg who require large volumes exceeding 100 cc. Furthermore, in a report by Conwell et al., obese children who had repeat FSIGTT on two or three different occasions had a 30–40% variability in their S_I [71]. Collectively, because of such inconsistencies in the FSIGTT protocol in the pediatric age group and the limited comparison to the gold standard of the glucose clamp technique, the reliability of the data remains questionable. Due to these limitations, suggestions for improvement have ensued [72, 73].

Oral Glucose Tolerance Test

The standard oral glucose tolerance test (OGTT) is one of the earliest employed measures to assess insulin sensitivity in vivo. Seminal work by Reaven and Miller revealed a heterogeneity of response, from mild glucose intolerance to severe diabetes, and formed the foundation of the modern diagnostic use of the OGTT [74]. As such, the OGTT is widely used in clinical practice to diagnose glucose intolerance and type 2 diabetes [75] and is recommended by the American Diabetes Association and the World Health Organization (WHO) as a diagnostic tool. The procedure begins after an overnight fast by ingesting (within 5–10 minutes) a standard oral glucose load (1.75 g/kg body weight; max. 75 g). Blood is sampled every 15–30 minutes for 2–5 hours for measurement of plasma glucose, insulin, and C-peptide concentrations. Conceptually, it is believed that the higher the glucose concentrations during the OGTT, the lower the insulin sensitivity. Though in theory this may seem sensible, there are a number of considerations that must be addressed. The OGTT is typically poorly reproducible, the rate of gastrointestinal absorption of glucose varies considerably from one person to another, glucose and insulin levels are constantly changing and are not "clamped," hepatic glucose production is unknown, and, lastly, glucose uptake by the peripheral tissues is affected independently by hyperglycemia and hyperinsulinemia [76–78]. In our hands, the reproducibility of repeat OGTT (interval between 2 OGTTs 1–25 days) in overweight 8- to 17-year-old otherwise healthy children was poor [79]. Fasting blood glucose had higher reproducibility compared with the 2-hour glucose. The percent positive agreement between the first and second OGTT was low, 22.2% for impaired fasting glucose and 27.3% for IGT. Among the youth with IGT during the first OGTT, only 30% had IGT during the second OGTT.

Surrogate Estimates of Insulin Sensitivity and Secretion

The aforementioned techniques, especially the clamp technique, require trained staff and are costly and labor intensive, invasive, and not feasible with large-scale epidemiological studies or interventional longitudinal studies with repeated measurements. Therefore, simple but reliable sur-

rogate measures of insulin sensitivity and secretion are warranted. These surrogate measures are based on insulin and/or glucose samples taken in the fasting state (Table 2.1).

Fasting Estimates of Insulin Sensitivity and Secretion

Surrogate indices of insulin sensitivity using fasting parameters include (1) fasting insulin (I_F), (2) the inverse of fasting insulin ($1/I_F$), (3) fasting glucose to insulin ratio (G_F/I_F), (4) HOMA for insulin sensitivity (HOMA-IS) or resistance (HOMA-IR) [14, 80] and the HOMA2 Calculator at http://www.dtu.ox.ac.uk, and (5) the quantitative insulin sensitivity check index (QUICKI) [13] (Table 2.1).

In an initial study of 156 children and adolescents with a spectrum of body mass index (BMI) from normal to obese, we demonstrated that insulin sensitivity measured using the hyperinsulinemic–euglycemic clamp correlated strongly with fasting insulin ($r = -0.92$), G_F/I_F ($r = 0.92$), HOMA-IS ($r = 0.91$), and QUICKI ($r = 0.91$) [12]. In a follow-up study of 188 obese adolescents spanning the spectrum of glucose tolerance from NGT to IGT to diabetes [11], $1/I_F$, G_F/I_F, HOMA-IS, and QUICKI strongly correlated with hyperinsulinemic–euglycemic clamp-measured insulin sensitivity in the total group ($r \geq 0.78$) and in each glucose tolerance category, as depicted in Fig. 2.4. In our studies and others, the calculation of HOMA or QUICKI did not have any added advantage over the simple measure of $1/I_F$ [11, 12, 81] because they did not show stronger correlations or a better utility for prediction of insulin sensitivity [11]. Other studies examining surrogate indices against clamp-derived measures in the pediatric population demonstrated the usefulness of fasting-derived surrogate indices in estimating insulin sensitivity in prepubertal, pubertal lean, overweight, and obese youth [12, 81–83]. It is critical to stress that, despite the strong correlations between surrogate estimates of insulin sensitivity and clamp measured insulin sensitivity, these are research tools and none of these surrogate measure should be used clinically to diagnose insulin resistance in any single individual (i.e., using cutoffs to define if a patient has insulin resistance), because fasting insulin concentrations and fasting surrogates overlap greatly across quartiles of clamp-measured insulin sensitivity [11] (Fig. 2.5).

In studies comparing surrogate indices against insulin sensitivity derived from MINMOD FSIVGTT, correlations with HOMA-IR ($r = -0.4$) and with fasting insulin ($r = -0.4$) were significant [84, 85]. In agreement with our findings [11], Rössner et al. [85] indicated that QUICKI showed similar results to HOMA-IR and, in a later publication, indicated that HOMA and QUICKI have an equal ability to predict insulin sensitivity [86]. In contrast, however, the correlations reported [85] for the MINMOD S_I were lower than reported in our hyperinsulinemic–euglycemic clamp studies. A relatively small study in 18 obese children (7 prepubertal and 11 pubertal) who repeated the FSIVGTT up to three times showed high correlations ($r > 0.85$) between MINMOD S_I and HOMA-IR, QUICKI, and G_F/I_F [71]. However, another study showed virtually no relationship between QUICKI and MINMOD S_I in lean, prepubertal children despite significant correlations between MINMOD S_I and HOMA-IR and IF [84]. In interpreting the reported differences in the correlations between surrogate estimates and insulin sensitivity, whether measured by clamp or FSIVGTT, factors that could potentially explain these differences include differing study populations (age, Tanner stage, race, glucose tolerance status) and differences in experimental methods.

It must be stressed, however, that there are limitations to using fasting glucose and insulin [87]. First, because of significant variability in insulin measurements across various laboratories, as demonstrated by the American Diabetes Association Task Force [88], there will be important differences in these estimates among different centers. Therefore, these estimates are center or laboratory specific and cannot be applied uniformly, particularly in the clinical setting. Second, whereas the fasting glucose–insulin ratio may be a good proxy of insulin sensitivity in nondiabetic children, this will not be the case when fasting

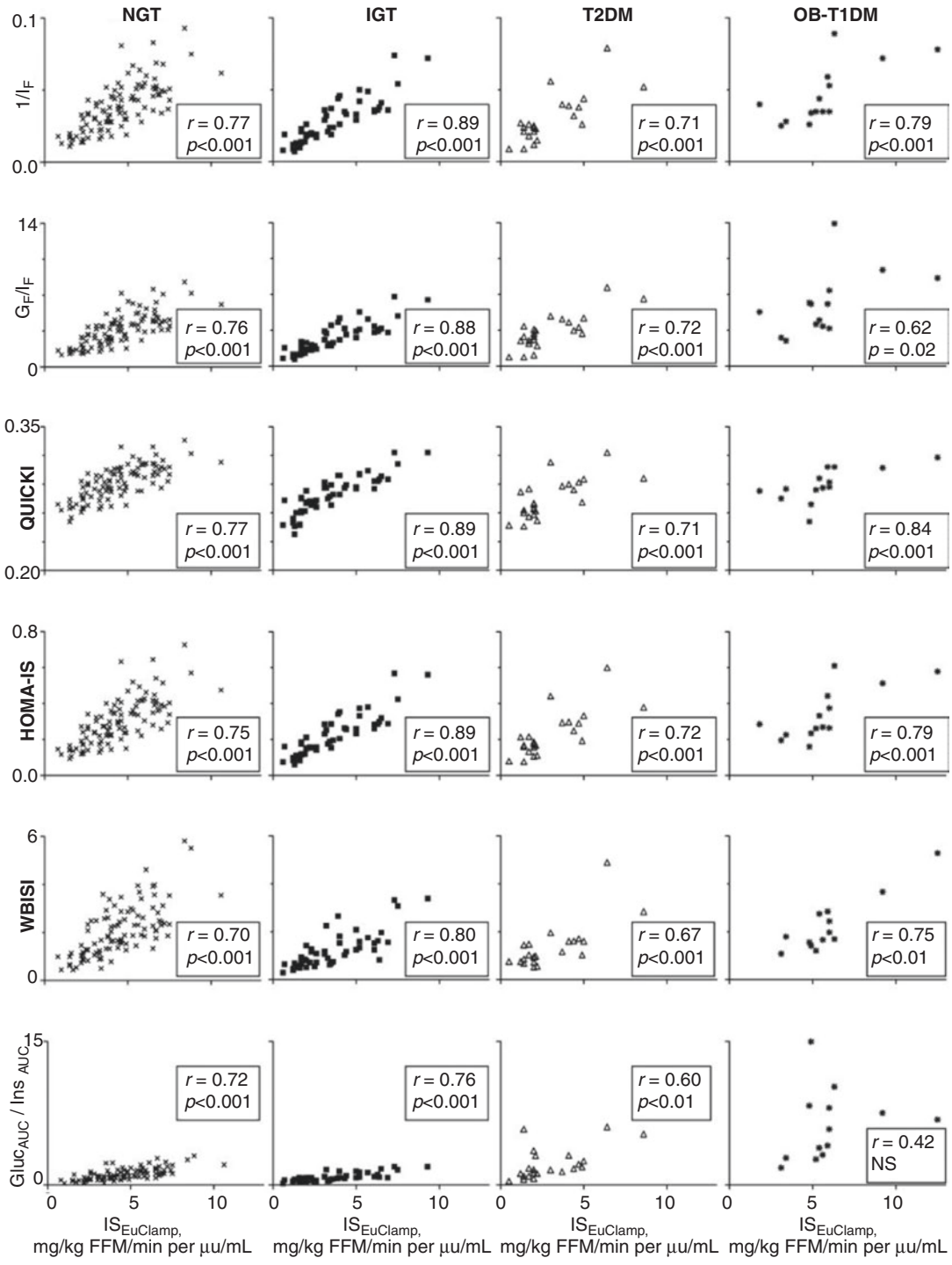

Fig. 2.4 Correlations between clamp-measured insulin sensitivity and surrogate estimates of fasting and OGTT-derived insulin sensitivity in overweight/obese adolescents shown separately in each group: NGT, IGT, T2DM, and OB-T1DM. *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance, *T2DM* type 2 diabetes mellitus, *OB-T1DM* obese type 1 diabetes mellitus. (Reproduced with permission from George et al. [11])

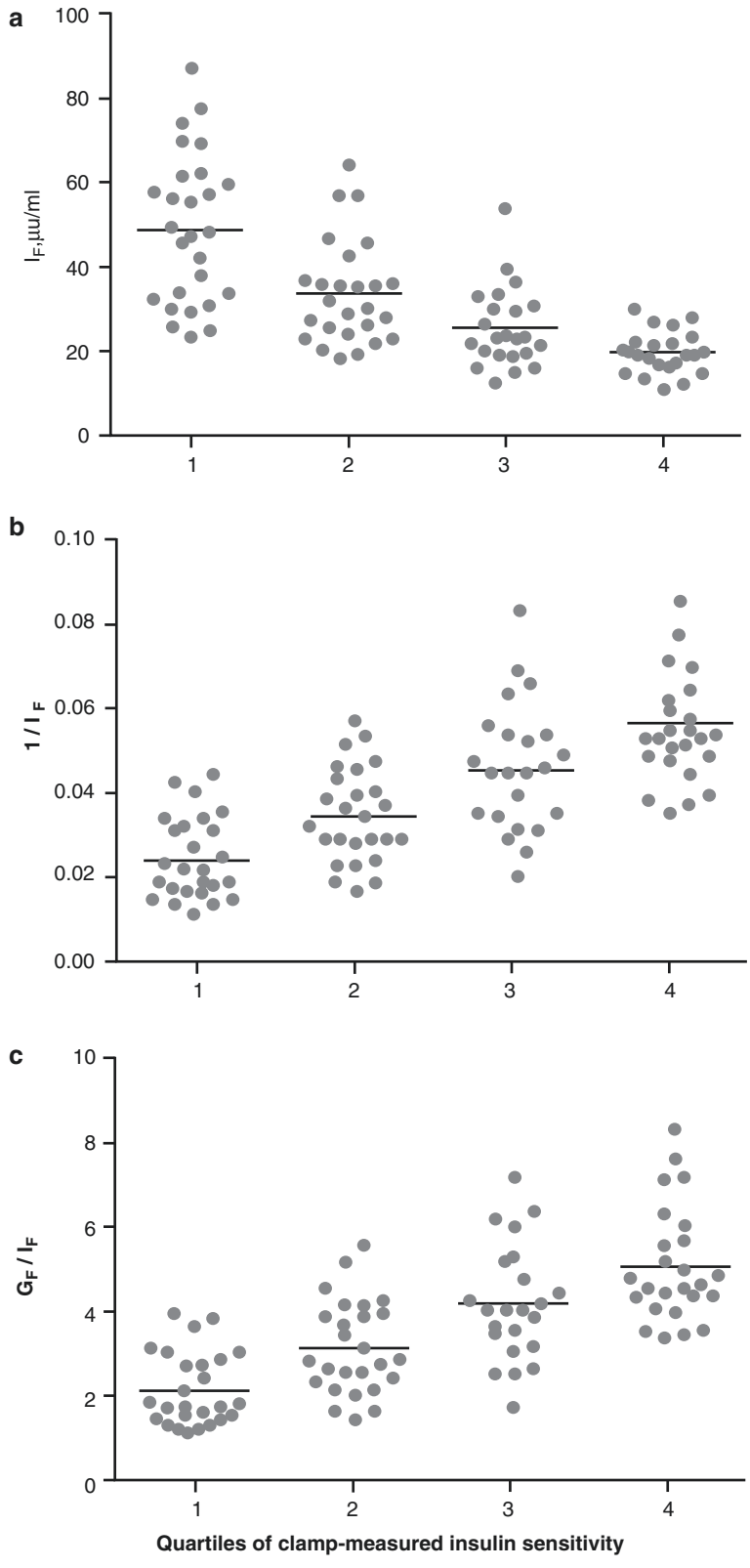

Fig. 2.5 Distribution of I_F (**a**), 1/ I_F (**b**), and G_F/I_F (**c**) according to quartiles of clamp-measured insulin sensitivity in normal glucose tolerance (NGT) subjects (n = 101). Black bars represent mean, and points represent individual values. Quartiles of clamp-measured insulin sensitivity were defined as quartile 1 (most insulin resistant), at or below 25th percentile (insulin sensitivity ≤3.1 mg/kg fat-free mass/min per μ[mu]U/ml); 2, higher than 25th to at or below 50th percentile (>3.1 to ≤4.4); 3, higher than 50th to at or below 75th percentile (>4.4 to ≤5.8); and 4 (most insulin sensitive), higher than 75th percentile (>5.8). (Reproduced with permission from George et al. [11])

glucose is elevated as in children with diabetes. This will result in a falsely elevated ratio that does not truly reflect increased insulin sensitivity [89]. Alternatively, as fasting insulin declines due to a progressively failing pancreas, the increased glucose–insulin ratio will give the false impression of improved insulin sensitivity [89].

Estimates of Insulin Sensitivity and Secretion During the Oral Glucose Tolerance Test

While the clinical use of the OGTT to determine glucose tolerance status is well accepted and widely utilized, its use for estimating indices of insulin sensitivity and secretion is mostly applicable to large-scale studies where more sophisticated methods are not feasible and where an OGTT is mandated per experimental protocol. The most frequently used OGTT-derived estimate of insulin sensitivity is the Whole-Body Insulin Sensitivity Index (WBISI) described by Matsuda and DeFronzo [15]. This index represents a composite of both hepatic and peripheral tissue sensitivity to insulin and is derived from plasma glucose (expressed in mg/dl) and insulin (expressed in μ[mu]U/ml) measurements from the fasting state and during the OGTT (Table 2.1). In adults, this index correlates strongly with the direct measure of insulin sensitivity derived from the euglycemic insulin clamp in normally glucose-tolerant individuals, but the correlation declines in individuals with IGT or type 2 diabetes [15]. In our study of 188 overweight/obese adolescents, WBISI showed a correlation coefficient of 0.77 with clamp insulin sensitivity, but the correlation was weakest ($r = 0.67$) in adolescents with type 2 diabetes (Fig. 2.4) [11]. Furthermore, the correlations between WBISI and clamp insulin sensitivity depends on gender, with males demonstrating the lowest association ($r = 0.55$) [11]. In a smaller study of 38 obese youth with NGT and IGT, the correlation between WBISI and glucose disposal during the clamp was 0.78 [82]. The reported correlations between insulin sensitivity derived from the FSIGTT and WBISI are weaker ($r = 0.67$) in overweight youth compared to those with clamp [90]. In 2010, a reduced sample WBISI using 0-, 60-, and 120-minute or 0- and 120-minute measurements provided strong correlations with the rate of insulin-stimulated glucose Rd during the euglycemic clamp in adults [91]. To our knowledge, this has not been evaluated in pediatrics.

Because an OGTT is routinely performed in most metabolic and epidemiological studies, another advantage is the readily available index of β(beta)-cell function. The insulinogenic index is calculated as the ratio of $\Delta(\text{Delta})I_{30}/\Delta(\text{Delta})G_{30}$ (i.e., the difference in insulin and glucose concentrations between 30 and 0 minutes) and the C-peptide index ($\Delta[\text{Delta}]C_{30}/\Delta[\text{Delta}]G_{30}$) [81, 92]. Other assessments of β(beta)-cell function include the 15-minute insulinogenic index and/or C-peptide index [16, 79], the area under the curve (AUC) [17], and the use of mathematical modeling [18, 73]. In a small study of 26 prepubertal normoglycemic children, the correlations between first-phase insulin or C-peptide secretion derived from the hyperglycemic clamp and OGTT-derived measures of insulin secretion were stronger for the 15-minute index than for the 30-minute index and stronger for C-peptide index ($r = 0.73$) than for insulin index ($r = 0.49$) [16]. In 185 overweigh/obese adolescents with varying glucose tolerance, we found that $\Delta(\text{Delta})I_{30}/\Delta(\text{Delta})G_{30}$ and $\Delta(\text{Delta})C_{30}/\Delta(\text{Delta})G_{30}$ decline progressively from NGT to IGT (~29% for both) and from IGT to type 2 diabetes (~57% for $\Delta[\text{Delta}]I_{30}/\Delta[\text{Delta}]G_{30}$ and ~40% for $\Delta[\text{Delta}]C_{30}/\Delta[\text{Delta}]G_{30}$) translating to an overall decline in insulinogenic index from NGT to type 2 diabetes of ~70% and in C-peptide index by ~57% [93].

Mathematical models to analyze standardized [37, 38, 94] and non-standardized tests [36, 56, 73, 95], such as the OGTT, have been developed to assess β(beta)-cell function. Parameters derived using the model developed by Mari and Ferrannini include β(beta)-cell glucose sensitivity, rate sensitivity, and potentiation. A model-based index of insulin sensitivity (derived from the OGTT, termed OGIS) may also be calculated using the plasma glucose and insulin concentrations [96], which have shown modest associations with insulin sensitivity derived from the

hyperinsulinemic–euglycemic clamp [97]. Using this model in 255 obese youth with NGT, IGT, and diabetes, we demonstrated that β(beta)-cell glucose sensitivity was 30% and 65% lower in youth with impaired glucose tolerance and type 2 diabetes whereas rate sensitivity was 40% lower in youth with type 2 diabetes [35].

Lastly, the OGTT provides the opportunity to express β(beta)-cell function (insulinogenic index or C-peptide index) relative to insulin sensitivity (fasting surrogates or WBISI) to calculate the oral disposition index (oDI). The oDI is demonstrated to be the strongest metabolic predictor of progression from IGT to diabetes in adults, even more than insulin sensitivity, fasting glucose, or OGTT alone [92, 98, 99]. In our study of 185 overweight/obese youth with varying glucose tolerance, oDI mirrored clamp-derived DI showing that β(beta)-cell function relative to insulin sensitivity declines from NGT to IGT to type 2 diabetes. Moreover, oDI correlated strongly with clamp DI in the overall group ($r \geq 0.74$) and within each glucose tolerance category [93]. Lastly, oDI predicted 2-hour OGTT glucose similar to that of clamp DI. Our observations in pediatrics regarding the utility of OGTT-calculated oDI advanced the scientific literature in pediatrics by providing the opportunity to use oDI in the TODAY (Treatment Options for type 2 diabetes in Adolescents and Youth) multicenter trial. These studies demonstrated that oDI, or β(beta)-cell function relative to insulin sensitivity, is an important determinant of HbA1c at randomization [100]. Moreover, initial β(beta)-cell reserve or oDI was an independent predictor of glycemic durability, with oDI declining rapidly (~27% by 6 months, ~56% by 24 months, and ~67% by 36 months) in those patients who failed to maintain glycemic control [101].

More complicated formulas evolved over the years using OGTT parameters to estimate insulin sensitivity [19–22] (Table 2.1). These are sparingly used in pediatrics because they do not necessarily add any advantage over the more frequently used and validated ones in the pediatric age group.

Methodologies Not Currently Practiced in Pediatrics

Some methodologies of assessing insulin sensitivity are not popular in pediatrics due to the risk that accompanies the protocol and/or the complexity of the protocol and hence will not be discussed. Suffice it to say that these methodologies include the insulin tolerance test, which examines the rate at which glucose falls and the nadir it reaches after a bolus of intravenous insulin [102]. Its major disadvantage is hypoglycemia and counterregulatory hormone response that affect glucose metabolism. Another is the insulin suppression test, which evaluates disposal of an intravenously administered glucose load by a constant and fixed level of hyperinsulinemia [103, 104]. The major disadvantage is that it requires a quadruple infusion of epinephrine, propranolol, insulin, and glucose. For a more thorough review of these methodologies, the reader is referred to the following reference [105].

Summary

Despite the multiplicity of methods available for adults, techniques applicable to children and adolescents are limited. In this chapter, we have discussed a variety of methods currently available for estimating insulin sensitivity/resistance and β(beta)-cell function in pediatrics. The state-of-the art clamp methodology—though time consuming, costly, and labor intensive—should be used in patient-oriented research where specific metabolic pathways of insulin action and mechanisms are being investigated. In large-scale epidemiological, observational, or interventional trials where repeated measurements are needed, surrogate estimates of insulin sensitivity and β(beta)-cell function, fasting or OGTT derived, which have been validated against the gold standard are more applicable. Other available methodologies (e.g., FSIVGTT) need to be refined, standardized, and validated across a spectrum of glucose tolerance in children.

References

1. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Phys. 1979;237(3):E214–23.
2. Morris AD, Ueda S, Petrie JR, Connell JM, Elliott HL, Donnelly R. The euglycaemic hyperinsulinaemic clamp: an evaluation of current methodology. Clin Exp Pharmacol Physiol. 1997;24(7):513–8.
3. Soop M, Nygren J, Brismar K, Thorell A, Ljungqvist O. The hyperinsulinaemic-euglycaemic glucose clamp: reproducibility and metabolic effects of prolonged insulin infusion in healthy subjects. Clin Sci. 2000;98(4):367–74.
4. Bokemark L, Froden A, Attvall S, Wikstrand J, Fagerberg B. The euglycemic hyperinsulinemic clamp examination: variability and reproducibility. Scand J Clin Lab Invest. 2000;60(1):27–36.
5. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13c nuclear magnetic resonance spectroscopy. N Engl J Med. 1990;322(4):223–8.
6. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metab Clin Exp. 1981;30(9):936–40.
7. Arslanian SA, Heil BV, Becker DJ, Drash AL. Sexual dimorphism in insulin sensitivity in adolescents with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1991;72(4):920–6.
8. Sheen AJ, Paquot N, Castillo MJ, Lefebvre PJ. How to measure insulin action in vivo. Diabetes Metab Rev. 1994;10:151–88.
9. Arslanian SA. Clamp techniques in paediatrics: what have we learned? Horm Res. 2005;64(Suppl 3):16–24.
10. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J. Hyperinsulinemia in african-american children: decreased insulin clearance and increased insulin secretion and its relationship to insulin sensitivity. Diabetes. 2002;51(10):3014–9.
11. George L, Bacha F, Lee S, Tfayli H, Andreatta E, Arslanian S. Surrogate estimates of insulin sensitivity in obese youth along the spectrum of glucose tolerance from normal to prediabetes to diabetes. J Clin Endocrinol Metab. 2011;96(7):2136–45.
12. Gungor N, Saad R, Janosky J, Arslanian S. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr. 2004;144(1):47–55.
13. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402–10.
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
15. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22(9):1462–70.
16. Bacha F, Gungor N, Arslanian SA. Measures of beta-cell function during the oral glucose tolerance test, liquid mixed-meal test, and hyperglycemic clamp test. J Pediatr. 2008;152(5):618–21.
17. Allison DB, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of areas under curves in diabetes research. Diabetes Care. 1995;18(2):245–50.
18. Mari A, Ferrannini E. Beta-cell function assessment from modelling of oral tests: an effective approach. Diabetes Obes Metab. 2008;10(Suppl 4):77–87.
19. Cederholm J, Wibell L. Insulin release and peripheral sensitivity at the oral glucose tolerance test. Diabetes Res Clin Pract. 1990;10(2):167–75.
20. Avignon A, Boegner C, Mariano-Goulart D, Colette C, Monnier L. Assessment of insulin sensitivity from plasma insulin and glucose in the fasting or post oral glucose-load state. Int J Obes Relat Metab Disord. 1999;23(5):512–7.
21. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295–301.
22. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract. 2000;47(3):177–84.
23. Arslanian S, Suprasongsin C. Glucose-fatty acid interactions in prepubertal and pubertal children: effects of lipid infusion. Am J Phys. 1997;272(4 Pt 1):E523–9.
24. Bacha F, Saad R, Gungor N, Arslanian SA. Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care. 2004;27(2):547–52.
25. Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab. 2002;283(6):E1135–43.
26. Arslanian S, Suprasongsin C. Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. J Clin Endocrinol Metab. 1997;82(10):3213–20.
27. Arslanian S, Suprasongsin C, Janosky JE. Insulin secretion and sensitivity in black versus white prepubertal healthy children. J Clin Endocrinol Metab. 1997;82(6):1923–7.
28. Arslanian SA, Lewy VD, Danadian K. Glucose intolerance in obese adolescents with polycystic

ovary syndrome: roles of insulin resistance and beta-cell dysfunction and risk of cardiovascular disease. J Clin Endocrinol Metab. 2001;86(1):66–71.
29. Hovorka R, Jones RH. How to measure insulin secretion. Diabetes Metab Rev. 1994;10(2):91–117.
30. Saad RJ, Danadian K, Lewy V, Arslanian SA. Insulin resistance of puberty in African-American children: lack of a compensatory increase in insulin secretion. Pediatr Diabetes. 2002;3(1):4–9.
31. Del Prato S, Ferrannini E, Defronzo RA. Evaluation of insulin sensitivity in man. In: Clarke WL, Larner J, Pohl SL, editors. Methods in diabetes research. New York: John Wiley & Sons, Inc; 1986. p. 36–76.
32. Bonadonna RC, Stumvoll M, Fritsche A, Muggeo M, Haring H, Bonora E, et al. Altered homeostatic adaptation of first- and second-phase beta-cell secretion in the offspring of patients with type 2 diabetes: studies with a minimal model to assess beta-cell function. Diabetes. 2003;52(2):470–80.
33. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from c-peptide levels. Comparison of individual and standard kinetic parameters for c-peptide clearance. Diabetes. 1992;41(3):368–77.
34. Polonsky KS. Lilly Lecture 1994. The beta-cell in diabetes: from molecular genetics to clinical research. Diabetes. 1995;44(6):705–17.
35. Michaliszyn SF, Mari A, Lee S, Bacha F, Tfayli H, Farchoukh L, et al. Beta-cell function, incretin effect, and incretin hormones in obese youth along the span of glucose tolerance from normal to prediabetes to type 2 diabetes. Diabetes. 2014;63(11):3846–55.
36. Mari A, Camastra S, Toschi E, Giancaterini A, Gastaldelli A, Mingrone G, et al. A model for glucose control of insulin secretion during 24 h of free living. Diabetes. 2001;50(Suppl 1):S164–8.
37. Cali AM, Bonadonna RC, Trombetta M, Weiss R, Caprio S. Metabolic abnormalities underlying the different prediabetic phenotypes in obese adolescents. J Clin Endocrinol Metab. 2008;93(5):1767–73.
38. Weiss R, Caprio S, Trombetta M, Taksali SE, Tamborlane WV, Bonadonna R. Beta-cell function across the spectrum of glucose tolerance in obese youth. Diabetes. 2005;54(6):1735–43.
39. Sjaarda L, Lee S, Tfayli H, Bacha F, Bertolet M, Arslanian S. Measuring beta-cell function relative to insulin sensitivity in youth: does the hyperglycemic clamp suffice? Diabetes Care. 2013;36(6):1607–12.
40. Uwaifo GI, Parikh SJ, Keil M, Elberg J, Chin J, Yanovski JA. Comparison of insulin sensitivity, clearance, and secretion estimates using euglycemic and hyperglycemic clamps in children. J Clin Endocrinol Metab. 2002;87(6):2899–905.
41. Saad RJ, Keenan BS, Danadian K, Lewy VD, Arslanian SA. Dihydrotestosterone treatment in adolescents with delayed puberty: does it explain insulin resistance of puberty? J Clin Endocrinol Metab. 2001;86(10):4881–6.
42. Wolfe RR. Stable isotope tracers in biomedicine. Principles and practice of kinetic analysis. New York: Wiley-Liss; 1992.
43. Kim IY, Suh SH, Lee IK, Wolfe RR. Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research. Exp Mol Med. 2016;48:e203.
44. Schutz Y. The basis of direct and indirect calorimetry and their potentials. Diabetes Metab Rev. 1995;11(4):383–408.
45. Arslanian SA, Kalhan SC. Correlations between fatty-acid and glucose-metabolism – potential explanation of insulin-resistance of puberty. Diabetes. 1994;43(7):908–14.
46. Lee S, Arslanian SA. Fat oxidation in black and white youth: a metabolic phenotype potentially predisposing black girls to obesity. J Clin Endocrinol Metab. 2008;93(11):4547–51.
47. Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. Am J Phys. 1979;236(6):E667–77.
48. Bergman RN. Lilly Lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes. 1989;38(12):1512–27.
49. Bergman RN, Hope ID, Yang YJ, Watanabe RM, Meador MA, Youn JH, et al. Assessment of insulin sensitivity in vivo: a critical review. Diabetes Metab Rev. 1989;5(5):411–29.
50. Saad MF, Steil GM, Kades WW, Ayad MF, Elsewafy WA, Boyadjian R, et al. Differences between the tolbutamide-boosted and the insulin-modified minimal model protocols. Diabetes. 1997;46(7):1167–71.
51. Yang YJ, Youn JH, Bergman RN. Modified protocols improve insulin sensitivity estimation using the minimal model. Am J Phys. 1987;253(6 Pt 1):E595–602.
52. Finegood DT, Hramiak IM, Dupre J. A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. J Clin Endocrinol Metab. 1990;70(6):1538–49.
53. Welch S, Gebhart SS, Bergman RN, Phillips LS. Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. J Clin Endocrinol Metab. 1990;71(6):1508–18.
54. Cobelli C, Pacini G, Toffolo G, Sacca L. Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled ivgtt. Am J Phys. 1986;250(5 Pt 1):E591–8.
55. Avogaro A, Bristow JD, Bier DM, Cobelli C, Toffolo G. Stable-label intravenous glucose tolerance test minimal model. Diabetes. 1989;38(8):1048–55.
56. Sunehag AL, Treuth MS, Toffolo G, Butte NF, Cobelli C, Bier DM, et al. Glucose production, gluconeogenesis, and insulin sensitivity in children and adolescents: an evaluation of their reproducibility. Pediatr Res. 2001;50(1):115–23.
57. Hoffman RP, Armstrong PT. Glucose effectiveness, peripheral and hepatic insulin sensitivity, in obese

and lean prepubertal children. Int J Obes Relat Metab Disord. 1996;20(6):521–5.
58. Steil GM, Volund A, Kahn SE, Bergman RN. Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model. Suitability for use in population studies. Diabetes. 1993;42(2):250–6.
59. Steil GM, Murray J, Bergman RN, Buchanan TA. Repeatability of insulin sensitivity and glucose effectiveness from the minimal model. Implications for study design. Diabetes. 1994;43(11):1365–71.
60. Cutfield WS, Bergman RN, Menon RK, Sperling MA. The modified minimal model: application to measurement of insulin sensitivity in children. J Clin Endocrinol Metab. 1990;70(6):1644–50.
61. Goran MI, Bergman RN, Avila Q, Watkins M, Ball GD, Shaibi GQ, et al. Impaired glucose tolerance and reduced beta-cell function in overweight latino children with a positive family history for type 2 diabetes. J Clin Endocrinol Metab. 2004;89(1):207–12.
62. Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH. Gender and tanner stage differences in body composition and insulin sensitivity in early pubertal children. J Clin Endocrinol Metab. 1995;80(1):172–8.
63. Travers SH, Jeffers BW, Eckel RH. Insulin resistance during puberty and future fat accumulation. J Clin Endocrinol Metab. 2002;87(8):3814–8.
64. Freemark M, Bursey D. The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. Pediatrics. 2001;107(4):E55.
65. Lindquist CH, Gower BA, Goran MI. Role of dietary factors in ethnic differences in early risk of cardiovascular disease and type 2 diabetes. Am J Clin Nutr. 2000;71(3):725–32.
66. Goran MI, Bergman RN, Gower BA. Influence of total vs. visceral fat on insulin action and secretion in African American and white children. Obes Res. 2001;9(8):423–31.
67. Cook JS, Hoffman RP, Stene MA, Hansen JR. Effects of maturational stage on insulin sensitivity during puberty. J Clin Endocrinol Metab. 1993;77(3):725–30.
68. Hoffman RP, Vicini P, Sivitz WI, Cobelli C. Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. Pediatr Res. 2000;48(3):384–8.
69. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, et al. Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab. 1997;82(2):402–6.
70. Hamilton J, Cummings E, Zdravkovic V, Finegood D, Daneman D. Metformin as an adjunct therapy in adolescents with type 1 diabetes and insulin resistance: a randomized controlled trial. Diabetes Care. 2003;26(1):138–43.
71. Conwell LS, Trost SG, Brown WJ, Batch JA. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. Diabetes Care. 2004;27(2):314–9.
72. Ferrannini E, Mari A. How to measure insulin sensitivity. J Hypertens. 1998;16(7):895–906.
73. Mari A. Mathematical modeling in glucose metabolism and insulin secretion. Curr Opin Clin Nutr Metab Care. 2002;5(5):495–501.
74. Reaven G, Miller R. Study of the relationship between glucose and insulin responses to an oral glucose load in man. Diabetes. 1968;17(9):560–9.
75. American Diabetes Association. Standards of medical care in diabetes-2017: summary of revisions. Diabetes Care. 2017;40(Suppl 1):S4–5.
76. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, et al. Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. Diabetologia. 1996;39(3):298–305.
77. Barrett-Connor E. The oral glucose tolerance test, revisited. Eur Heart J. 2002;23(16):1229–31.
78. Davidson MB. Counterpoint: the oral glucose tolerance test is superfluous. Diabetes Care. 2002;25(10):1883–5.
79. Libman IM, Barinas-Mitchell E, Bartucci A, Robertson R, Arslanian S. Reproducibility of the oral glucose tolerance test in overweight children. J Clin Endocrinol Metab. 2008;93(11):4231–7.
80. Trout KK, Homko C, Tkacs NC. Methods of measuring insulin sensitivity. Biol Res Nurs. 2007;8(4):305–18.
81. Uwaifo GI, Fallon EM, Chin J, Elberg J, Parikh SJ, Yanovski JA. Indices of insulin action, disposal, and secretion derived from fasting samples and clamps in normal glucose-tolerant black and white children. Diabetes Care. 2002;25(11):2081–7.
82. Yeckel CW, Weiss R, Dziura J, Taksali SE, Dufour S, Burgert TS, et al. Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. J Clin Endocrinol Metab. 2004;89(3):1096–101.
83. Schwartz B, Jacobs DR Jr, Moran A, Steinberger J, Hong CP, Sinaiko AR. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. Diabetes Care. 2008;31(4):783–8.
84. Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hofman PL. Evaluation of homa and quicki as measures of insulin sensitivity in prepubertal children. Pediatr Diabetes. 2003;4(3):119–25.
85. Rossner SM, Neovius M, Montgomery SM, Marcus C, Norgren S. Alternative methods of insulin sensitivity assessment in obese children and adolescents. Diabetes Care. 2008;31(4):802–4.
86. Rossner SM, Neovius M, Mattsson A, Marcus C, Norgren S. HOMA-IR and QUICKI: decide on a general standard instead of making further comparisons. Acta Paediatr. 2010;99(11):1735–40.

87. Quon MJ. Limitations of the fasting glucose to insulin ratio as an index of insulin sensitivity. J Clin Endocrinol Metab. 2001;86(10):4615–7.
88. Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, et al. Report of the American diabetes association's task force on standardization of the insulin assay. Diabetes. 1996;45(2):242–56.
89. Saad R, Gungor N, Arslanian S. Progression from normal glucose tolerance to type 2 diabetes in a young girl: longitudinal changes in insulin sensitivity and secretion assessed by the clamp technique and surrogate estimates. Pediatr Diabetes. 2005;6(2):95–9.
90. Weigensberg MJ, Cruz ML, Goran MI. Association between insulin sensitivity and post-glucose challenge plasma insulin values in overweight Latino youth. Diabetes Care. 2003;26(7):2094–9.
91. DeFronzo RA, Matsuda M. Reduced time points to calculate the composite index. Diabetes Care. 2010;33(7):e93.
92. Lyssenko V, Almgren P, Anevski D, Perfekt R, Lahti K, Nissen M, et al. Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. Diabetes. 2005;54(1):166–74.
93. Sjaarda LG, Bacha F, Lee S, Tfayli H, Andreatta E, Arslanian S. Oral disposition index in obese youth from normal to prediabetes to diabetes: relationship to clamp disposition index. J Pediatr. 2012;161(1):51–7.
94. Michaliszyn SF, Bonadonna RC, Sjaarda LA, Lee S, Farchoukh L, Arslanian SA. Beta-cell lipotoxicity in response to free fatty acid elevation in prepubertal youth: African American versus Caucasian contrast. Diabetes. 2013;62(8):2917–22.
95. Cobelli C, Dalla Man C, Toffolo G, Basu R, Vella A, Rizza R. The oral minimal model method. Diabetes. 2014;63(4):1203–13.
96. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care. 2001;24(3):539–48.
97. Mari A, Pacini G, Brazzale AR, Ahren B. Comparative evaluation of simple insulin sensitivity methods based on the oral glucose tolerance test. Diabetologia. 2005;48(4):748–51.
98. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J, Carr DB, Boyko EJ, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care. 2009;32(2):335–41.
99. Abdul-Ghani MA, Williams K, DeFronzo RA, Stern M. What is the best predictor of future type 2 diabetes? Diabetes Care. 2007;30(6):1544–8.
100. Bacha F, Pyle L, Nadeau K, Cuttler L, Goland R, Haymond M, et al. Determinants of glycemic control in youth with type 2 diabetes at randomization in the today study. Pediatr Diabetes. 2012;13(5):376–83.
101. The TODAY Study Group Effects of metformin, metformin plus rosiglitazone, and metformin plus lifestyle on insulin sensitivity and beta-cell function in today. Diabetes Care. 2013;36(6):1749–57.
102. Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, et al. Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. J Clin Endocrinol Metab. 1989;68(2):374–8.
103. Shen SW, Reaven GM, Farquhar JW. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. J Clin Invest. 1970;49(12):2151–60.
104. Harano Y, Hidaka H, Takatsuki K, Ohgaku S, Haneda M, Motoi S, et al. Glucose, insulin, and somatostatin infusion for the determination of insulin sensitivity in vivo. Metab Clin Exp. 1978;27(9 Suppl 1):1449–52.
105. Muniyappa R, Madan R, Quon MJ. Assessing insulin sensitivity and resistance in humans. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. Endotext. South Dartmouth: MDText.com, Inc.; 2000.

Insulin Resistance in Chronic Disease

3

Uri Hamiel and Orit Pinhas-Hamiel

Introduction

The metabolic syndrome (MetS) is a complex disorder defined by a cluster of interconnected factors, including abdominal obesity, hypertension, dyslipidemia, and insulin resistance [1]. The MetS has been proposed to identify patients at high cardiovascular risk. In addition to their underlying disease, some patients with chronic diseases develop insulin resistance and the MetS; these conditions may aggravate their morbidity. Several routes may result in an increased risk of developing insulin resistance and the MetS. This increased risk may result secondary to increased obesity that is associated with a number of chronic diseases, such as asthma and type 1 diabetes. In addition, weight gain and metabolic impairment can occur as an integral component of the disease itself, such as in Prader-Willi syndrome, sometimes secondary to medications administered to treat chronic diseases, or secondary to disability associated with a disease, such as in multiple sclerosis. Often, the increased risk is a combination of more than one route (Fig. 3.1). In the current chapter, we review the prevalence of the MetS in several chronic diseases and the impact on long-term morbidity. Prevalent diseases were chosen to highlight the possible bidirectional relationships between obesity and chronic diseases, mainly in children and youth.

Asthma

Asthma is the most common chronic disease in children. The dramatic increase in the prevalence of asthma over the past few decades has paralleled an increase in obesity. This observation has triggered investigation of an association, possibly bidirectional, between these two conditions. On one hand, many children with asthma avoid exercise, increase sedentary time, and receive treatment with oral corticosteroid medications—three factors that promote weight gain [2]. On the other hand, obesity has also been implicated as a significant risk factor for asthma prevalence and severity in children and adolescents [3, 4]. Since obesity is recognized as a potential risk factor for cardiometabolic conditions—such as insulin resistance and the MetS, with further tracking to type 2 diabetes (T2D), dyslipidemia, and cardiovascular diseases—the impact of obesity in patients with asthma is paramount [5].

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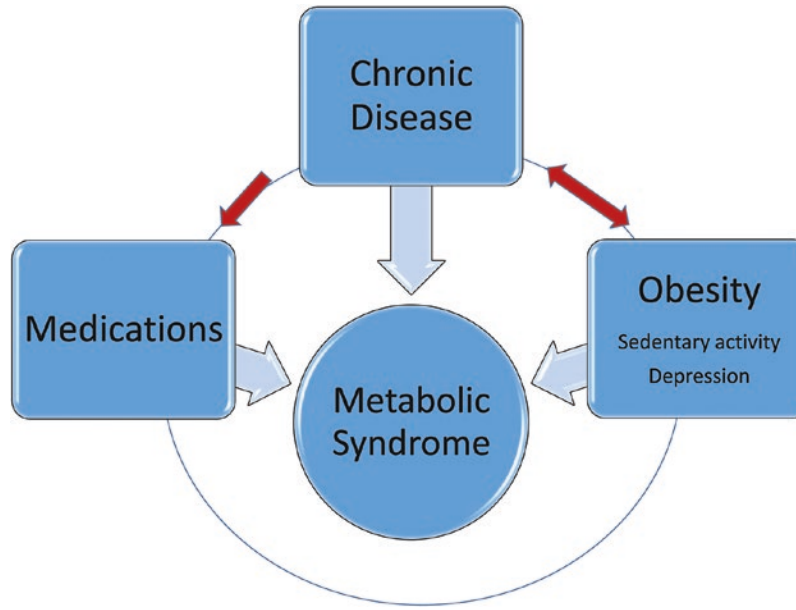

Fig. 3.1 Increased risk of developing metabolic syndrome is often a combination of more than one route

Obesity Can Predate Asthma

Longitudinal data demonstrate a pattern in which obesity predates and increases the risk for incident asthma [2]. In a meta-analysis that included 333,102 adults, overweight and obesity conferred a 51% increased odds of incident asthma [2]. A dose–response effect of elevated body mass index (BMI) on asthma incidence was observed; risks of incident asthma for overweight and obese persons were 1.4 and 1.9, respectively, compared with a normal-weight group. Thus, an atypically non-atopic child with early life weight gain may subsequently develop asthma-like symptoms. The underlying mechanism may prove to impair lung growth and alter airflow perception. Similarly, data from 12,050 children and adolescents of 8 European birth cohorts on asthma and allergies were combined to investigate whether the course of BMI predicts incident asthma in childhood [6]. Children with a rapid change in BMI-standard deviation score gain in the first 2 years of life had a higher risk for incident asthma up to age 6 years than did children with a less pronounced weight gain slope in early childhood. Rapid BMI gain between 2 and 6 years of age did not significantly enhance the risk of asthma [6].

Obesity in Persons with Asthma

In a study of 472 children with persistent asthma, 49% were overweight/obese; the overweight/obese children experienced more asthma symptoms, greater limitation in activity, and more visits to the emergency department or hospitalization for any reason than did normal-weight children [7]. Similarly, among children aged 2–18 years, obesity was significantly associated with higher odds of using mechanical ventilation, higher mean total hospital charges, and longer mean length of hospital stay compared [8].

Asthma and Insulin Resistance

The pro-inflammatory state of insulin resistance has been speculated to contribute to the pathogenesis of asthma in obese patients. Studies in adults showed associations of obesity and insulin resistance with asthma and aeroallergen sensitization [9, 10]. In one study, insulin resistance was found to be significantly associated with asthma among morbidly obese children and adolescents (odds ratio 4) [11]. A smaller study showed children with allergic asthma to be more likely to show an insulin resistance profile [12]. Indeed, in

a study of 71 prepubertal children who underwent spirometry and bronchial hyperresponsiveness testing, obese asthmatic children with confirmed insulin resistance (homeostatic model assessment of insulin resistance [HOMA-IR] ≥ 2.5) had significantly greater bronchial hyperresponsiveness than did obese children without insulin resistance [7]. This suggests that obesity per se does not correlate with airway hyperreactivity unless it is accompanied by glucose intolerance and insulin resistance.

Data from a randomized controlled trial on the safety and direct effects of inhaled human insulin showed that those treated with inhaled insulin were more likely to exhibit respiratory symptoms, including cough and mild dyspnea, along with deterioration in lung function [13]. This finding supports the notion that insulin may exert a direct effect on human airways. Indeed, insulin dysregulation may negatively affect the airway through multiple routes, to cause lung function impairment [13]. Insulin induces hypercontractility in airway smooth muscle. In addition, hyperinsulinemia was shown to result in vagally mediated bronchoconstriction in animal models [13].

Asthma and the Metabolic Syndrome

The MetS has been associated with asthma in large cross-sectional and longitudinal studies in adults. Among 9942 individuals aged 40–69 years, asthma-like symptoms (wheeze, resting dyspnea, and post-exercise dyspnea) were increased in those with MetS. Among the components of the MetS, abdominal obesity and hypertension were the risk factors for asthma-like symptoms [14]. In a Norwegian prospective cohort study of participants who were asthma-free at baseline ($n = 23{,}191$), MetS was a risk factor for incident asthma (adjusted OR 1.57, 95% CI 1.31–1.87). Among the components of the MetS, high-waist circumference and elevated glucose or diabetes were associated with incident asthma, after adjustment for the other metabolic components [15]. While the association was clear in adults, data from the 2007–2010 National Health and Nutrition Examination Survey (NHANES) of 1429 adolescents aged 12–17 years showed no statistically significant differences in indicators of metabolic syndrome and insulin resistance between adolescents with and without asthma [16]. However, adolescents with insulin resistance and low high-density lipoprotein (HDL) cholesterol had lower airway obstruction and lower residual volumes. Truncal obesity was predictive of lower residual volume, which conferred additional risk, beyond general adiposity [17]. Each MetS component was shown to contribute to lung disease. In addition, adipokine dysregulation is a potential mechanism for obesity-mediated airway changes in asthma. Furthermore, hyperlipidemia and lung function impairment in adults were reported to be associated with asthma risk in children [16].

Significance and Treatment

If insulin resistance and obesity can be considered modifiable risk factors, interventions that affect weight loss could be associated with a decrease in asthma incidence. In studies conducted among adults ($n = 197$), such interventions included supervised physical activity, low-calorie diet, and weight loss medications [18]. Considering certain methodological problems, a statistically significant reduction in asthma symptom scores was reported in treatment compared to control groups. Further, short-term analysis showed a reduction in doses of rescue medication in treatment compared to control groups. Weight loss was associated with some improvement in forced expiratory volume in 1 second (FEV1) and in forced vital capacity (FVC); the latter was statistically significant, but clinically unimportant [18].

Patients with concurrent asthma and diabetes who were treated with metformin (444) were compared to metformin non-users (1333) of the same age and gender. Patients were followed for 3 years to measure the occurrence of asthma-related outcomes. Compared with non-users, metformin users had a lower risk of asthma-related hospitalization (OR = 0.21, 95%

CI: 0.07–0.63) and asthma exacerbation (OR = 0.39, 95% CI: 0.19–0.79) [17]. Thus, targeting insulin resistance may represent a novel therapeutic strategy for obese patients with asthma.

Type 1 Diabetes Mellitus

Type 1 diabetes (T1DM) is one of the most common chronic diseases in children. A bidirectional association between T1DM and obesity is suggested. On one hand, the accelerator hypothesis argues that the obesity epidemic results in an increase in insulin resistance, which leads to glucotoxicity. This accelerates β(beta)-cell apoptosis and, in a subset of genetically predisposed individuals, results in overt diabetes. On the other hand, recent studies demonstrate that overweight and obesity are highly prevalent among individuals with T1DM. Moreover, several investigations have recently evaluated the prevalence of MetS and its components in individuals with T1DM. Importantly in T1DM, the impaired fasting glucose criterion is irrelevant since it is automatically fulfilled. In addition, the widespread use of antihypertensive and lipid-lowering medications for cardiac and renal prevention can contribute to overestimations of the prevalence of raised blood pressure and elevated triglycerides [19].

Obesity Predates the Development of Type 1 Diabetes Mellitus

Obesity confers an increased risk of developing T1DM throughout the life cycle. In a meta-analysis that included 12 807 individuals with T1DM, children with birth weights from 3.5 to 4.0 kg had a 6% increased risk of diabetes and children with birth weights greater than 4.0 kg had a 10% increased risk, compared to children who weighed 3.0–3.5 kg at birth [20]. This corresponded to a linear increase in diabetes risk of 3% per 500 g increase in birthweight. In addition, weight increase during the first year of life was shown to be positively associated with T1DM [21]. Similarly, another meta-analysis demonstrated a continuous association between childhood BMI and subsequent T1DM, thus supporting a dose–response relationship [22].

Obesity Among Children and Adolescents with Type 1 Diabetes Mellitus

Among 326 children and young adults (aged 5–30 years), with mean diabetes duration of 8.7 ± 5.0 years, females aged 15 to <18 and 18 to <25 years were significantly overweight compared to healthy females in the same age groups: 33% vs. 13% and 26% vs. 8%, respectively. Among males of all age groups, prevalence of overweight and obesity did not differ compared with healthy males in the general population [23]. Similarly, in the SEARCH study, youth with T1DM had a higher prevalence of overweight, but not of obesity, than did nondiabetic youth [24]. The prevalence of overweight among youth with T1DM was significantly higher than among those without diabetes overall (22.1% vs. 16.1%) [24].

Insulin Resistance Among Type 1 Diabetes Mellitus Individuals

Insulin resistance is defined as a state in which the biologic response to a given concentration of insulin is reduced. In the setting of T1DM, insulin resistance has been attributed to chronic hyperglycemia, which results in glucotoxicity, thus creating downregulation of glucose transport systems and post-transport steps of insulin action. The relationship of insulin resistance to cardiovascular disease (CVD) risk in youth with T1DM is evident even in the pediatric age group. In the Determinants of Macrovascular Disease in Adolescents with T1DM study, reduced insulin sensitivity was associated with CVD risk factors, such as waist circumference, cholesterol, blood pressure, and C-reactive protein.

Metabolic Syndrome Among Type 1 Diabetes Mellitus Individuals

MetS was detected in 35% of obese, 8% of overweight, and 5% of normal-weight children and young adults with T1DM (n = 326), mean age 18.5 ± 6.0 years [23]. Among 477 adolescents with T1DM, aged 16–19 years, the prevalence of MetS was 9.5%. Abdominal obesity was the most common risk factor (20%) in females, while hypertension was the most common risk factor in males (25%) [19]. Similarly, among younger children, median age 12.8 years, 38.5% were overweight or obese. Among the overweight/obese children with T1DM, 25.7% had the MetS vs. 6.3% in the normal-weight group. A high triglyceride level was detected in 17.3%, high low-density lipoprotein (LDL) cholesterol in 28.6%, low HDL cholesterol in 21.2%, and hypertension in 13.1% of the patients [19].

Significance and Treatment

Insulin resistance, rather than hyperglycemia, was the more significant determinant of coronary artery calcifications, which is an important marker of CV events. Similarly, insulin resistance in normal-weight adolescents with T1DM compared with weight-matched controls was associated with echocardiographic abnormalities (left ventricular hypertrophy and diastolic dysfunction) [19]. Therefore, insulin resistance and glycemia are recognized targets for treatment of T1DM, for prevention of microvascular and macrovascular complications. Identifying diabetes patients at increased risk for cardiovascular complications and early mortality is crucial from a prevention standpoint. A meta-analysis showed that the addition of metformin resulted in decreased total insulin daily dose and reduced BMI values and BMI z-scores; however, HbA1c remained the same [19].

Cancer Survivors

Hematopoietic stem cell transplantations (HSCT) in pediatric patients have considerably increased in recent years for treatment of both malignant and nonmalignant diseases. Improved supportive care and greater experience in patient care have led to a decreased mortality rate. The growing number of long-term survivors has increased concern regarding late complications and long-term consequences. Several important observations arise from studies among HSCT survivors.

Components of the Metabolic Syndrome May Be Detected Within the First Years After Hematopoietic Stem Cell Transplantations

A substantial proportion of the patient population presents with features of the MetS at the time of the procedure. Among 38 patients, with median age at HSCT of 8.5 years, evaluated at a median of 3.9 years post-HSCT, 10.5% met the criteria of MetS and 31.6% had at least one component of the MetS [25]. Similarly among 69 patients, median age of 13 years at the time of diagnosis and a median time of follow-up posttransplant of 4 years, 32% had the MetS [26]. Among 170 adults, evaluated at a mean age of 25 ± 5 years, with follow-up duration 14.5 years, the incidence of the MetS increased with age [27].

Overweight, Obesity, Sarcopenic Obesity, and the Metabolic Syndrome

Overweight and Obesity at the Time of HSCT were found to confer a risk for the MetS. For each additional unit in BMI-z score at the time of transplantation, the risk for development of the MetS was 57% higher [27]. Furthermore, patients with BMI above the 50th percentile at HSCT already showed an increased risk compared to those with lower BMI [25]. This suggests that even BMI within the accepted normal range (50th to 84th percentiles) was associated with increased morbidity.

Overweight and Obesity at the Time of Evaluation of the MetS In general, survivors of HSCT are not more likely to be obese than

their siblings despite having been exposed to radiation, steroids, and prolonged periods of inactivity [28]. Nonetheless, HCT survivors, even with normal BMI, develop significantly altered body composition that results in both an increase in total percent fat mass and a reduction in lean body mass [28]. This condition is termed "sarcopenic obesity" and results in a loss of myocyte insulin receptors and an increase in adipocyte insulin receptors; the latter are less efficient in binding insulin and clearing glucose and ultimately contribute to insulin resistance [2]. Indeed, among 45 survivors of hematological malignancies aged 14 ± 5 years, only one was obese. However, abnormal glucose tolerance by oral glucose tolerance test (OGTT) was present in 15.6%. Nonetheless, overweight and obesity are risk factors that are associated with the development of MetS [26]; overweight and obesity at the time of evaluation of the MetS were associated with a 4.3-fold increased risk (P = 0.05) for developing components of the MetS (P = 0.007) [25].

Factors Associated with Increased Risk of Developing the Metabolic Syndrome

Among 160 patients with a median age at transplant of 5 years, who had been followed for a median of 7 years, total body irradiation and younger age at HSCT were associated with the highest burden of long-term effects. Female sex was more significantly associated than male sex with MetS-related dysfunction [29]. Among 69 patients, with median age at the time of diagnosis of 12.9 years, and median time of follow-up post-transplant 4 years, 32% had MetS; the most common MetS features were abdominal obesity (73%), hypertriglyceridemia (91%), and a low HDL-C level (96%) [30]. In another study, the most significant risk factor for MS was corticosteroid therapy use [26].

Of note, increased risk of developing the MetS was also observed among acute leukemia survivors treated without HSCT. Among 650 adult patients, mean age at evaluation was 24 years; mean follow-up after leukemia diagnosis was 16 years; and the prevalence of MetS increased with age and was 1.3%, 6.1%, 10.8%, and 22.4% at ages 20, 25, 30, and 35 years, respectively [31]. Irradiated and nonirradiated patients exhibited different patterns of metabolic abnormalities, with more frequent abdominal obesity in irradiated patients and more frequent hypertension in nonirradiated patients. Thus, survivors of childhood acute leukemia are at risk of the MetS, even when treated without HSCT or central nervous system irradiation. Risk factors that were significantly associated with the MetS were male sex, age at last evaluation, and BMI at diagnosis. In this cohort, the cumulative steroid dose was not a significant risk factor [31].

Significance

Long-term survivors after HSCT in childhood are at a high risk of insulin resistance, impaired glucose tolerance, and T2D, even when at a normal weight and young age. This was shown in a study of long-term survivors, in which the median age of patients with allogeneic HSCT at the last follow-up was 39 years and the median follow-up period was 9 years [32]. An arterial event was experienced by 6.8% of patients after allogeneic HSCT and by 2.1% patients after autologous HSCT. The cumulative incidence in the 15 years subsequent to allogeneic HSCT was 7.5%, compared to 2.3% after autologous HSCT. After adjusting for age, the risk of an arterial event was sevenfold higher after allogeneic HSCT (RR: 6.92; P = 0.009). In multivariate analysis, allogeneic HSCT (RR: 14.5; P = 0.003), together with at least two of four cardiovascular risk factors (hypertension, dyslipidemia, diabetes, obesity) (RR: 12.4; P = 0.02), was associated with a higher incidence of arterial events after HSCT [32].

Psychiatric Disorders

Obesity and psychiatric disorder may also have a bidirectional association. On one hand, many obese children suffer from depression and

anxiety and often require antipsychotic medications. On the other hand, psychiatric disorders have been implicated as a significant risk factor for obesity. Medications appear to be an important mediator in these relationships. Prescriptions for antipsychotic medications among children and adolescents are reported to have increased greatly in recent years. First-generation antipsychotics, also known as typical antipsychotics, were first developed in the 1950s. They have considerable potential to cause extrapyramidal side effects and tardive dyskinesia. Second-generation antipsychotics, also known as atypical antipsychotics, were approved for use in the 1990s as treatments for schizophrenia and bipolar disorder, and some of them for the treatment of irritability in children with autism spectrum disorders. However, most antipsychotic medications used for children and adolescents are "off-label," either for indications or for age ranges that are not approved by the US Food and Drug Administration (FDA) [33]. About 60% of youth treated with antipsychotics were found to have no claim that indicated a mental disorder diagnosis. Youth who use atypical antipsychotics are more susceptible to acute and long-term adverse effects of these medications than are adults [33].

Obesity Predates Psychiatric Disorders

Data from 41,654 respondents in the National Epidemiologic Survey revealed that the continuous variable of BMI was significantly associated with most mood, anxiety, and personality disorders [34]. When persons were classified into BMI categories, those classified as obese and extremely obese had significantly increased odds of any mood, anxiety, or personality disorder; of major depression, dysthymia, or manic episode; and of antisocial, avoidant, schizoid, paranoid, and obsessive-compulsive personality disorders. Similarly, longitudinal studies of adolescents and young adults show that the prospective risk of developing depression in obese individuals was significantly higher in females [35].

Persons with Psychiatric Disease Are at Higher Risk of Being Obese

The increased risk of overweight and obesity in patients with severe mental illness is likely to be a composite of decreased physical inactivity and poor diet, which are common in this population, as well as the inherent biological risk associated with mental illnesses and its treatment. In this scenario, weight gain usually appears faster and to a greater extent in children than in adults. In a historical prospective study of 146 adolescent patients, mean age 15 ± 2 years, treated in an adolescent day unit and discharged after 141 ± 76 days, a significant increase in BMI-z score was observed at admission and discharge (0.5 ± 1.2 vs. 0.7 ± 1.1, respectively, $P < 0.001$) [36]. Males were 3.5-fold more prone to weight gain than were females. In a systematic review and meta-analysis of randomized controlled trials of atypical antipsychotic drugs vs. placebo in 2455 children, the mean increase in weight, relative to placebo, was greatest with olanzapine (3.5 kg), intermediate with risperidone (1.8 kg), and least with aripiprazole (0.9 kg). Olanzapine was also associated with increased glucose and total cholesterol levels [8].

The Metabolic Syndrome in Psychiatric Disorders

Among adults, the prevalence of the MetS was 20–40% [37–39]. Factors predicting the MetS are BMI, type of psychiatric diagnosis—bipolar I disorder (47%), bipolar II disorder (25%), major depressive disorder (22%), and anxiety-only disorders (17%)—and no mood and/or anxiety disorders (14%) [37]. In addition, the use of antipsychotics and mood stabilizers independently predicted a higher risk of the MetS, after controlling for demographic variables and psychiatric diagnoses. In another study, family history of diabetes and hyperlipidemia predicted MetS. Compared to matched healthy controls ($n = 253$), patients with a first episode of psychosis, aged 9–35 years ($n = 335$), showed poorer metabolic profiles at diagnosis [40]. In addition,

2 years after the first episode of psychosis, individuals showed progressive worsening and higher mean levels of glucose, triglycerides, and diastolic blood pressure and lower HDL-C, together with higher body weight, BMI, and waist circumference compared to the control group. The rate of the MetS increased in the psychosis group, from 6.6% to 14.6% during 2 years, whereas it remained steady at 3.4% in the control group. Male sex; the presence of affective symptoms or an early-onset, antipsychotic polypharmacy; and the use of antidepressants or mood stabilizers were associated with higher risk [40].

Data are scarce regarding antipsychotics and the risk of T2D in children and adolescents. A meta-analysis was conducted on 13 retrospective and prospective studies, which included 185 105 youth exposed to antipsychotics and 310 438 patient-years [41]; the mean age at diagnosis was 14.1 ± 2.1 years. During a relatively short mean follow-up time of 1.7 years, patients exposed to antipsychotics had a three times higher incidence of T2D than did healthy controls and almost twice the incidence of that of nonexposed psychiatric patients. The smaller difference with psychiatrically ill patients than with healthy controls may reflect contributions of unhealthy lifestyle behaviors and other pharmacological treatments associated with psychiatric disorders to the risk of weight gain and T2D [41]. Greater cumulative T2D risk was associated with longer follow-up ($P < 001$), olanzapine prescription, and male sex.

Significance and Treatment

The preponderance of evidence suggests that patients with major depression, bipolar disorder, and schizophrenia are at significantly higher risk for cardiovascular morbidity and mortality than are their counterparts in the general population [42]. Moreover, in these patients, CVD is the commonest cause of death [42]. It is therefore critical to prevent weight gain and associated comorbidities. A meta-analysis of 12 published studies, with a total of 743 patients treated with antipsychotics, found that metformin treatment resulted in significantly better anthropometric and metabolic parameters than did placebo [43]. The mean difference in weight was −3.3 kg. Metformin compared to placebo resulted in a significant reduction in BMI, by −1.1 kg/m^2, and in insulin resistance, but not in fasting blood sugar. A small body of research suggests that children who gain weight with atypical antipsychotics could lose some of this weight with adjunctive treatment with metformin (1000–1700 mg/d) [44]. It is not clear, however, whether glucose and lipid metabolism also improves [45].

Epilepsy

Patients with epilepsy have high incidences of cardiovascular mortality and morbidity compared to the general population [46].

Are Overweight and Obesity More Prevalent in Individuals with Epilepsy?

Newly diagnosed children with epilepsy, aged 2–18 years, and not previously treated with antiepileptic medication ($n = 251$), were compared to a healthy control group [47]. Overall, 39% of the epilepsy groups was considered overweight or obese (BMI ≥85th percentile for age), compared with 28% of the healthy control group. Mean BMI-z scores differed significantly between the epilepsy cohort and the standard US Centers for Disease Control (CDC) growth curve ($P < 0.0001$). Obesity correlated with increasing age, idiopathic etiology, and the absence of concomitant medication. It is unclear if the elevated rate of obesity in the epilepsy population is coincidental or a result of a common mechanism.

Several antiepileptic drugs (i.e., valproic acid [VPA], carbamazepine, gabapentin, and vigabatrin) promote weight gain. Since antiepileptic therapy is often prescribed for years, and sometimes for an entire lifetime, it is crucial to understand the impact of the drugs, throughout the therapy course, on weight status, insulin resistance, and the MetS.

Among individuals with epilepsy, increased weight gain and the occurrence of overweight seem to be greater with increased age. Eighty-seven patients, mean age at initiation of VPA therapy 4.8 ± 0.8 years, were followed for 3 years [48]. The mean change from baseline in BMI-z scores was 0.80 ($P = 0.001$). The percentage of overweight children at baseline was 6.9% and rose to 16% by the final visit ($P = 0.08$). Among 114 epileptic children, mean age 10.1 ± 4.7 years, treated with VPA monotherapy for at least 24 months, 40% had a considerable increase in body weight at the end of follow-up and 17% developed obesity [49]. Similarly, among 43 children aged 10–17 years, mild-to-moderate weight gain was observed in 58% of those who were treated with VPA and 14% were classified as overweight or obese. Overweight at initiation was a predictor of overweight at follow-up. Among adults with epilepsy, 57–71% gained weight [50]. A long duration of therapy was associated with significant gain, which persisted after the first rapid increase of weight during the first months of therapy. Women seemed to be more prone to weight gain during VPA therapy than men.

Insulin Resistance and Epilepsy

Data are inconsistent regarding insulin concentrations in individuals treated with VPA. Among those with newly diagnosed idiopathic epilepsy, insulin concentrations and the insulin resistance index did not differ before and after 6 and 12 months of treatment [51]. Insulin concentrations were compared between 81 adult patients, mean age 30 ± 11 years, treated with VPA for 6.5 ± 4.9 years and 51 healthy controls aged 37 ± 7 years. Forty-nine of the epileptic patients and the control group were obese. The mean insulin concentration was higher in the VPA-treated than in the control group, despite similar BMI. Furthermore, serum insulin concentrations were also higher among lean males and lean females than among lean controls of the same sex [51]. Adult patients treated with VPA ($n = 51$) had fasting hyperinsulinemia, although their fasting serum proinsulin and C-peptide concentrations were similar to values in healthy controls ($n = 45$). This suggests that VPA does not induce insulin secretion but may interfere with insulin metabolism in the liver, resulting in higher insulin concentrations in peripheral circulation [52].

Metabolic Syndrome in Epilepsy

Of children and adolescents who became obese since the initiation of VPA therapy, 40% developed the MetS [49]. Other smaller studies did not find a statistically significant increased risk of MetS among patients on VPA, despite an increased risk of hyperinsulinemia [53–55]. One of those studies observed a small positive correlation between the development of MetS and both the HOMA index and VPA dosage [54]. MetS prevalence was compared between 118 patients with epilepsy who received VPA monotherapy for a median duration of 6 years and 492 individuals in the same geographic region [52]. VPA-treated patients had higher serum insulin concentrations than did controls, independent of BMI [52]. However, the risk of MS was not increased among VPA-treated patients with epilepsy compared to the general population. Among epileptic patients with at least 3 years of varied antiepileptic treatment in northern India, 29.5% of those younger than age 50 with epilepsy had MetS, compared to 21.9% of similar aged persons in the general population [56]. This difference did not reach statistical significance overall ($P = 0.066$), but was significant in males (32.5% vs. 17.41%, $P = 0.001$). The study specifically showed that the use of VPA was associated with a significantly increased risk of the prevalence of MetS [56]. The reason that VPA might cause MetS in some, but not all, patients remains unclear and controversial. Genetic factors that influence energy homeostasis, such as the insulin receptor signaling pathway or lipid metabolism, might represent possible explanations for the development of MetS in a certain proportion of obese patients [57, 58]. The metabolic changes reported in epileptic patients treated with VPA are secondary to excess fat mass, since these

changes are not present in epileptic patients treated with VPA who do not gain weight [59]. This suggests that MetS is not directly caused by VPA medication but may be related to the weight gain induced by VPA therapy.

Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory chronic disease. First symptoms generally appear in young adults and disability progresses over time. In individuals with multiple sclerosis, both disability and steroid treatment may account for an increased prevalence of the MetS. Among 130 adults with MS who had disabilities, mean age 55 ± 6 years and disease duration 18 ± 10 years, obesity was present in 18.5% and overweight in 34.6% [60]. Compared to the general population, adult disabled MS patients had lower rates of obesity and overweight, as assessed by BMI. Despite these reduced rates, the prevalence of the MetS was 30%, with no difference between the sexes, and was similar to that of the general population. To better explain this paradox, it is important to understand the components of MetS that result in its high prevalence in patients with MS. Fifty-six percent of MS patients had waist circumference consistent with abdominal obesity; this rate is higher than the previously reported rates of 21% and 39% in males and females of the same age groups in the general population. Thus, BMI may be misleading in terms of health risk in disabled patients. This finding is supported by previous observations of lower ratios of muscle to fat in MS patients and in individuals with other disabilities. The ENRICA study showed a MetS prevalence of 22.7% (95% CI: 21.7–23.7%) in a sample of 11,143 adults in Spain [61].

Abdominal obesity is a major risk factor for coronary heart disease and T2D. Indeed, patients with MS have been reported to have a 2.4-fold increased rate of death related to CVD than people without MS. Of note, the occurrence of MetS was not associated with possible risk factors, such as disease duration, degree of disability, and the number of steroid courses received [60]. A study of 222 MS patients from two regions of Spain, mean age 45 years, provides support for the presence of comorbidities and MetS in MS patients, as well as for a trend for increasing comorbidity with increasing MS disability. Depression, dyslipidemia, hypertension, and obesity were the most frequently observed comorbidities [61].

Classical Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

Congenital adrenal hyperplasia (CAH) is lethal in its most severe forms, if not treated with glucocorticoids. However, glucocorticoids may increase the risk of cardiovascular and metabolic morbidity. Childhood obesity rates in CAH exceed the high rates seen in normal children and may potentially increase the risk of CVD. Abdominal adiposity, particularly visceral adipose tissue, is strongly associated with MetS and CVD [41]. A cross-sectional study matched 15 females and 13 males with CAH, mean age 15.6 ± 3.2 years, for age, sex, ethnicity, and BMI to healthy controls [41]. CAH adolescents and young adults had increased abdominal adiposity, with a higher proportion of visceral adipose tissue. HOMA-IR and LDL-C were found to correlate with abdominal adiposity. In addition, measures of inflammation, plasminogen activator inhibitor-1, high-sensitivity C-reactive protein, and leptin correlated strongly with visceral adipose tissue.

Using the Swedish national registry, 588 patients with CAH due to 21-hydroxylase deficiency were compared to matched controls ($n = 58{,}800$); the median age was 26 years (0–90) [62]. In the CAH group, the risk of any cardiovascular disease or metabolic disorder was significantly increased (OR, 3.9; 95% CI, 3.1–5.0). Separate analyses of individual disorders showed higher frequencies in CAH of hypertension, hyperlipidemia, atrial fibrillation, venous thromboembolism, obesity, diabetes (mainly type 2), obstructive sleep disorder, thyrotoxicosis, and hypothyroidism. The risk was almost four times

higher for females but not significantly higher for males. Similarly, diabetes and hypertension were more prevalent in CAH patients, particularly in females. In the general population, the higher prevalence of CVD in men versus women has been attributed to their higher testosterone concentrations. Similarly, higher testosterone concentrations among CAH females were proposed as one of the reasons for their increased CVD risk [62]. Alternatively, due to their greater likelihood to manifest symptoms of hyperandrogenism than CAH males, CAH females may be exposed to higher doses of corticosteroids, resulting in obesity, insulin resistance, and CVD. Finally, some genetic subgroups seemed to be more affected.

Steroids

Up to 0.9% of the adult Western population reportedly receive systemic glucocorticoid (GC) therapy [63]. GCs are potent drugs that are important components of rational therapy for a number of common diseases in pediatrics. Diabetes mellitus, dyslipidemia, central obesity, and hypertension are well-known and common sequelae of glucocorticoid treatment [64] and are dependent on the administered dose and duration of treatment. Impaired glucose tolerance is a very consistent finding in clinical studies after GC exposure; the numerous mechanisms suggested for its development are as follows: direct inhibition of insulin signaling in skeletal muscle, induction of protein catabolism, enhancement of whole-body lipolysis, increased hepatic glucose production, alteration of hepatic lipid metabolism, increased body fat content and alteration of body fat distribution, modulation of adipose tissue biology, and impairment of insulin secretion. It has been suggested that GC may induce β(beta)-cell dysfunction and, thus, determine the progression from insulin resistance to hyperglycemia [65, 66].

Features of the MetS show a correlation to increased GC activity [67], though a clear association between excess plasma levels of GCs and the MetS has yet to be established. Only a few studies are available on patients who are treated with systemic glucocorticoids and who have MetS. However, one study of patients with rheumatoid arthritis demonstrated an association of previous exposure to oral prednisone and high doses of pulsed GCs with decreased insulin sensitivity. GC use was not associated with obesity, hypertension, or dyslipidemia in that study [68]. Another study found an association of cumulative GC dose with glucose tolerance and insulin sensitivity, but not with other comparable metabolic parameters [69]. Patients with polymyositis and MetS had received more cumulative prednisolone doses ($P < 0.050$).

A randomized controlled trial in patients needing GC therapy showed a beneficial effect of metformin on glycemic control. This suggests that metformin may be a promising drug for preventing metabolic side effects during systemic GC treatment [70]. Another such drug is an 11β(beta)-HSD type 1 inhibitor, which is now in clinical trials. Local GC action depends on intracellular GC metabolism by 11β(beta)-hydroxysteroid dehydrogenases (11β[beta]-HSD). While 11β(beta)-HSD type 1 amplifies GC activity, 11β(beta)-HSD type 2 reduces it. Several studies in both rodents and humans have shown a positive association of 11β(beta)-HSD type 1 expression and activity in subcutaneous adipose tissue with insulin resistance and obesity [64, 71].

In conclusion, GC action appears to mediate certain aspects of the MetS. Prospects of targeting key players in GC action might show benefit in treatment of features of the MetS.

Other Common Medications Associated with the Metabolic Syndrome

The possibility that oral contraceptive pills (OCPs) may be associated with cardiometabolic parameters remains controversial. In a longitudinal population-based study that followed 3160 women, OCPs appeared to have no cardiometabolic effects when used for less than 3 years. However, the odds ratio of hypercholesterolemia

was significantly higher in women who used OCPs for >36 months compared to non-OCP users [8].

A number of medications other than GCs—antepileptics, antidepressants, growth hormone, beta-blockers, and antihypertensive agents—have been associated with increases in the risk of the MetS, either by promoting weight gain or by altering lipid or glucose metabolism [72].

Summary

Patients with several chronic diseases are at increased risk of developing obesity secondary to lifestyle factors associated with their chronic diseases, as well as due to their medications. Obesity has been considered a crucial component in the MetS. Furthermore, patients with chronic disease may develop components of the MetS even with normal BMI, as a result of an increase in the total percent of fat mass, as well as a reduction in lean body mass. Patients with chronic diseases carry an increased risk of morbidity due to coronary heart disease, which may be associated with the risk for MetS. Beneficial effects of metformin have been demonstrated in small studies in some disorders, and targeting of insulin resistance may be a promising approach for preventing cardiometabolic side effects in some chronic diseases.

References

1. Gingras V, Leroux C, Fortin A, Legault L, Rabasa-Lhoret R. Predictors of cardiovascular risk among patients with type 1 diabetes: a critical analysis of the metabolic syndrome and its components. Diabetes Metab. 2017;43:217–22. https://doi.org/10.1016/j.diabet.2016.10.007.
2. Baker KS, Chow E, Steinberger J. Metabolic syndrome and cardiovascular risk in survivors after hematopoietic cell transplantation. Bone Marrow Transplant. 2012;47:619–25. https://doi.org/10.1038/bmt.2011.118.
3. Gilliland FD, Berhane K, Islam T, McConnell R, Gauderman WJ, Gilliland SS, et al. Obesity and the risk of newly diagnosed asthma in school-age children. Am J Epidemiol. 2003;158:406–15. https://doi.org/10.1093/aje/kwg175.
4. Guerra S, Wright AL, Morgan WJ, Sherrill DL, Holberg CJ, Martinez FD. Persistence of asthma symptoms during adolescence. Am J Respir Crit Care Med. 2004;170:78–85. https://doi.org/10.1164/rccm.200309-1224OC.
5. Wilson PWF, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005;112:3066–72. https://doi.org/10.1161/CIRCULATIONAHA.105.539528.
6. Rzehak P, Wijga AH, Keil T, Eller E, Bindslev-Jensen C, Smit HA, et al. Body mass index trajectory classes and incident asthma in childhood: results from 8 European Birth Cohorts–a Global Allergy and Asthma European Network initiative. J Allergy Clin Immunol. 2013;131:1528–36. https://doi.org/10.1016/j.jaci.2013.01.001.
7. Wiesenthal EN, Fagnano M, Cook S, Halterman JS. Asthma and overweight/obese: double trouble for urban children. J Asthma. 2016;53:485–91. https://doi.org/10.3109/02770903.2015.1108435.
8. Farahmand M, Ramezani Tehrani F, Rostami Dovom M, Hashemi S, Azizi F. The impact of oral contraceptives on cardiometabolic parameters. J Endocrinol Investig. 2016;39:277–83. https://doi.org/10.1007/s40618-015-0346-z.
9. Thuesen BH, Husemoen LL, Hersoug LG, Pisinger C, Linneberg A. Insulin resistance as a predictor of incident asthma-like symptoms in adults. Clin Exp Allergy. 2009;39:700–7. https://doi.org/10.1111/j.1365-2222.2008.03197.x.
10. Husemoen LLN, Glümer C, Lau C, Pisinger C, Mørch LS, Linneberg A. Association of obesity and insulin resistance with asthma and aeroallergen sensitization. Allergy. 2008;63:575–82. https://doi.org/10.1111/j.1398-9995.2007.01613.x.
11. Al-Shawwa BA, Al-Huniti NH, DeMattia L, Gershan W. Asthma and insulin resistance in morbidly obese children and adolescents. J Asthma. 2007;44:469–73. https://doi.org/10.1080/02770900701423597.
12. Arshi M, Cardinal J, Hill RJ, Davies PS, Wainwright C. Asthma and insulin resistance in children. Respirology. 2010;15:779–84. https://doi.org/10.1111/j.1440-1843.2010.01767.x.
13. Baffi CW, Wood L, Winnica D, Strollo PJ Jr, Gladwin MT, Que LG, et al. Metabolic syndrome and the lung. Chest. 2016;149:1525–34. https://doi.org/10.1016/j.chest.2015.12.034.
14. Lee EJ, In KH, Ha ES, Lee KJ, Hur GY, Kang EH, et al. Asthma-like symptoms are increased in the metabolic syndrome. J Asthma. 2009;46:339–42. https://doi.org/10.1080/02770900802660931.
15. Brumpton BM, Camargo CA, Romundstad PR, Langhammer A, Chen Y, Mai XM. Metabolic syndrome and incidence of asthma in adults: the HUNT study. Eur Respir J. 2013;42(6):1495–502. http://erj.ersjournals.com/content/42/6/1495.long. Accessed 29 Jul 2017.
16. Forno E, Han YY, Muzumdar RH, Celedón JC. Insulin resistance, metabolic syndrome, and lung function in

US adolescents with and without asthma. J Allergy Clin Immunol. 2015;136:304–11.e8. https://doi.org/10.1016/j.jaci.2015.01.010.
17. Rastogi D, Bhalani K, Hall CB, Isasi CR. Association of pulmonary function with adiposity and metabolic abnormalities in urban minority adolescents. Ann Am Thorac Soc. 2014;11:744–52. https://doi.org/10.1513/AnnalsATS.201311-403OC.
18. Adeniyi FB, Young T. Weight loss interventions for chronic asthma. Cochrane Database Syst Rev. 2012;(7):CD009339. https://doi.org/10.1002/14651858.CD009339.pub2.
19. Al Khalifah RA, Alnhdi A, Alghar H, Alanazi M, Florez ID. The effect of adding metformin to insulin therapy for type 1 diabetes mellitus children: a systematic review and meta-analysis. Pediatr Diabetes. 2017;18(7):664–73. https://doi.org/10.1111/pedi.12493.
20. Cardwell CR, Stene LC, Joner G, Davis EA, Cinek O, Rosenbauer J, et al. Birthweight and the risk of childhood-onset type 1 diabetes: a meta-analysis of observational studies using individual patient data. Diabetologia. 2010;53:641–51. https://doi.org/10.1007/s00125-009-1648-5.
21. Magnus MC, Olsen SF, Granström C, Joner G, Skrivarhaug T, Svensson J, et al. Infant growth and risk of childhood-onset type 1 diabetes in children from 2 Scandinavian birth cohorts. JAMA Pediatr. 2015;169:e153759. https://doi.org/10.1001/jamapediatrics.2015.3759.
22. Verbeeten KC, Elks CE, Daneman D, Ong KK. Association between childhood obesity and subsequent type 1 diabetes: a systematic review and meta-analysis. Diabet Med. 2011;28:10–8. https://doi.org/10.1111/j.1464-5491.2010.03160.x.
23. Pinhas-Hamiel O, Levek-Motola N, Kaidar K, Boyko V, Tisch E, Mazor-Aronovitch K, et al. Prevalence of overweight, obesity and metabolic syndrome components in children, adolescents and young adults with type 1 diabetes mellitus. Diabetes Metab Res Rev. 2015;31:76–84. https://doi.org/10.1002/dmrr.2565.
24. Liu LL, Lawrence JM, Davis C, Liese AD, Pettitt DJ, Pihoker C, et al. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. Pediatr Diabetes. 2010;11:4–11. https://doi.org/10.1111/j.1399-5448.2009.00519.x.
25. Bielorai B, Weintraub Y, Hutt D, Hemi R, Kanety H, Modan-Moses D, et al. The metabolic syndrome and its components in pediatric survivors of allogeneic hematopoietic stem cell transplantation. Clin Transpl. 2017;31(3). https://doi.org/10.1111/ctr.12903.
26. Paris C, Yates L, Lama P, Zepeda AJ, Gutiérrez D, Palma J. Evaluation of metabolic syndrome after hematopoietic stem cell transplantation in children and adolescents. Pediatr Blood Cancer. 2012;59:306–10. https://doi.org/10.1002/pbc.24104.
27. Oudin C, Auquier P, Bertrand Y, Contet A, Kanold J, Sirvent N, et al. Metabolic syndrome in adults who received hematopoietic stem cell transplantation for acute childhood leukemia: an LEA study. Bone Marrow Transplant. 2015;50:1438–44. https://doi.org/10.1038/bmt.2015.167.
28. Baker KS, Ness KK, Steinberger J, Carter A, Francisco L, Burns LJ, et al. Diabetes, hypertension, and cardiovascular events in survivors of hematopoietic cell transplantation: a report from the bone marrow transplantation survivor study. Blood. 2007;109:1765–72. https://doi.org/10.1182/blood-2006-05-022335.
29. Bajwa R, Skeens M, Garee A, Miao Y, Soni S, Pietryga D, et al. Metabolic syndrome and endocrine dysfunctions after HSCT in children. Pediatr Transplant. 2012;16:872–8. https://doi.org/10.1111/petr.12002.
30. Bizzarri C, Pinto RM, Ciccone S, Brescia LP, Locatelli F, Cappa M. Early and progressive insulin resistance in young, non-obese cancer survivors treated with hematopoietic stem cell transplantation. Pediatr Blood Cancer. 2015;62:1650–5. https://doi.org/10.1002/pbc.25603.
31. Saultier P, Auquier P, Bertrand Y, Vercasson C, Oudin C, Contet A, et al. Metabolic syndrome in long-term survivors of childhood acute leukemia treated without hematopoietic stem cell transplantation: an L.E.A. study. Haematologica. 2016;101(12):1603–10. https://doi.org/10.3324/haematol.2016.148908.
32. Tichelli A, Bucher C, Rovó A, Stussi G, Stern M, Paulussen M, et al. Premature cardiovascular disease after allogeneic hematopoietic stem-cell transplantation. Blood. 2007;110(9):3463–71.
33. Penfold RB, Stewart C, Hunkeler EM, Madden JM, Cummings JR, Owen-Smith AA, et al. Use of antipsychotic medications in pediatric populations: what do the data say? Curr Psychiatry Rep. 2013;15:426. https://doi.org/10.1007/s11920-013-0426-8.
34. Petry NM, Barry D, Pietrzak RH, Wagner JA. Overweight and obesity are associated with psychiatric disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Psychosom Med. 2008;70:288–97. https://doi.org/10.1097/PSY.0b013e3181651651.
35. Rajan T, Menon V. Psychiatric disorders and obesity: a review of association studies. J Postgrad Med. 2017;63:182. https://doi.org/10.4103/jpgm.JPGM_712_16.
36. Landau Z, Hadi-Cohen R, Boaz M, Krivoy A, Amit BH, Zalsman G, et al. Risk factors for weight gain and metabolic syndrome in adolescents with psychiatric disorders: a historical prospective study. J Child Adolesc Psychopharmacol. 2015;25:160–7. https://doi.org/10.1089/cap.2014.0098.
37. Hung CI, Liu CY, Hsiao MC, Yu NW, Chu CL. Metabolic syndrome among psychiatric outpatients with mood and anxiety disorders. BMC Psychiatry. 2014;14:185. https://doi.org/10.1186/1471-244X-14-185.
38. Alosaimi FD, Abalhassan M, Alhaddad B, Alzain N, Fallata E, Alhabbad A, et al. Prevalence of metabolic syndrome and its components among patients with various psychiatric diagnoses and treatments: a cross-

sectional study. Gen Hosp Psychiatry. 2017;45:62–9. https://doi.org/10.1016/j.genhosppsych.2016.12.007.
39. Kawada T. Relationship between metabolic syndrome and psychiatric disorders in patients with bipolar disorder or schizophrenia. Bipolar Disord. 2015;17:233–4. https://doi.org/10.1111/bdi.12279.
40. Bioque M, García-Portilla MAP, García-Rizo C, Cabrera B, Lobo A, González-Pinto A, et al. Evolution of metabolic risk factors over a two-year period in a cohort of first episodes of psychosis. Schizophr Res. 2018;193:188–96.
41. Galling B, Roldán A, Nielsen RE, Nielsen J, Gerhard T, Carbon M, et al. Type 2 diabetes mellitus in youth exposed to antipsychotics: a systematic review and meta-analysis. JAMA Psychiat. 2016;73(3):247–59. https://doi.org/10.1001/jamapsychiatry.2015.2923.
42. DE Hert M, Correll CU, Bobes J, Cetkovich-Bakmas M, Cohen D, Asai I, et al. Physical illness in patients with severe mental disorders. I. Prevalence, impact of medications and disparities in health care. World Psychiatry. 2011;10:52–77. http://www.ncbi.nlm.nih.gov/pubmed/21379357. Accessed 5 Aug 2017.
43. de Silva VA, Suraweera C, Ratnatunga SS, Dayabandara M, Wanniarachchi N, Hanwella R. Metformin in prevention and treatment of antipsychotic induced weight gain: a systematic review and meta-analysis. BMC Psychiatry. 2016;16:341. https://doi.org/10.1186/s12888-016-1049-5.
44. Almandil NB, Liu Y, Murray ML, Besag FM, Aitchison KJ, Wong IC. Weight gain and other metabolic adverse effects associated with atypical antipsychotic treatment of children and adolescents: a systematic review and meta-analysis. Paediatr Drugs. 2013;15:139–50. https://doi.org/10.1007/s40272-013-0016-6.
45. Anagnostou E, Aman MG, Handen BL, Sanders KB, Shui A, Hollway JA, et al. Metformin for treatment of overweight induced by atypical antipsychotic medication in young people with autism spectrum disorder: a randomized clinical trial. JAMA Psychiat. 2016;73:928–37. https://doi.org/10.1001/jamapsychiatry.2016.1232.
46. Janszky I, Hallqvist J, Tomson T, Ahlbom A, Mukamal KJ, Ahnve S. Increased risk and worse prognosis of myocardial infarction in patients with prior hospitalization for epilepsy-the Stockholm Heart Epidemiology Program. Brain. 2009;132:2798–804. https://doi.org/10.1093/brain/awp216.
47. Daniels ZS, Nick TG, Liu C, Cassedy A, Glauser TA. Obesity is a common comorbidity for pediatric patients with untreated, newly diagnosed epilepsy. Neurology. 2009;73:658–64. https://doi.org/10.1212/WNL.0b013e3181ab2b11.
48. Grosso S, Mostardini R, Piccini B, Balestri P. Body mass index and serum lipid changes during treatment with valproic acid in children with epilepsy. Ann Pharmacother. 2009;43:45–50. https://doi.org/10.1345/aph.1L414.
49. Verrotti A, di Corcia G, Salladini C, Trotta D, Morgese G, Chiarelli F. Serum insulin and leptin levels and valproate-associated obesity. Epilepsia. 2003;44:1606. http://www.ncbi.nlm.nih.gov/pubmed/14636339.
50. Corman CL, Leung NM, Guberman AH. Weight gain in epileptic patients during treatment with valproic acid: a retrospective study. Can J Neurol Sci. 1997;24:240–4. http://www.ncbi.nlm.nih.gov/pubmed/9276111.
51. Abaci A, Saygi M, Yis U, Demir K, Dirik E, Bober E. Metabolic alterations during valproic acid treatment: a prospective study. Pediatr Neurol. 2009;41:435–9. https://doi.org/10.1016/j.pediatrneurol.2009.06.003.
52. Pylvänen V, Pakarinen A, Knip M, Isojärvi J. Characterization of insulin secretion in valproate-treated patients with epilepsy. Epilepsia. 2006;47:1460–4. https://doi.org/10.1111/j.1528-1167.2006.00546.x.
53. Rakitin A, Eglit T, Kõks S, Lember M, Haldre S. Comparison of the metabolic syndrome risk in valproate-treated patients with epilepsy and the general population in Estonia. PLoS One. 2014;9:e103856. https://doi.org/10.1371/journal.pone.0103856.
54. Fang J, Chen S, Tong N, Chen L, An D, Mu J, et al. Metabolic syndrome among Chinese obese patients with epilepsy on sodium valproate. Seizure. 2012;21:578–82. https://doi.org/10.1016/j.seizure.2012.06.001.
55. Pylvänen V, Pakarinen A, Knip M, Isojärvi J. Insulin-related metabolic changes during treatment with valproate in patients with epilepsy. Epilepsy Behav. 2006;8:643–8. https://doi.org/10.1016/j.yebeh.2006.02.008.
56. Nair SS, Harikrishnan S, Sarma PS, Thomas SV. Metabolic syndrome in young adults with epilepsy. Seizure. 2016;37:61–4. https://doi.org/10.1016/j.seizure.2016.03.002.
57. Virkamäki A, Ueki K, Kahn CR. Protein–protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest. 1999;103:931–43. https://doi.org/10.1172/JCI6609.
58. Kohen-Avramoglu R, Theriault A, Adeli K. Emergence of the metabolic syndrome in childhood: an epidemiological overview and mechanistic link to dyslipidemia. Clin Biochem. 2003;36:413–20. http://www.ncbi.nlm.nih.gov/pubmed/12951167. Accessed 24 Jul 2017.
59. Verrotti A, Manco R, Agostinelli S, Coppola G, Chiarelli F. The metabolic syndrome in overweight epileptic patients treated with valproic acid. Epilepsia. 2010;51:268–73. https://doi.org/10.1111/j.1528-1167.2009.02206.x.
60. Pinhas-Hamiel O, Livne M, Harari G, Achiron A. Prevalence of overweight, obesity and metabolic syndrome components in multiple sclerosis patients with significant disability. Eur J Neurol. 2015;22:1275–9. https://doi.org/10.1111/ene.12738.
61. Sicras-Mainar A, Ruíz-Beato E, Navarro-Artieda R, Maurino J. Comorbidity and metabolic syndrome in patients with multiple sclerosis from Asturias and Catalonia, Spain. BMC Neurol. 2017;17:134. https://doi.org/10.1186/s12883-017-0914-2.

62. Falhammar H, Frisén L, Hirschberg AL, Norrby C, Almqvist C, Nordenskjöld A, et al. Increased cardiovascular and metabolic morbidity in patients with 21-hydroxylase deficiency: a Swedish population-based national cohort study. J Clin Endocrinol Metab. 2015;100:3520–8. https://doi.org/10.1210/JC.2015-2093.
63. van Staa TP, Leufkens HGM, Abenhaim L, Begaud B, Zhang B, Cooper C. Use of oral corticosteroids in the United Kingdom. QJM. 2000;93:105–11. https://doi.org/10.1093/qjmed/93.2.105.
64. van Raalte DH, Ouwens DM, Diamant M. Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options? Eur J Clin Investig. 2009;39:81–93. https://doi.org/10.1111/j.1365-2362.2008.02067.x.
65. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. J Clin Invest. 1997;99:414–23. https://doi.org/10.1172/JCI119175.
66. Larsson H, Ahrén B. Insulin resistant subjects lack islet adaptation to short-term dexamethasone-induced reduction in insulin sensitivity. Diabetologia. 1999;42:936–43. https://doi.org/10.1007/s001250051251.
67. Wang M. The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. Nutr Metab. 2005;2(1):3. https://doi.org/10.1186/1743-7075-2-3.
68. Dessein PH, Joffe BI, Stanwix AE, Christian BF, Veller M. Glucocorticoids and insulin sensitivity in rheumatoid arthritis. J Rheumatol. 2004;31:867–74. http://www.ncbi.nlm.nih.gov/pubmed/15124244.
69. de Souza FHC, Shinjo SK. The high prevalence of metabolic syndrome in polymyositis. Clin Exp Rheumatol. 2014;32:82–7. http://www.ncbi.nlm.nih.gov/pubmed/24144101.
70. Seelig E, Meyer S, Timper K, Nigro N, Bally M, Pernicova I, et al. Metformin prevents metabolic side effects during systemic glucocorticoid treatment. Eur J Endocrinol. 2017;176:349–58. https://doi.org/10.1530/EJE-16-0653.
71. Kershaw EE, Morton NM, Dhillon H, Ramage L, Seckl JR, Flier JS. Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. Diabetes. 2005;54:1023–31. http://www.ncbi.nlm.nih.gov/pubmed/15793240.
72. Wofford MR, King DS, Harrell TK. Drug-induced metabolic syndrome. J Clin Hypertens (Greenwich). 2006;8:114–9. http://www.ncbi.nlm.nih.gov/pubmed/16470080. Accessed 3 Aug 2017.

Part II

Pathophysiology

Molecular Mechanisms of Insulin Resistance

4

Boris Draznin

Even though insulin resistance has emerged as an enormous healthcare problem, intersecting 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 β(beta)-cells postprandially 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, the main physiological task of insulin is to suppress glucose production by the liver. Thus, if any one of these two 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 total body insulin resistance.

On a cellular level, insulin plays an important role not only in carbohydrate metabolism but also in protein and lipid synthesis, ion fluxes, and cell growth and differentiation, as well as in 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 a 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].

If insulin binding to its receptor is the first step in the mechanism of insulin action, propagation of the signal generated by this binding event downstream to the signaling molecules represents the next steps in implementation of cellular response to insulin. Insulin signaling constitutes a complex system, and while signals are transmitted downstream via multiple phosphorylation and dephosphorylation steps, there are several feedback regulatory steps that guard against overstimulation [18, 19]. Furthermore, enzymes

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and nonenzymatic proteins, as well as other signaling molecules involved in propagation of insulin signaling, can affect and frequently are affected by other intracellular mediators, including inflammatory molecules and lipids, which mainly result in inhibition of insulin action [20, 21].

Even though there is redundancy in multiple sites of the insulin signaling system, defects in the insulin signaling cascade consisting of single inherited or multiple acquired mutations that block insulin action have been described in humans [22], resulting in various phenotypic expressions of hyperglycemia and diabetes. In some cases, impairment of insulin action can lead to the subtle clinical manifestations of mild insulin resistance and minimal hyperglycemia, while in other cases patients may exhibit severe insulin resistance with or without overt diabetes.

Insulin action is initiated by an interaction of insulin with its cell surface receptor [23, 24]. The insulin receptor is a heterotetrameric protein that consists of two extracellular α(alpha) subunits and two transmembrane β(beta) subunits connected by disulfide bridges [25–27]. Insulin binding to the extracellular α(alpha) subunit induces conformational changes of the insulin receptor that activate the tyrosine kinase (TK) domain of the intracellular portion of the β(beta) subunit [28–31]. Once the TK of insulin receptors is activated, it promotes autophosphorylation of the β(beta) subunit itself, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity [32, 33]. 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, IRS-2, IRS-3, and IRS-4 and several Shc (Src-homology collagen) proteins (52, 46, and 64 kD isoforms) [34, 35] that, 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 the majority of patients lies at the post-receptor level of insulin signaling [36–38]. 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 [39], using in vivo magnetic resonance spectroscopy 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 [23, 24]. 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, thus accounting for the multitude of insulin's biological effects [23, 24].

Most, if not all, of the metabolic and antiapoptotic 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 generation of phosphatidylinositol triphosphate, PIP_3, or phosphoinositide 3,4,5-P_3, which in turn phosphorylates a serine kinase Akt [40]. Posttranslationally, Akt binds to heat shock protein 90, protecting the inactive Akt from proteasomal degradation [41]. Akt is then recruited to the cell membrane through PIP_3 [40, 42]. 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 [43].

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 [44, 45], 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 [19, 46–49], and can also lead to an accelerated degradation of IRS-1 protein [50]. 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 4.1) [51–75].

Table 4.1 IRS-1 serine phosphorylation can be caused by

1. mTOR – p70S6 kinase – Amino acids, hyperinsulinemia, TSC1–2 depletion, nutrition [51–54]
2. JNK – Stress, hyperlipidemia, inflammation [55–59]
3. IKK – Inflammation [60–63]
4. TNF-α(alpha) – Obesity, inflammation [64–68]
5. Mitochondrial dysfunction [69–71]
6. PKCθ(theta) – Hyperglycemia, diacylglycerol, inflammation [58, 72–75]

A cellular nutrient sensor, mTOR, has been identified as a critical element integrating cellular metabolism with growth factor signaling [76–80]. In response to insulin and amino acids, mTOR, which is a serine/threonine kinase, phosphorylates and modulates activities of p70 S6 kinase (S6K1 kinase) and is an inhibitor of translational initiation, eIF-4E-binding protein (eIF-4EBP) [81, 82]. mTOR interacts with two scaffolding proteins, Raptor [83, 84] and Rictor [85–87]. The Raptor/mTOR complex is rapamycin sensitive and regulates growth via S6K1 and eIF-4EBP [83, 84]. The Rictor/mTOR complex is insensitive to rapamycin and regulates cellular proliferation via Akt [88], PKCα(alpha) [87], and small molecular weight GTPases [85]. While insulin activates mTOR and S6K1 kinase via the IRS-1/PI 3-kinase/Akt pathway [89, 90], amino acids seem to exert their effect through a direct influence on mTOR [78, 91, 92]. 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. 4.1a). In contrast to wild-type littermates, transgenic mice lacking S6K1 kinase (S6K1-deficient mice) displayed a strong resistance to age- and diet-induced obesity and insulin resistance [47]. 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 [47, 93, 94].

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

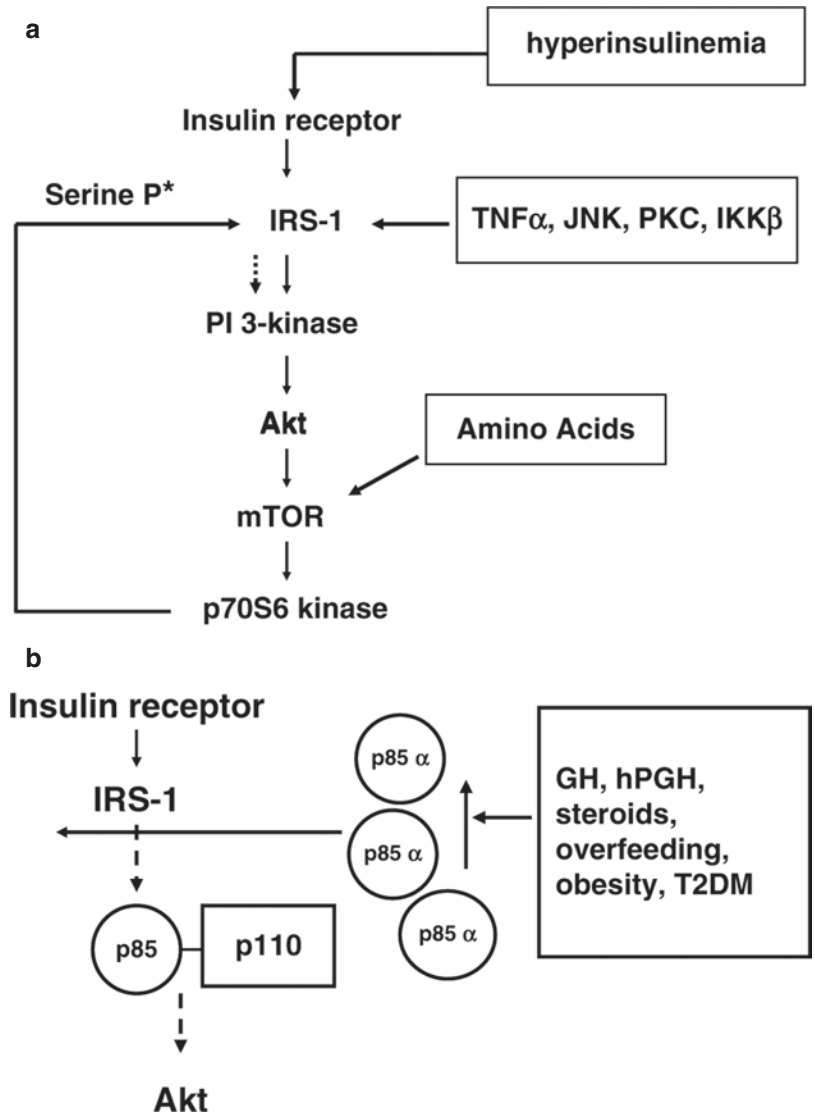

Fig. 4.1 Inhibition of the metabolic insulin signaling. IRS-1 is phosphorylated by the tyrosine kinase of the insulin receptor in response to insulin binding. Protein/lipid kinase, PI 3-kinase, binds to the specific MYMX motifs of IRS-1, containing phosphorylated tyrosine residues. PI 3-kinase is then 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(beta), and TNFα(alpha). (**b**) Increased expression of p85α(alpha) 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(alpha) expression. (See text for details and references)

isoform) [95]. 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 the 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(alpha) (TNFα[alpha]), and PKC, among others, can promote serine phosphorylation of IRS-1 (Table 4.1 and Fig. 4.1a) [51–75].

Activation of JNK by free fatty acids (FFA), stress, and inflammation [55–58] has been shown to increase serine phosphorylation of IRS-1, with a resulting decline in the strength of insulin signaling along the metabolic pathway [96, 97]. Blocking JNK activation rescued the cellular and molecular defects induced by FFA [95]. Furthermore, JNK-1 knockout mice were found to be resistant to diet-induced obesity and insulin resistance [55]. Treatment of cells with a specific JNK activator, anisomycin, was reported to elicit IRS-1 phosphorylation on Ser 307 [98]. Increased serine 307 phosphorylation of IRS-1 has been found in liver of the wild-type but not JNK-1-defficient mice [99].

Similarly, activation of the pro-inflammatory kinase that phosphorylates the inhibitor of NF-κ(kappa)B, inhibitor kappa B kinase β(beta) (IKKβ[beta]), has been shown to induce insulin resistance [60, 61, 99, 100]. In an unstimulated state, NF-κ(kappa)B dimers are restrained in the cytoplasm in association with inhibitory proteins Iκ(kappa)Bs. In response to pro-inflammatory stimuli, such as TNFα(alpha), IKKβ(beta) is activated and phosphorylates two serine residues of the Iκ(kappa)B. Phosphorylated Iκ(kappa)B is rapidly degraded by proteasomes, releasing NF-κ(kappa)B for translocation to the nucleus where it activates transcription of target genes. Inhibition of IKKβ(beta) with salicylates has been shown to prevent and reverse diet- and obesity-induced insulin resistance [62, 63].

Activation of IKKβ(beta) in skeletal muscle is associated with impaired IRS-1–PI 3-kinase signaling [101]. Furthermore, Kim et al. [102] have demonstrated that activation of IKKβ(beta) by hyperglycemia played an important role in impaired insulin-stimulated nitric oxide production in endothelial cells. Overexpression of wild-type IKKβ(beta) recapitulated the deleterious effect of hyperglycemia on insulin-mediated activation of endothelial nitric oxide synthase [63]. Taken together, available evidence implicates IKKβ(beta) in the pathogenesis of insulin resistance via a mechanism that involves impairment in the IRS-1–PI 3-kinase signaling pathway.

TNFα(alpha), 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(alpha) appeared to reverse insulin resistance in animal models, it has been suggested that TNFα(alpha) plays an important role in the pathogenesis of insulin resistance in obesity [64–66]. Furthermore, mice lacking TNFα(alpha) function were protected from obesity-induced insulin resistance [67]. More recently, TNFα(alpha) has been shown to block insulin signaling by promoting serine phosphorylation of IRS-1 [68], with a resultant decline in IRS-1-associated PI 3-kinase activity.

Recently, a hypothesis that mitochondrial dysfunction or reduced mitochondrial content accompanied by decreased mitochondrial fatty acid oxidation and accumulation of fatty acid acyl CoA and diacylglycerol can cause insulin resistance has gained substantial experimental support [69–71]. The mechanism of insulin resistance in these cases has been suggested to involve activation of a novel PKC that either by itself or via IKKβ(beta) or JNK-1 could lead to increased serine phosphorylation of IRS-1.

The pro-inflammatory novel PKCθ(theta) has been found to cause serine phosphorylation of IRS-1 [72, 73], while PKCθ(theta) knockout mice have been shown to be protected from fat-induced insulin resistance [74]. Increased activity of PKCθ(theta) along with increased activity of JNK has also been found in skeletal muscle of obese and type 2 diabetic subjects [58, 75], supporting a potential role of these serine kinases in the pathogenesis of insulin resistance.

Increased Expression of p85α(Alpha)

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 [103]. PI 3-kinase belongs to the class 1a 3-kinases [104], which exist as heterodimers, consisting of a regulatory subunit (p85) that is tightly associated with a catalytic subunit, p110. The regulatory p85 subunit is encoded by at least three genes that generate highly homologous products. Two isoforms are termed p85α(alpha) (PIK3R1) and p85β(beta) (products of the two genes). Three splice variants of p85α(alpha) have been reported, including p85α(alpha) itself, p55α(alpha), and p50α(alpha). The third gene product is p55γ(gamma). The p85α(alpha), however, appears to be the most abundant isoform [104].

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 [105]. The p85 regulatory subunit stabilizes the p110 subunit [106–108] and maintains it in a low activity state [105]. 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 [109–111]. The nSH2 domain of p85 inhibits p110 activity, and its interaction with a phosphopeptide disinhibits the p85–p110 heterodimer's activity [109–111].

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 shift this balance in favor of either free p85 or p85–p110 complexes [112–115]. 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. 4.1b). This possibility has been recently supported by studies in insulin-resistant states induced by human placental growth hormone [116], obesity, and type 2 diabetes [58] and by short-term overfeeding of lean nondiabetic women [117].

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 [118]. This treatment significantly reduced PI 3-kinase activity, despite an almost fourfold increase in expression of p85α(alpha) (no change in p85β[beta]) and only a minimal increase in p110. The authors concluded that p85α(alpha) competes with the p85–p110 heterodimer, thus reducing PI 3-kinase activity (Table 4.2) [58, 116, 117, 119, 120].

Subsequently, animals with a targeted disruption of *p85α(alpha)* ($p85^{+/-}$ heterozygous mice) have been found to have a higher ratio of p85–p110 dimer to free p85 and to be more sensitive to insulin [103, 119, 121]. In order to determine this ratio, the investigators immunodepleted p110 and blotted both the immunoprecipitates and the supernatant with p85 antibody. The amounts of

Table 4.2 An imbalance between PI 3-kinase subunits can be caused by

1. Steroids [119]
2. Growth hormone (GH) [120]
3. Human placental (hPGH) [116, 120]
4. Short-term overfeeding [117]
5. Obesity and diabetes [58]

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 p85α(alpha) in cultured cells. This overexpression significantly inhibited the PI 3-kinase activity [114, 115, 122]. Overexpression of p50α(alpha) or p55α(alpha) did not inhibit PI 3-kinase activity to the same extent. These experimental results were consistent with the competition hypothesis.

Recently, Barbour et al. [116, 120] demonstrated that insulin resistance of pregnancy is likely due to increased expression of skeletal muscle p85 in response to increasing concentrations of human placental growth hormone (hPGH). Furthermore, women remaining insulin-resistant postpartum have been found to display higher levels of p85 in the muscle [123]. Thus, results reported in the literature support the hypothesis that the p85 monomer completes 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. [117] were able to show that 3 days of overfeeding (50% above usual caloric intake) led to a significant increase in expression of p85α(alpha), the ratio of p85α(alpha) to p110, and 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 [117], suggesting that increased expression of p85α(alpha) may be an early molecular step in the pathogenesis of the 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(alpha) 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(alpha) and increased serine phosphorylation of IRS-1 is needed to induce clinically apparent insulin resistance. Further studies are needed in order to evaluate this hypothesis.

References

1. Olefsky JM. The insulin receptor: a multifunctional protein. Diabetes. 1990;39:1009–16.
2. Reaven GM. Role of insulin resistance in human disease. Diabetes. 1998;37:1595–607.
3. DeFronzo RA. The triumvirate: beta-cell, muscle, liver. A collision responsible for NIDDM. Diabetes. 1988;37:667–87.
4. Bell GI, Burant CF, Takeda J, Gould GW. Structure and function of mammalian facilitative sugar transporters. J Biol Chem. 1993;268:19161–4.
5. Shepherd PR, Kahn BB. Glucose transporters and insulin action. N Engl J Med. 1999;341:248–57.
6. Cheatham B. Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70S6 kinase, DNA synthesis, and glucose transporter translocation. Mol Cell Biol. 1994;14:4902–11.
7. Shepherd PR, Nave BT, Siddle K. Insulin stimulation of glycogen synthesis and glycogen synthase activity is blocked by wortmannin and rapamycin in 3T3-L1 adipocytes: evidence for the involvement of phosphoinositide 3-kinase and p70 ribosomal protein-S6 kinase. Biochem J. 1995;305:25–8.
8. Lazar D. Mitogen-activated kinase kinase inhibition does not block the stimulation of glucose utilization by insulin. J Biol Chem. 1995;270:20801–7.
9. Sutherland C, Waltner-Law M, Gnudi L, Kahn BB, Granner DK. Activation of the Ras Mitogen-activated protein kinase-ribosomal protein kinase pathway is not required for the repression of phosphoenolpyruvate carboxykinase gene transcription by insulin. J Biol Chem. 1998;273:3198–204.
10. Bandyopadhyay GK, Standaert ML, Zhao L, Yu B, Avignon A, Galloway L, Karnam P, Moscat J,

Farese RV. Activation of protein kinase (α, β, and ξ) by insulin in 3T3-L1 cells: transfection studies suggest a role for PKC-zeta in glucose transport. J Biol Chem. 1997;272:2551–8.
11. Jiang ZY, Lin Y-W, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. J Clin Invest. 1999;104:447–57.
12. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest. 2000;105:311–20.
13. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, Johansen M, Kucik DF, Quon MJ, Draznin B. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic action of insulin in endothelial cells. J Biol Chem. 2002;277:1794–9.
14. Wang C, Gurevich I, Draznin B. Insulin affects vascular smooth muscle cell phenotype and migration via distinct signaling pathways. Diabetes. 2003;52:2562–9.
15. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci U S A. 2003;100:7265–70.
16. Ginsberg H. Insulin resistance and cardiovascular disease. J Clin Invest. 2000;106:453–8.
17. Shulman GI. Cellular mechanisms of insulin resistance in humans. Am J Cardiol. 1999;84:3J–10J.
18. Birnbaum MJ. Turning down insulin signaling. J Clin Invest. 2001;108:655–9.
19. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem. 2002;277:1531–7.
20. Pessin JE, Saltiel AR. Signaling pathways in insulin action: molecular targets of insulin resistance. J Clin Invest. 2000;106:165–9.
21. White MF. Insulin signaling in health and disease. Science. 2003;302:1710–1.
22. Semple RK. How does insulin resistance arise, and how does it cause disease? Human genetic lessons. Eur J Endocrinol. 2016;174:R209–23.
23. Cheatham B, Kahn CR. Insulin action and the insulin signaling network. Endocr Rev. 1995;16:117–41.
24. Kahn CR. Insulin action, diabetogenes, and the cause of type 2 diabetes. Diabetes. 1994;43:1066–84.
25. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli IM, Dull TJ, Gray A, Coussens L, Liao Y-C, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature. 1985;313:756–61.
26. Ebina Y, Ellis L, Jarnagin K, Edery M, Grat L, Clauser E, Ou J-H, Masiarz F, Kan YW, Goldfine ID, Roth RA, Rutter WJ. The human insulin receptor cDNA. The structural basis for hormone-activated transmembrane signaling. Cell. 1985;40:747–58.
27. Seino S, Seino M, Nishi S, Bell GI. Structure of human insulin receptor gene and characterization of its promoter. Proc Natl Acad Sci U S A. 1989;86:114–8.
28. Kasuga M, Karisson FA, Kahn CR. Insulin stimulates the phosphorylation of the 95,000 Dalton subunit of its own receptor. Science. 1982;215:185–7.
29. Wilden PA, Siddle K, Haring E, Backer JM, White MF, Kahn CR. The role of insulin receptor-kinase domain autophosphorylation in receptor-mediated activities. J Biol Chem. 1992;267:13719–27.
30. De Meyts P, Chistoffersen CT, Torquist H, Seedorf K. Insulin receptors and insulin action. Curr Opin Endocrinol Diabetes. 1996;3:369–77.
31. Rhodes CJ, White MF. Molecular insights into insulin action and secretion. Eur J Clin Invest. 2002;32(Suppl 3):3–13.
32. White MF, Shoelson SE, Keutmann H, Kahn CR. A cascade of autophosphorylation in the beta subunit activates phosphotransferase of the insulin receptor. J Biol Chem. 1988;263:2969–80.
33. Tornqvist HE, Avruch J. Relationship of site-specific beta subunit tyrosine autophosphorylation to insulin activation of the insulin receptor (tyrosine) protein kinase activity. J Biol Chem. 1988;263:4593–601.
34. Myers MG Jr, White MF. Insulin signal transduction and the IRS proteins. Annu Rev Pharmacol Toxicol. 1996;36:615–58.
35. Paz K, Voliovitch H, Hadari YR, Roberts CT, LeRoith D, Zick Y. Interaction between the insulin receptor and its downstream effectors. J Biol Chem. 1996;271:6998–7003.
36. Kolterman OG, Insel J, Saekow M, Olefsky JM. Mechanisms of insulin resistance in human obesity: evidence for receptor and post-receptor defects. J Clin Invest. 1980;65:1272–84.
37. Marshal S, Olefsky JM. Effects if insulin incubation on insulin binding, glucose transport, and insulin degradation by isolated rat adipocytes. Evidence for hormone-induced desensitization at the receptor and post-receptor level. J Clin Invest. 1980;66:763–72.
38. Haring HU. The insulin receptor: signaling mechanism and contribution to the pathogenesis of insulin resistance. Diabetologia. 1991;34:848–61.
39. Petersen KF, Shulman GI. Etiology of insulin resistance. Am J Med. 2006;119:10S–6S.
40. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer. 2002;2:489–501.
41. Munster PN, Marchion DC, Basso AD, Rosen N. Degradation of HER2 by ansamycins includes growth arrest and apoptosis in cells with HER2 overexpression via HER3, phosphatidylinositol 3′-kinase-Akt-dependent pathway. Cancer Res. 2002;62:3132–7.
42. Amaravadi R, Thompson GB. The survival kinases AKT and PIM as potential pharmacological targets. J Clin Invest. 2005;115:2618–24.

43. Ali IU, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. J Natl Cancer Inst. 1999;91:1922–32.
44. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000;106:473–81.
45. LeRoith D, Zick Y. Recent advances in our understanding of insulin action and insulin resistance. Diabetes Care. 2001;24:588–97.
46. Qiao L, Goldberg JL, Russell JC, Sun XJ. Identification of enhanced serine kinase activity in insulin resistance. J Biol Chem. 1999;274:10625–32.
47. Um SH, Frogerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature. 2004;431:200–5.
48. Patti M-E, Kahn BB. Nutrient sensor links obesity with diabetes risk. Nat Med. 2004;10:1049–50.
49. Qiao L, Zhande R, Jetton TL, Zhou G, Sun XJ. In vivo phosphorylation of insulin receptor substrate 1 at serine 789 by a novel serine kinase in insulin-resistant rodents. J Biol Chem. 2002;277:26530–9.
50. Shah OJ, Wang Z, Hunter T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol. 2004;14:1650–6.
51. Ueno M, Carvalheira JBC, Tambascia RC, Bezzera RMN, Amara ME, Carneiro EM, Folli F, Franchini KG, Saad MJA. Regulation of insulin signaling by hyperinsulinemia: role of IRS-1/2 serine phosphorylation and mTOR/p70 S6K pathway. Diabetologia. 2005;48:506–18.
52. Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, Olefsky JM, Kobayashi M. A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol Endocrinol. 2000;14:783–94.
53. Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle. J Biol Chem. 2001;276:38052–60.
54. Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR, Gout I, Downes CP, Lamb RE. The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. J Cell Biol. 2004;166:213–23.
55. Hirosumi J, Tuncman G, Chang L, Gorzun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. Nature. 2002;420:333–6.
56. Gao Z, Zhang X, Zuberi A, Hwang D, Quon MJ, Lefevre M, Ye J. Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. Mol Endocrinol. 2004;18:2024–34.
57. Nguyen MTA, Satoh H, Favelyukis S, Babendure JL, Imamura T, Sbodio JI, Zalevsky J, Dahiyat B, Chi N-W, Olefsky JM. JNK and TNF-α mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. J Biol Chem. 2005;280:35361.
58. Bandyopadhyay GK, Yu JG, Ofrecio J, Olefsky JM. Increased p85/55/50 expression and decreased phosphatidylinositol 3-kinase activity in insulin-resistant human skeletal muscle. Diabetes. 2005;54:2351–9.
59. Lee YH, Giraud J, Davis RJ, White MF. C-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem. 2003;278:2896–902.
60. Perseghin G, Petersen K, Shulman GI. Cellular mechanism of insulin resistance: potential links with inflammation. Int J Obes Relat Metab Disord. 2003;27(Suppl 3):S6–11.
61. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, Ye J. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kB kinase complex. J Biol Chem. 2002;277:48115–21.
62. Kim JK, Kim Y-J, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI. Prevention of fat-induced insulin resistance by salicylate. J Clin Invest. 2001;108:437–46.
63. Hundal RS, Petersen KF, Mayerson AB, Rahdhawa PS, Inzucchi S, Shoelson SE, Shulman GI. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest. 2002;109:1321–6.
64. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes. 1994;43:1271–8.
65. Qi C, Pekala PH. Tumor necrosis factor-alpha-induced insulin resistance in adipocytes. Proc Soc Exp Biol Med. 2000;223:128–35.
66. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259:87–91.
67. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature. 1997;389:610–4.
68. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha and obesity-induced insulin resistance. Science. 1996;271:665–8.
69. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science. 2005;307:384–7.
70. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science. 2003;300:1140–2.
71. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-

resistant offspring of patients with type 2 diabetes. N Engl J Med. 2004;350:664–71.
72. Li Y, Soos TJ, Li X, Wu J, Degennaro M, Sun X, Littman DR, Birnbaum MJ, Polakiewicz RD. Protein kinase θ inhibits insulin signaling by phosphorylating IRS1 at Ser(1101). J Biol Chem. 2004;279:45304–7.
73. Bell KS, Shcmitz-Peiffer C, Lim-Fraser M, Biden TJ, Cooney GJ, Kraegen EW. Acute reversal of lipid-induced muscle insulin resistance is associated with rapid alteration in PKC-θ localization. Am J Physiol Endocrinol Metab. 2000;279:E1196–201.
74. Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim DW, Liu ZX, Soos TJ, Cline GW, O'Brien WR, Littman DR, Shulman GI. PKC-θ knockout mice are protected from fat-induced insulin resistance. J Clin Invest. 2004;114:823–7.
75. Itani SI, Pories WJ, Macdonald KG, Dohm GL. Increased protein kinase C θ in skeletal muscle of diabetic patients. Metabolism. 2001;50:553–7.
76. Raught B, Gingras AC, Sonenberg N. The target of rapamycin (TOR) proteins. Proc Natl Acad Sci U S A. 2001;98:7037–44.
77. Rohde J, Heitman J, Cardenas ME. The TOR kinases link nutrient sensing to cell growth. J Biol Chem. 2001;276:9583–6.
78. Khamzina L, Veilleux A, Bergeron S, Marette A. Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. Endocrinology. 2005;146:1473–81.
79. Trembley F, Gagnon A, Veilleux A, Sorisky A, Marette A. Activation of the mammalian target of rapamycin pathway acutely inhibits insulin signaling to Akt and glucose transport in 3T3-L1 and human adipocytes. Endocrinology. 2005;146:1328–37.
80. Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM. RAFT1 phosphorylation of the translational regulators p70S6 kinase and 4E-BP1. Proc Natl Acad Sci U S A. 1998;95:1432–7.
81. Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng OP, Kasuga M, Nishimoto I, Avruch J. Regulation of eIF-4E BP1 phosphorylation by mTOR. J Biol Chem. 1997;272:26457–63.
82. Isotani S, Hara K, Tokunaga C, Inoue H, Avruch J, Yonezawa K. Immunopurified mammalian target of rapamycin phosphorylates and activates p70S6 kinase α in vitro. J Biol Chem. 1999;274:34493–8.
83. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell. 2002;110:177–89.
84. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell. 2002;110:163–75.
85. Jacinto E, Loewith R, Scmidt A, Lin S, Ruegg MA, Hall A, Hall MN. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin sensitive. Nat Cell Biol. 2004;6:1122–8.
86. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell. 2002;10:457–68.
87. Sabrassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol. 2004;14:1296–302.
88. Sabrassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science. 2005;307:1098–101.
89. Nave BT, Ouwens M, Withers DJ, Alessi DR, Shepherd PR. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. Biochem J. 1999;344:427–31.
90. Scott PH, Brunn GJ, Kohn AD, Roth RA, Lawrence JC Jr. Evidence of insulin-stimulated phosphorylation and activation of the mammalian target of rapamycin mediated by a protein kinase B signaling pathway. Proc Natl Acad Sci U S A. 1998;95:7772–7.
91. Hinault C, Mothe-Satney I, Gautier N, Lawrence JC Jr, Van Obberghen E. Amino acids and leucine allow insulin activation of the PKB/mTOR pathway in normal adipocytes treated with wortmannin and in adipocytes from *db/db* mice. FASEB J. 2004;18:1894–6.
92. Pham P-TT, Heydrick SJ, Fox HL, Kimball SR, Jefferson LS Jr, Lynch CJ. Assessment of cell-signaling pathways in the regulation of mammalian target of rapamycin (mTOR) by amino acids in rat adipocytes. J Cell Biochem. 2000;79:427–41.
93. Pende M, Kozma SC, Jaquet M, Oorshcot V, Burcelin R, Le Marchand-Brustel Y, Klumperman J, Thorens B, Thomas G. Hypoinsulinemia, glucose intolerance and diminished β-cell size in S6K1-deficient mice. Nature. 2000;408:994–7.
94. Tremblay F, Krebs M, Dombrowski L, Brehm A, Bernroider E, Roth E, Nowotny P, Waldhausl W, Marette A, Roden M. Overactivation of S6 kinase 1 as a cause of human insulin resistance during increased amino acid availability. Diabetes. 2005;54:2674–84.
95. Tzatsos A, Kandor KV. Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via Raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. Mol Cell Biol. 2006;26:63–76.
96. Mussig K, Fiedler H, Staiger H, Weigert C, Lehmann R, Schleicher ED, Haring H-U. Insulin-induced

stimulation of JNK and the PI 3-kinase/mTOR pathway leads to phosphorylation of serine 318 of IRS-1 in C2C12 myotubes. Biochem Biophys Res Commun. 2005;335:819–25.
97. Hiratani K, Haruta T, Tani A, Kawahara J, Usui I, Kobayashi M. Roles of mTOR and JNK in serine phosphorylation, translocation, and degradation of IRS-1. Biochem Biophys Res Commun. 2005;335:836–42.
98. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of ser307. J Biol Chem. 2000;275:9047–54.
99. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. Nature. 2002;410:333–6.
100. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKK-beta. Science. 2001;293:1673–7.
101. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon M, Ye J. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. J Biol Chem. 2002;277:48115–21.
102. Kim F, Tysseling KA, Rice J, Gallis B, Haji L, Giachelli CM, Raines EW, Corson MA, Schwartz MW. Activation of IKKβ by glucose is necessary and sufficient to impair insulin signaling and nitric oxide production in endothelial cells. J Mol Cell Cardiol. 2005;39:327–34.
103. Ueki K, Fruman DA, Brachmann SM, Tseng YH, Cantley LC, Kahn CR. Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. Mol Cell Biol. 2002;22:965–77.
104. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signaling. Biochem J. 1998;333:471–90.
105. Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM. Regulation of the p85/p110 phosphatidylinositol 3′-kinase: stabilization and inhibition of the p110 alpha catalytic subunit by the p85 regulatory subunit. Mol Cell Biol. 1998;18:1379–87.
106. Dhand R, Hara K, Hiles I, Bax B, Gout I, Panayotou G, Fry MJ, Yonezawa K, Kasuga M, Waterfield MD. PI 3-kinase: structural and functional analysis of intersubunit interactions. EMBO J. 1994;13:511–21.
107. Klippel A, Escobedo JA, Hirano M, Williams LT. The interaction of small domains between the subunits of phosphatidylinositol 3-kinase determines enzyme activity. Mol Cell Biol. 1994;14:2675–85.
108. Hu P, Mondino A, Skolnik EY, Schlessinger J. Cloning of a novel, ubiquitously expressed human phosphatidylinositol 3-kinase and identification of its binding site on p85. Mol Cell Biol. 1993;13:7677–88.
109. Yu J, Wjasow C, Backer JM. Regulation of the p85/p110α phosphatidylinositol 3′-kinase. J Biol Chem. 1998;273:30199–203.
110. Shekar SC, Wu H, Fu Z, Yip S-C, Nagajyothi, Cahill SM, Girvin ME, Backer JM. Mechanism of constitutive phosphoinositide 3-kinase activation by oncogenic mutants of the p85 regulatory subunit. J Biol Chem. 2005;280:27850–5.
111. Fu Z, Aronoff-Spencer E, Wu H, Gerfen GJ, Backer JM. The iSH2 domain of PI 3-kinase is a rigid tether for p110 and not a conformational switch. Arch Biochem Biophys. 2004;432:244–51.
112. Terauchi Y, Tsuji Y, Satoh S, Minoura H, Murakami K, Okuno A, Inukai K, Asano T, Kaburagi Y, Ueki K, Nakajima H, Hanafusa T, Matsuzawa Y, Sekihara H, Yin Y, Barrett JC, Oda H, Ishikawa T, Akanuma Y, Komuro I, Suzuki M, Yamamura K, Kodama T, Suzuki H, Kadowaki T. Increased insulin sensitivity and hypoglycaemia in mice lacking the p85α subunit of phosphoinositide 3-kinase. Nat Genet. 1999;21:230–5.
113. Ueki K, Algenstaedt P, Mauvais-Jarvis F, Kahn CR. Positive and negative regulation of phosphoinositide 3-kinase-dependent signaling pathways by three different gene products of the p85α regulatory subunit. Mol Cell Biol. 2000;20:8035–46.
114. Mauvais-Jarvis F, Ueki K, Fruman DA, Hirshman MF, Sakamoto K, Goodyear LJ, Iannacone M, Accili D, Cantley LC, Kahn CR. Reduced expression of the murine p85α subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. J Clin Invest. 2000;109:141–9.
115. Ueki K, Fruman DA, Yballe CM, Fasshauer M, Klein J, Asano T, Cantley LC, Kahn CR. Positive and negative roles of p85α and p85β regulatory subunits of phosphoinositide 3-kinase in insulin signaling. J Biol Chem. 2003;278:48453–66.
116. Barbour LA, Shao J, Qiao L, Leitner W, Anderson M, Friedman JE, Draznin B. Human placental growth hormone increases expression of p85 regulatory unit of phosphatidylinositol 3-kinase and triggers severe insulin resistance in skeletal muscle. Endocrinology. 2004;145:1144–50.
117. Cornier M-A, Bessesen DH, Gurevich I, Leitner JW, Draznin B. Nutritional up-regulation of p85α expression is an early molecular manifestation of insulin resistance. Diabetologia. 2006;49:748–54.
118. Giorgino F, Pedrini MT, Matera L, Smith RJ. Specific increase in p85α expression in response to dexamethazone is associated with inhibition of insulin-like growth factor-I stimulated phosphatidylinositol 3-kinase activity in cultured muscle cells. J Biol Chem. 1997;272:7455–63.
119. Lamia KA, Peroni OD, Kim Y-B, Rameh LE, Kahn BB, Cantley LC. Increased insulin sensitivity and reduced adiposity in phosphatidylinositol 5-phosphate 4-kinase $\beta^{-/-}$ mice. Mol Cell Biol. 2004;24:5080–7.
120. Barbour L, Rahman SM, Gurevich I, Leitner JW, Fisher S, Roper M, Knotts T, Vo Y, Yakar S, LeRoith

D, Kahn CR, Cantley L, Friedman J, Draznin B. Increased P85alpha is a potent negative regulator of skeletal muscle insulin signaling and induces in vivo insulin resistance associated with growth hormone excess. J Biol Chem. 2005;280:37489–94.
121. Ueki K, Yballe CM, Brachmann SM, Vicent D, Watt JM, Kahn CR, Cantley LC. Increased insulin sensitivity in mice lacking p85β subunit of phosphoinositide 3-kinase. Proc Natl Acad Sci U S A. 2002;99:419–24.
122. Luo J, Field SJ, Lee JY, Engelman JA, Cantley LC. The p85 regulatory subunit of phosphoinositide 3-kinase down-regulates IRS-1 signaling via the formation of a sequestration complex. J Cell Biol. 2005;170:455–64.
123. Kirwan J, Varastehpour A, Jing M, Presley L, Shao J, Friedman JE, Catalano PM. Reversal of insulin resistance post-partum is linked to enhanced skeletal muscle insulin signaling. J Clin Endocrinol Metab. 2004;89:4678–84.

5 Insulin Resistance in Pregnancy: Implications for Mother and Offspring

Teri L. Hernandez, Jacob E. Friedman, and Linda A. Barbour

Introduction

Insulin resistance (IR) in pregnancy is a critical and beneficial adaptation of human physiology to ensure the viability and growth of the fetus. In normal pregnancy, maternal metabolism transforms to one characterized by IR to promote nutrient availability to the fetus while supporting maternal metabolic needs. Although the shift in maternal metabolism to IR during pregnancy is physiologic, the rising global epidemic of obesity and overweight that approaches 60% in some countries has not spared women of childbearing age [1, 2], resulting in obesity-related IR in these women entering pregnancy. Pre-pregnancy IR compounded by pregnancy IR results in an intrauterine environment characterized by nutrient excess in the form of glucose, lipids, and amino acids in parallel with metabolic derangements, including inflammation and oxidative stress. When a sedentary lifestyle, poor nutrition, and excess gestational weight gain (GWG) are added, the maternal metabolome and microbiome are further altered. Consequently, what is a normal adaptive mechanism to guarantee the survival of the next generation can cause excessive growth and altered development in the offspring of the obese mother. This alteration has an impact on cellular, molecular, and epigenetic pathways in both the placenta and fetus. The fetal response to excess maternal IR and over-nutrition results in fetal hyperinsulinemia and may be accompanied by alterations in appetite regulation, mitochondrial function, and growth and development that may predispose the offspring to later cardiometabolic disease. Thus, the implications of IR on the mother and fetus are complex and far-reaching and can have consequences for both maternal and offspring health over the life span.

Obesity is now recognized as the leading health concern in pregnant women [3, 4], carrying significant risks to both the mother and the infant. According to the 2011–2012 National Health and Nutrition Examination survey (NHANES), nearly two-thirds of all US women of childbearing age are overweight or obese. The proportion of women of childbearing age who are overweight or obese varies by ethnic group, from 26% of Asian women, 55% of non-Hispanic white women, 70% of Hispanic women, and 75%

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of non-Hispanic black women [5]. Obesity rates have tripled from 9.3% in 1960 to 32% in 2010 [6], and there has been a marked increase in Class 3 (body mass index [BMI] ≥ 40 kg/m^2) obesity rates [7]. Accordingly, in this chapter, the components of IR during pregnancy will be discussed in the context of normal pregnancy and those affected by obesity. The influences of IR during pregnancy on both maternal and offspring health will be presented, and future directions for research will be suggested.

Overview of Insulin Resistance During Pregnancy

Pregnancy is an elaborate metabolic state that involves remarkable alterations in the hormonal milieu in addition to changes in adipokines, inflammatory cytokines, and the gut microbiome. The placenta generates the widest array and greatest production of hormones of any endocrine gland. These hormones reprogram maternal physiology to become insulin resistant in the second and third trimesters to ensure an adequate supply of nutrients is available to support fetal growth [8]. Placental hormones that markedly influence maternal metabolism include high concentrations of estrogen, progesterone, prolactin, cortisol, human chorionic gonadotropin, human placental growth hormone (hPGH), human placental lactogen (hPL), and leptin. Tumor necrosis factor-α(alpha) (TNF-α[alpha]) also appears to be generated by the placenta and other tissues locally and may contribute to IR and oxidative stress. In adipose tissue, decreased adiponectin contributes to excess maternal IR by the second trimester [9]. Data also suggest that the maternal intestinal microbiome may dramatically shift to an "obesigenic" colonization pattern over the first to third trimester in normal pregnancy, heightening the pro-inflammatory state of pregnancy that may contribute to increased energy harvest and a potential increase in inflammation, further contributing to IR [10]. A less recognized comorbidity in obesity, and one likely to worsen maternal IR, is sleep-disordered breathing. Both frequent awakenings and hypopneas are common in pregnancy and may result in elevated cortisol, catecholamines, and worsened IR. The factors may lead to changes in both glucose and lipid metabolism and increased risk for gestational diabetes mellitus (GDM) [11]. Their influence on maternal metabolism and fetal growth deserves further study.

Typical carbohydrate metabolism in pregnancy is characterized by impaired insulin sensitivity with advancing gestation, compensatory pancreatic β(beta)-cell response with hyperinsulinemia, and a slightly elevated postprandial plasma glucose [12] but a decrease in fasting glucose due to fetal-placental glucose demands (Fig. 5.1). In addition, there are increases in circulating free fatty acids (FFA), triglycerides (TG), total cholesterol, and phospholipids [12, 13]. These changes occur as a maternal adaptation to increasing fetal metabolic and growth needs [13], powered by increasing insulin resistance and high levels of estrogen that increase very low-density lipoprotein–triglycerides (VLDL-TG) synthesis. They are also necessary to prepare the pregnant mother for delivery of the fetus and consequent lactation [12]. In particular, the second and third trimesters of pregnancy are characterized by a nearly 50% decrease in insulin-mediated glucose disposal and a 200–300% compensatory increase in insulin secretion as a response to glucose [14].

Glucose is the main source of fuel for the developing fetus. It is transferred across the placenta facilitated by glucose transporter (GLUT)-1 (non-insulin-mediated) transporters via a gradient, such that when maternal glucose concentrations rise to greater than fetal levels, glucose is transported across the placenta to the fetus. This, in combination with heightened maternal IR to ensure glucose availability, serves to meet fetal metabolic demands, which require 80% of energy as glucose. The placental and fetal demands for glucose are considerable. It is estimated that ~6% of maternal glucose per liter of blood that perfuses the placenta is extracted and 10% of glucose per liter of blood in the fetal-placental circulation is consumed by the fetus [15]. Glucose transport to the fetus occurs in direct proportion to maternal glucose levels and

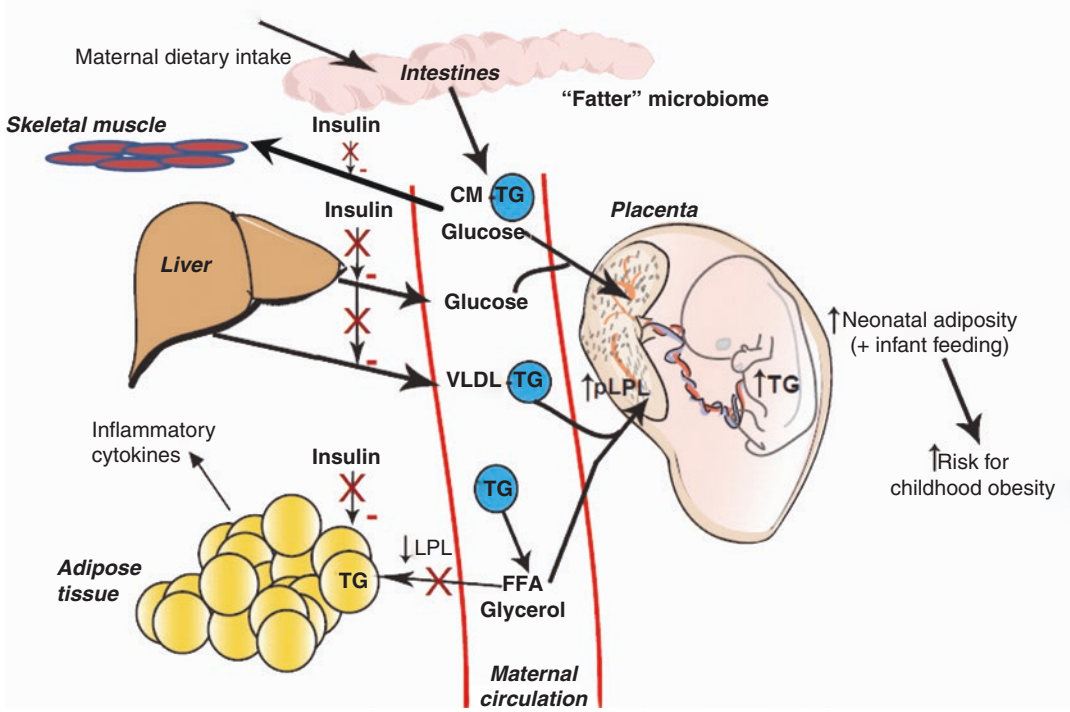

Fig. 5.1 Contribution of maternal glucose and lipid metabolism to fetal fuel availability and consequent neonatal adiposity. Pre-existing maternal obesity in combination with the adaptive insulin resistance (IR) of pregnancy results in increased glucose, lipid (triglycerides [TG]/free fatty acids [FFA]), and amino acids (AA) availability to the fetus. Enhanced hepatic IR contributes to heightened very low-density lipoprotein–triglycerides (VLDL-TG) and glucose production in both fasting and postprandial conditions. Peripheral IR enhances hydrolysis of maternal TG into FFA and glycerol via lipolysis. Decreased maternal adipose tissue lipoprotein lipase (LPL) activity and increased placental LPL (pLPL) activity favors partitioning of FFA to the placenta, augmenting fetal TG storage. Increased inflammatory cytokines contribute to worsening maternal IR. The maternal intestinal microbiome may shift to an "obesigenic" colonization pattern over the first to third trimester in normal pregnancy, heightening the pro-inflammatory state of pregnancy that may contribute to increased energy harvest and a potential increase in inflammation, further contributing to IR

is augmented by a fivefold increase in placental glucose transporters (GLUT-1) with advancing pregnancy, which facilitates transplacental glucose flux even in the absence of maternal hyperglycemia [16] and a lower fasting glucose than in the non-pregnant state.

In the fasting state, pregnant women deplete their glycogen stores more quickly than outside of pregnancy due to the fetoplacental glucose demands. As a result, the switch from carbohydrate to fat metabolism occurs within 12 hours, resulting in increased lipolysis and ketone production [17–19], further increasing maternal IR and serving to shunt available nutrients to the fetus. Freinkel introduced this phenomenon as "accelerated starvation of pregnancy"[20]. In addition, the maternal basal metabolic rate increases by ~150–300 kcal/day in the third trimester, depending on the amount of GWG—the latter further driving IR. These increased nutritional needs place the mother at risk for ketosis, which occurs much earlier than usual in the absence of adequate oral or intravenous nutrients.

The placenta's role in promoting maternal IR in the second and third trimesters of pregnancy appears to be due to an increase in human placental growth hormone (hPGH) [9, 21] in combination with human placental lactogen (hPL), progesterone, and TNFα(alpha), the latter

correlating with maternal IR measured by hyperinsulinemic-euglycemic clamp [22]. We demonstrated that hPGH is a metabolically active hormone capable of causing severe IR in transgenic mice that express it at levels comparable to the third trimester of pregnancy [23]. Human placental growth hormone may mediate IR similar to the effect of excess pituitary growth hormone (pitGH) when it is administered or expressed chronically. In fact, hPGH differs from pitGH by only 13 amino acids. It almost completely replaces pitGH in the maternal circulation by 20 weeks, and it is unregulated by growth hormone releasing hormone [21]. The major metabolic role of hPL is to mobilize lipids as free fatty acids (FFA) to support maternal metabolism. When maternal glucose falls, hPL rises and stimulates lipolysis, liberating FFA from storage to be used as energy. Thus, hPL leads to an increase in circulating FFA as a source of maternal fuel so that glucose and amino acids are spared for the fetus. Conversely, when maternal glucose levels rise, levels of hPL fall [8, 24]. Human placental lactogen also appears to play a key role in stimulating insulin production in human islets [25] by promoting the synthesis of serotonin in pancreatic β(beta)-cells, which in turn drives β(beta)-cell proliferation and glucose-stimulated insulin secretion [26]. The mechanisms for this β(beta)-cell expansion, which appears to be unique in human pregnancy, remain uncertain but adiponectin may play a role; lower levels may in part predict the development of gestational diabetes [27–30].

The IR of pregnancy alters multiple maternal fuels in synchronized coordination (Fig. 5.1). In addition to glucose, TG, cholesterol, and FFA are increased. Free fatty acids may serve to further promote the IR of pregnancy [18, 31, 32] and provide an important fuel supply for fetal fat accretion in the third trimester. During the first trimester of pregnancy, insulin sensitivity is modestly increased for unclear physiologic reasons, but this drives lipogenesis, resulting in increased maternal subcutaneous fat mass that is mainly centrally distributed and in the flanks. However, later in pregnancy, coincident with IR, the ability of insulin to suppress whole body lipolysis is reduced, resulting in an increase in circulating FFA that can also be used as fuel by the mother and fetoplacental unit [18]. The placenta utilizes its own lipases, including lipoprotein lipase (placental LPL), a major TG hydrolase enzyme, enabling maternal TG to be hydrolyzed to FFA for fetal and placental use. FFA can then be transported across the placenta to increase fetal fat deposition. Our group has recently published that placental LPL activity is correlated with an increased percentage of newborn body fat at birth (% newborn fat) [33, 34], suggesting that placental LPL plays an important role in providing fuels for fetal fat accretion. As detailed in a later section, there are many studies supporting the influence of elevated maternal TG and FFA as important substrates that contribute to excess fetal fat accretion [31, 35, 36].

Specific Changes in Glucose, Lipids, and Amino Acids in Pregnancy as a Consequence of Maternal Insulin Resistance

Changes in Glucose Metabolism

By the late second and early third trimesters, maternal metabolism has shifted to favor catabolism of maternal energy stores as IR increases markedly with each gestational week; the metabolic milieu allows for increased nutrient shunting away from the mother to support fetal growth. The fetus responds to the increased nutrient flux with fetal hyperinsulinemia since maternal insulin does not cross the placenta; the fetal hyperinsulinemia is a practical clinical biomarker for the detection of excess nutrient flux corresponding to excess fetal growth and metabolic derangements (Fig. 5.1). Specific changes in patterns of glycemia associated with pregnancy have been identified to inform the contemporary understanding of glucose metabolism in pregnancy.

Our group set out to characterize patterns of glycemia in normal pregnancy unaffected by obesity via archiving data from studies that had occurred over ~50 years [37]. In the seminal con-

trolled studies, Freinkel and others [20] identified that fasting glucose levels are lower in normal pregnancy due to fetal placental demands—previously discussed and coined by Freinkel et al. as *accelerated starvation*. However, they further identified that postprandial glucose is higher due to heightened IR and a possible effect on the first-phase insulin secretion, coined *facilitated anabolism*. In essence, one can imagine that maternal postprandial glucose responses "feed" the fetus, making use of the accentuated postprandial glucose response, which raises maternal glucose (making a larger gradient) and facilitates transport of glucose across the placenta (via GLUT-1 transporters, as previously discussed).

After a reduction in fasting glucose during the first and early second trimesters, fasting glucose slightly rises due to increasing IR, but it tends to remain lower than in the non-pregnant state. Our analysis of the data [37] from all plasma blood and fingerstick glucoses, or from continuous glucose monitoring (CGM) data, demonstrated that, when the data were pooled and graphed together for the first time, the patterns of glycemia were markedly lower than were previously appreciated. Normal pregnant women (BMI 22–28) during the third trimester (~34 weeks) demonstrate a fasting glucose of 71 ± 8 mg/dL (mean ± SD), a 1-hour postprandial value of 109 ± 13 and a 2-hour postprandial value of 99 ± 10 mg/dL, and a 24-hour mean glucose of 88 ± 10 mg/dL [37]. Gestational age and maternal BMI affect "normal" glucose levels. A longitudinal CGM study of 32 healthy, normal-weight women between 16 weeks gestation to 6 weeks postpartum demonstrated a rise in mean glucose from 16 weeks (82.3 mg/dL) to 36 weeks (94.0 mg/dL), which was maintained at 6 weeks postpartum (93.7 mg/dL) [38]. Moreover, with advancing gestation and increasing IR, the 2-hour postprandial glucose increased from 95.7 mg/dL at 16 weeks to a peak of 110.6 mg/dL at 36 weeks.

Two small studies characterizing glycemic profiles by CGM in obese pregnant women without GDM [31, 39] displayed [39] wide variance (up to ±8 weeks) in the gestational week of measurement, rendering the data difficult to interpret. We demonstrated using a controlled, provided diet that obese non-GDM women have 24-hour glycemic patterns that are shifted higher throughout the day and night compared to normal-weight women both early and late in pregnancy [31]. In fact, the 24-hour glucose area under the curve (AUC; a metric for total potential glucose availability to the fetoplacental unit) was ~8% higher in the obese women both early and late in pregnancy. Across meals during late pregnancy, postprandial glucose was ~13% higher in the obese vs. normal-weight mothers (1-hour, 115 vs. 102 mg/dL; 2-hour, 107 vs. 96 mg/dL, respectively). Thus, although the obese mothers did not meet diagnostic criteria for GDM, there was clearly a higher glucose gradient over 24 hours compared to the normal-weight controls. This sub-clinical pattern of mild hyperglycemia, which remains occult and untreated, may explain, in part, why the prevalence of large-for-gestational-age (LGA) offspring and macrosomia is highest in maternal obesity [2].

Changes in Lipid Metabolism

The changes in maternal glucose that occur over gestation are paralleled by an increase in maternal lipids and lipoproteins, which in normal pregnancy moderates fetal steroidogenesis and growth [13, 23, 40]. These changes are prompted by maternal estrogen, which increase VLDL-TG in addition to hPL, and are orchestrated by maternal IR. Importantly, the increase in lipids and lipoproteins, FFA (secondary to the IR-induced decrease in insulin suppression of lipolysis), and maternal ketones in fasting conditions (accelerated starvation) all serve to support maternal metabolism while sparing glucose for fetal utilization [41]. In recent years, mounting evidence further supports the role of maternal lipids as potent substrates for fetal growth.

In normal pregnancy, there is a two- to threefold increase in TG accompanied by a 25–50% increase in total and LDL cholesterol [18, 42] and increased VLDL and HDL cholesterol. In pregnancies affected by obesity, the change in maternal lipids is shifted higher with lower HDL compared to normal controls [41]. While the

association between maternal glucose and fetal growth has long been appreciated [20], a number of investigators have shown that in many cases, maternal TG and FFA have been strongly or more strongly correlated with excess fetal growth and LGA [31, 36, 43], supporting their role in excess fetal fat accretion. In fact, we demonstrated in obese and normal-weight women who were given fixed diets, both early and late in pregnancy, that maternal TG and FFA were much higher in the obese women. Moreover, TG and FFA correlated more strongly with infant adiposity than the differences in glycemic patterns between the groups [31]. Our group has specifically demonstrated that the change in maternal TG from early to late pregnancy are correlated with fetal growth and adiposity [44] and much more than glucose measures. Most recently, our group demonstrated that in obese women, fasting and postprandial TG in early pregnancy (14–16 weeks) was the strongest predictor of newborn % fat (r = 0.71 for early 1-hour postprandial TG) in contrast with normal-weight women, in which the increase in fasting and postprandial TG from early to later pregnancy was the strongest predictor [34].

Changes in Amino Acid Metabolism

Early investigations in which amino acids (AA) were measured in the serum of normal pregnant women and those with GDM [45, 46] demonstrated that GDM women had higher AA concentrations (including branched-chain amino acids associated with ketone formation and alanine associated with increased hepatic glucose output) during fasting conditions compared to normal pregnant women. More recently, changes in AA from the second and third trimesters were reported, but there were no differences in branched-chain AA between normal-weight and obese women [42, 47]. Outside of pregnancy, the synergy of increased lipids and branched-chain AA, particularly in combination with a higher-fat diet, has been implicated in IR states and the evolution of type 2 diabetes [48]. The contribution of maternal AA to fetal growth and fat accretion is recognized, but there is a need for more controlled studies in normal pregnancy and those affected by obesity that control for maternal diet.

Cellular Mechanisms of Insulin Resistance in Normal and Obese Pregnancies: Skeletal Muscle and Adipose Tissue

In the third trimester of normal pregnancy, there is decreased expression of the GLUT-4 glucose transporter protein in maternal adipose tissue and decreased translocation of GLUT-4 to the plasma membrane in skeletal muscle, both of which contribute to the IR of pregnancy. Mechanistically, insulin-stimulated glucose transport in human skeletal muscle fibers from obese women is suppressed in late pregnancy and more so in skeletal muscle of women who develop gestational diabetes mellitus (GDM). In transgenic mice, and later in human pregnancy, our group showed that hPGH is a major driver of the normal IR of pregnancy by increasing the p85 regulatory subunit of PI3-kinase, which acts in a dominant negative manner, resulting in suppression of the IRS-1-associated PI3-kinase insulin signaling cascade in skeletal muscle. Other skeletal muscle signaling changes characterizing IR include reduced tyrosine phosphorylation of the insulin receptor and decreased expression of IRS-1, all resulting in reduced GLUT-4 translocation to the plasma membrane and reduced glucose uptake [9].

The molecular changes in obese adipose tissue during pregnancy include a reduction in the transcription factor peroxisome proliferator-activated receptor (PPAR)-γ(gamma)1 [49]. PPAR-γ(gamma)1 binds to several adipose-specific genes and is a central regulator of the adipogenic transcriptional cascade. PPAR-γ(gamma)1 is normally highly expressed in adipose tissue and plays an essential role in fat cell differentiation, insulin sensitivity, and lipid storage. PPAR-γ(gamma) is also strongly implicated in the regulation of systemic insulin sensitivity

[50]. Unlike in skeletal muscle, we did not find a similar reduction in IRS-1 or GLUT-4 and increased p85-PI3-kinase in omental adipose tissues of obese pregnant mothers, but the function of these proteins in response to insulin was not tested [49]. Lappas et al. [51] demonstrated that maternal obesity and GDM is accompanied by defects in the uptake, synthesis, and breakdown of lipids. In subcutaneous and omental adipose tissues obtained at delivery, maternal obesity and GDM are associated with decreased expression of genes involved in FA uptake and intracellular transport, TG biosynthesis, triacylglyceride (TAG) biosynthesis (MGAT1,7 MGAT2, and DGAT1), lipogenesis (FASN), and lipolysis (PNPLA2, HSL, and MGLL), compared to normal-weight pregnant women, suggesting the obese mother may have reduced expandability of lipid stores compared to normal-weight mothers at term. The reduced lipolysis genes may be a compensatory mechanism that maintains fat mass in obese mothers. Importantly, decreased gene expression was also observed for the transcription factors involved in lipid synthesis and insulin sensitivity (LXRα[alpha], PPARα[alpha], PPARδ[delta], PPARγ[gamma], RXRα[alpha], and SREBP1c), while gene expression of the adipokines, TNF-α(alpha), IL-1β(beta), and/or leptin was increased in adipose tissue from obese and GDM women [51]. Whether these changes are associated with differential weight gain or maternal infant adiposity remains to be determined.

Adiponectin levels normally fall with advancing gestation, consistent with IR, and are lower in obese women and those with GDM [52, 53]. However, the role of adiponectin as a driver of maternal fuel metabolism is undergoing a re-examination. Potential roles of adiponectin in pregnancy include an insulin-sensitizing effect, pancreatic β(beta)-cell adaptive response, influence on hepatic gluconeogenesis, lipid metabolism, and placental signaling; these properties are being actively investigated [54, 55]. Adiponectin deficiency in pregnant mice resulted in decreased β(beta)-cell mass, glucose intolerance, increased hepatic glucose and TG production rates, increased adipose tissue lipolysis, and increased birth weight in the absence of changes in insulin sensitivity [56]. Adiponectin appears to be important, along with prolactin and HPL, for the expansion of β(beta)-cell mass in pregnancy [30]; in fact, lower levels in the first trimester have been associated with the development of GDM [27–29]. Adiponectin decreases throughout the course of normal pregnancy, although there is some suggestion that a transient increase in early pregnancy in normal-weight women might account for the early and short-lived increase in insulin sensitivity that is sometimes observed prior to 16 weeks [52]. Adiponectin does not appear to be made by the placenta and, thus, cord blood changes in fetal adiponectin do not correlate with that of the mother [57]. Women who are obese or who develop GDM have lower circulating levels of adiponectin, which is associated with increased fetal growth compared to normal-weight women [28, 53, 54, 57]. Declining adiponectin in GDM women is also associated with β(beta)-cell decompensation and development of type 2 DM postpartum [29, 58].

The most biologically active fraction of adiponectin appears to be the high molecular weight (HMW) oligomers. These isoforms are negatively correlated with TG, postulated to be due to an increased catabolism of VLDL- and CM-TG in part by stimulating LPL activity in skeletal muscle and fat, and are negatively correlated with IR across populations [59]. In humans, decreases in the HMW fractions are most highly associated with the increased IR of pregnancy [52, 60]. A recent trial in 300 women (STORK study) showed that adiponectin appeared to be an important predictor of birth weight (independent of maternal BMI, GWG, and the homeostatic model of IR index [HOMA-IR]); the largest decrease in adiponectin from early to late pregnancy occurred in mothers who gave birth to large-for-gestational-age (LGA) offspring [61]. In a multiple regression model, the ratio of HMW to total adiponectin was the strongest inverse predictor of birth weight compared to indices of IR or GWG [60].

The Microbiome in Normal and Obese Pregnancies: Influence on Mother and Offspring

Maternal obesity, excessive GWG, and changes in dietary intake during gestation can influence the composition of the maternal microbiome and may have a causative role in advancing the IR of pregnancy. Collado et al. [62] found that women who were obese prior to pregnancy had significantly different gut microbiota compositions compared to normal-weight women during both the first and third trimester of pregnancy. Furthermore, women with excessive GWG, regardless of pre-pregnancy BMI, had significant differences in their microbiota composition compared to women who had normal-weight gain. Obesity and its associated metabolic disorders are characterized by a systemic low-grade inflammation. The source of this inflammation is incompletely understood, but increased circulating levels of the bacterial derived endotoxin lipopolysaccharide (LPS) are believed to play a role. Alterations in microbial composition have been associated with increased gut permeability, resulting in translocation of LPS, which is a natural ligand for toll-like receptors (TLRs). Binding of LPS to TLR triggers the release of cytokines and an associated inflammatory response. Increased systemic levels of LPS have been observed in mothers with obesity [63] and correlate with increased systemic and adipose tissue inflammation. A seminal study underscoring the effect of normal maternal IR of pregnancy on the microbiome and the possible implications described the effect on a germ-free mouse. When a first trimester (insulin-sensitive) versus third trimester (insulin-resistant) maternal microbiome was transplanted into the germ-free mouse [10], the mouse receiving the third trimester microbiome became fatter. Moreover, those mice demonstrated inflammation and insulin resistance compared to germ-free mice who received the first trimester microbiome. While the exact changes that occur in microbiome of obese pregnant women are unclear, the important thing to note is that the microbiome of obese women may contribute to maternal IR and that differences could be passed onto the offspring at birth.

The exposure of the fetal intestine to maternal microbes, possibly even through amniotic fluid, is an important contributor to gut maturation and, by extension, to infant health [64]. However, functional studies of the microbiota inhabiting the placenta or amniotic fluid are lacking. Many studies have only shown the presence of bacterial DNA and have yet to characterize the virulence, metabolic characteristics, or other aspects of these bacteria and how these traits could relate to the establishment of the early microbiome. The exposure to antibiotics, cesarean section versus vaginal delivery, and breast milk versus formula feeding have been shown to play a critical role in shaping the early infant gut microbiota. While many factors have been shown to alter the microbiome throughout life, how the microbiome begins may have lifelong implications for disease risk [65–67]. Animal and human data strongly suggest that the composition of the neonatal gut microbiota is dependent both on maternal obesity and maternal diet during pregnancy and lactation [65] and on mode of delivery [68].

Studies in primates have shown distinct effects of maternal high-fat diet on offspring microbiota, such as decreased *Campylobacter*, *Helicobacter*, and *Bacteroides* as well as a decrease in overall bacterial diversity when compared to primates fed a control diet [65, 67, 69]. While the exact implications of these changes in microbiota are not fully known, it has been shown that decreased bacterial diversity is associated with adiposity, insulin resistance, dyslipidemia, and low-grade inflammation in humans [70]. Interestingly, a well-crafted study in primates has shown that after weaning, dysbiosis in juvenile animals is only partially corrected by a controlled low-fat diet [65, 67], demonstrating the lasting effects of a maternal high-fat diet on the microbiome of the offspring. Perhaps surprisingly, studies have also shown that maternal high-fat diet-induced offspring dysbiosis is independent of maternal obesity [65, 67, 69]. This is particularly interesting, as it suggests a more complex mechanism of maternal high-fat diet influence on the early microbiome than previously thought. Our human studies showed that the microbiome from infants at 2 weeks of age born to obese mothers exhibited less gammaproteobacteria, an early colonizing

bacteria essential for the development of immune tolerance, as well as a trend for higher bacilli class in the *Firmicutes* phylum, a high consumer of choline that has been associated with the development of nonalcoholic fatty liver disease (NAFLD) [71]. We have also shown that the breast milk of obese women demonstrated higher levels of insulin and leptin. Milk insulin and leptin may be able to pass through more permeable intestinal gap junctions in the newborn, potentially affecting appetite regulation, microbiome development, immune tolerance, and infant body composition and growth [72].

Dysbiosis of the gut microbiome has been correlated with NAFLD in children and adults; how the early life microbial composition influences hepatic fat accumulation and inflammation before the disease occurs is unclear. Early microbes from infants born to obese or GDM mothers may contribute to long-term health risks by triggering pro-inflammatory remodeling of the innate and adaptive immune system as well as other organs and tissues in the neonate. However, the critical microbes involved and whether maternal diet can alter the microbiome and the early immune system are unknown. In addition, it has been shown that the microbiome varies with genetic background and that differences in genetics are often associated with different groups of microorganisms rather than single species changes [73]. Viable therapeutic treatments (such as maternal diet) and the extent to which alterations in a mother's microbial population are related to clinical outcomes remain unstudied, but critically important.

Insulin Resistance, Obesity, and the Role of Excess Gestational Weight Gain on Maternal Outcomes

As noted, obese mothers enter pregnancy with increased IR compared to normal-weight mothers [14]. Figure 5.2 demonstrates the complex interplay of factors discussed in the following sections that are rooted in maternal IR within the vicious intergenerational cycle of metabolic dysfunction. The IR of pregnancy further exacerbates mothers' pre-existing metabolic perturbations, and their IR remains higher postpartum [14]. Catalano et al. demonstrated a ~40% decrease in insulin sensitivity in normal-weight and obese women over pregnancy and higher IR in obese women throughout pregnancy and postpartum compared to normal-weight controls [42].

Maternal obesity increases the risk of many maternal disorders characterized by increased IR, including hypertensive disorders, NAFLD, sleep disordered breathing, sleep apnea with pulmonary hypertension, thromboembolism, and cardiomyopathy [3, 74]. Obesity, commonly with co-existing IR, increases the risk of labor induction, failed induction of labor, cesarean delivery, anesthesia complications, postoperative wound infections, and lactation failure. Women with Class III obesity (BMI > 40 kg/m^2) have improved pregnancy outcomes if they undergo bariatric surgery before becoming pregnant; surgery has been shown to decrease IR, resulting in less diabetes, hypertension, and macrosomia (birth weight [BW] ≥ 4000 g) [75, 76].

Studies have demonstrated a marked increased risk of GDM in overweight and obese women [77–79], and early excess GWG is also associated with heightened risk for GDM. In fact, it was demonstrated, although retrospectively, that early rapid weight gain before 24 weeks gestation was associated with increased risk for GDM in overweight and obese women [80]. Several studies have demonstrated the independent effect of excess GWG on the increased likelihood of cesarean delivery [81–83]. Other studies have shown an association between excess GWG and preeclampsia [84, 85], at least in part due to the effect of GWG on worsening IR.

Influence of Insulin Resistance, Obesity, Excess Gestational Weight Gain, and Diet on Offspring Outcomes

Short-Term Offspring Outcomes

Maternal obesity independently increases the risk of first trimester and recurrent pregnancy losses. Moreover, the incidences of congenital

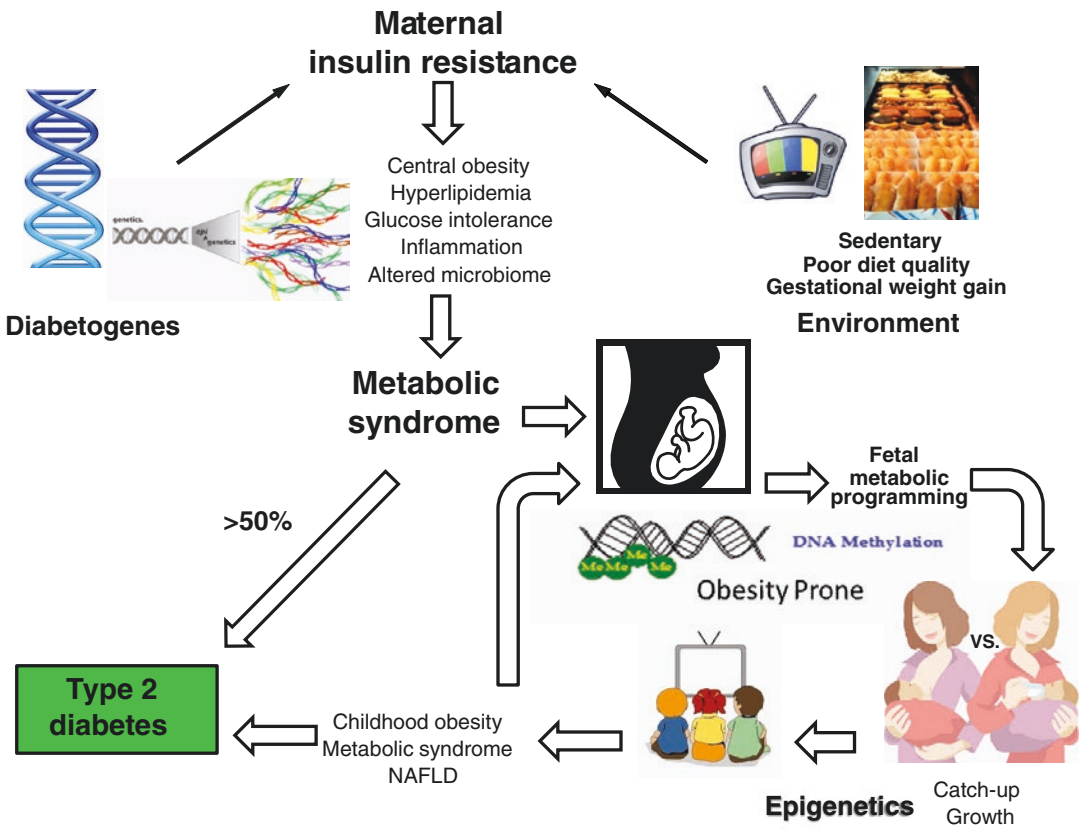

Fig. 5.2 Maternal insulin resistance (IR) begets childhood metabolic dysregulation. Genetic and epigenetic factors work in synergy with obesity-promoting environmental factors to create multiple exposures over years before pregnancy, resulting in pre-existing maternal IR that is compounded by the metabolic adaptations of pregnancy. Altered maternal fuels secondary to exacerbated IR in pregnancy are thought to program the fetus for heightened risk of childhood obesity. In combination with postnatal exposures such as excessive feeding, a sedentary lifestyle, and poor diet quality, the risk for childhood obesity and nonalcoholic fatty liver disease (NAFLD) are increased. Particularly in young women, these exposures create the context upon which their pregnancy is conceived, and the vicious cycle is propagated

malformations—including central nervous system (CNS), cardiac, and gastrointestinal (GI) defects and cleft palate—are associated with maternal obesity. One study estimated that for every unit increase in BMI, the relative risk of a neural tube defect increased 7% [86–88]. Obesity is also strongly associated with increased rate of large-for-gestational-age infants (infant BW ≥ 90th percentile for gestational age) and macrosomia and may pose a nearly equivalent risk as GDM for excess fetal growth, resulting in higher rates of shoulder dystocia and meconium aspiration. In the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) trial of >25 000 women worldwide [89], 78% of the LGA infants were born to mothers without GDM. In fact, obesity (OR = ~1.7) was nearly equivalent to GDM (OR ~ 2.2) as a risk factor for LGA [90, 91]. Overweight and obese women are at twofold increased risk of delivering a macrosomic infant. Because the prevalence of overweight and obese women is ~10 times that of GDM, maternal body habitus is likely to have the strongest attributable risk on the prevalence of macrosomia [92]. Obesity also quadruples the relative risk of perinatal mortality in part due to the greater maternal IR and fetal overgrowth [2] and in part due to the risk of a fetus outgrowing its blood supply and becoming ischemic.

Distinguishing the independent contribution of IR on adverse maternal and infant outcomes is difficult, largely because precise measurement of insulin action in pregnancy is challenging. The "gold standard" for measuring whole body insulin sensitivity is the hyperinsulinemic-euglycemic clamp technique [93]. However, use of this method is labor-intensive, impractical, and expensive and carries a slight risk to the mother of hypoglycemia. The frequently sampled intravenous glucose tolerance test (FSIGTT) [94–96], although less precise, is an alternative method for measuring insulin sensitivity and is methodologically simpler. However, intravenous injection of glucose bypasses absorption in the stomach, eliminating the effect of incretin hormones, which limits generalizability of the data. Most frequently, estimates of insulin action in pregnancy have relied on the use of fasting glucose and insulin, which represent only basal and non-stimulated conditions, usually with calculation of the homeostatic model of IR index (HOMA-IR). Although HOMA-IR is widely used even in small study samples, it was designed and validated for use in large epidemiologic studies [97, 98] and is difficult to interpret with any degree of maternal glucose intolerance. In a large observational study of 804 maternal-infant pairs, in mothers from ages 16–40 years with a wide range of BMI, IR early in pregnancy by HOMA-IR was correlated with newborn % fat by air-displacement plethysmography independent of maternal BMI, but the gestational age at which the samples were taken early (11–20 weeks) and later (20–34 weeks) spanned across a wide range [99]. Although imperfect, we have adopted use of a 75 g oral glucose tolerance test (OGTT) and calculation of the Matsuda Index. This technique includes measures of glucose and insulin during basal and glucose-stimulated conditions and is practical for use, and the calculated Matsuda Index has been shown to agree well with clamp-estimated measures of insulin action [100–103]. The disposition index, a mathematically determined measure of insulin secretion corrected for the degree of insulin sensitivity, is a better estimation of insulin secretory capacity relative to demand and has been shown to be very useful in predicting which women will develop GDM and which GDM women will progress to type 2 diabetes over time [104].

Emerging data suggest that excess maternal IR could be an important predictor of neonatal adiposity independent of maternal obesity [105, 106], as it is in rodents [107]. In a study of 301 infants of women with GDM, mild glucose intolerance, or normal glucose tolerance, maternal IR by the Matsuda Index predicted offspring weight gain and adiposity from 0 to12 months but pre-pregnancy BMI did not [105]. Greater maternal resistance to insulin has the capacity to make all nutrients more available to the fetus, including glucose, TG, FFA, and AA, all of which can contribute to excess fetal growth [108].

Excess GWG also amplifies IR, increases the risk for LGA and increased infant adiposity, and is a significant risk factor for childhood obesity and metabolic syndrome [109]. In a study of 4496 children ages 14–22 years in the National Longitudinal Survey of Youth [110], GWG was correlated with postpartum weight retention, LGA, and child obesity. In one study of 306 infants [111], in which adiposity was measured by air displacement plethysmography, the offspring of overweight mothers with excess GWG had the greatest difference in infant fat mass compared to the offspring of mothers who gained appropriate weight. It has been demonstrated that even infants born to mothers with GDM who are average weight for gestational age have increased adiposity at the expense of lean mass [112]. However, obese mothers gave birth to infants with the highest mean fat mass regardless of weight gain, which underscores the importance of pre-pregnancy BMI as a risk factor for increased neonatal adiposity. In a large retrospective cohort in which birth certificate data were extracted between 2004 and 2008 to assess the influence of maternal BMI versus GWG versus GDM on delivering an LGA infant [113], excessive GWG contributed most to LGA; however, maternal pre-pregnancy BMI was self-reported and likely underestimated. In the Healthy Start cohort of >800 mother-infant pairs, maternal pre-pregnancy BMI and GWG, including period-specific GWG, were positively and independently associated with neonatal adiposity [114].

Maternal diet is also very likely to influence LGA and infant adiposity, but has been inadequately studied in pregnancy. Although there are compelling data to support the influence of maternal diet on offspring in animal studies and nonhuman primates, most of the data from randomized trials in humans are in women with GDM. Moreover, across the trials, the outcomes are confounded by use of medication for glycemic control and poor diet adherence [115, 116]. Outside of pregnancy, diets higher in fat have been shown to promote IR [117]. Studies in mothers during late pregnancy demonstrated that elevating FFA with lipid infusion (mimicking high-fat postprandial conditions) contributes to worsening peripheral and hepatic IR [32]. Epidemiologic data have shown that healthier pre-pregnancy diet quality—characterized by eating patterns that include more leafy green vegetables, fiber, fruit, poultry, and fish—is associated with an up to 26% reduction in GDM risk, which indirectly supports the link between maternal diet and IR [118, 119]. There are also suggestions that eating patterns including foods with lower glycemic index are effective in mitigating GWG [120]. Overall, evidence supports that all pregnant women can benefit from minimizing fats, avoiding simple carbohydrates and excessive protein, and liberalizing fiber and complex carbohydrates. Higher-quality carbohydrates tend to be more nutrient dense (with more vitamins/minerals), have higher fiber, and are lower in calories and glycemic index/glycemic load [121]. Our published randomized controlled trial (RCT) in GDM mothers demonstrated that a higher complex carbohydrate/lower-fat diet controlled 24-hour and postprandial glycemia to similar levels compared to a carbohydrate-restricted/higher-fat diet, supporting the liberalization of higher-quality complex carbohydrates. Moreover, when women remained on the diets for 6–7 weeks through delivery, the higher complex carbohydrate/lower-fat diet was associated with attenuation of worsening IR, lower fasting glucose and FFA, and less newborn % fat [122, 123].

Long-Term Offspring Outcomes

In humans, it is difficult to separate out the independent contribution of maternal obesity from other potent intrauterine influences on long-term metabolic outcomes in the offspring. It is clear that obesity prevalence in children has paralleled the growth in maternal obesity. Approximately a quarter of 2- to 5-year-olds and one-third of school-age children (including adolescents) are now overweight or obese in the USA [5]. Furthermore, half of childhood obesity occurs among children who are obese by age 5 [124], signaling very early risk factors in the genesis of childhood obesity. In fact, the number of overweight/obese *infants* tripled from 1990 to 2012 [125]. In addition to pre-pregnancy BMI, other metabolic influences that affect long-term outcomes in the offspring include GWG, maternal diet, glucose, and lipids, all of which could lead to nutrient excess and fetal hyperinsulinemia [126]. Several epidemiologic studies have found that higher GWG is associated with higher weight for height and fat mass in childhood and adolescence, as well as dysfunctional metabolic and vascular traits [127, 128]. It is likely that maternal obesity acts in synergy with these potent factors. Moreover, the degree to which maternal and paternal genetics, pre-pregnancy factors, and early infant postnatal exposures such as breast feeding and early nutrition contribute to long-term metabolic outcomes is also a challenge to separately discern in human studies. When cultured in vitro, the blastocysts from obese mice have reduced levels of mitochondrial DNA [129]. In animal models, the negative effects of maternal obesity can be improved by treatment with insulin sensitizers administered at the time of conception, suggesting a causal role for IR and that the pre-conception or peri-conceptional period may represent a window for interventions that improve insulin sensitivity.

Separating out the influence of pre-pregnancy obesity and IR from exposure to maternal hyperglycemia or hypertriglyceridemia independent of IR on long-term offspring outcomes is challenging. In nonhuman primates, a high-fat diet

fed to normal-weight mothers was associated with fetal steatosis and oxidative stress in the third trimester [130]. However, in a longitudinal analysis of the juvenile offspring, only those infants exposed to a high-fat diet showed persistent hepatic steatosis and evidence of inflammatory changes as juveniles, both hallmarks of pediatric NAFLD [131]. Importantly, these changes were evident in juveniles even after switching to a healthy diet at the time of weaning, suggesting a programming effect of exposure to a high-fat diet during pregnancy and lactation. In a longitudinal study of 421 mother-daughter pairs, girls exposed to GDM had a higher risk of increased adiposity, but the risk was highest in the offspring of mothers with pre-pregnancy obesity [132]. This intergenerational transmission of obesity and IR may be partially mediated in utero by epigenetic modifications and directly mediated by the influence of fetal hyperinsulinemia or other factors, such as inflammation, oxidative stress, or excess lipid exposure. Importantly, all systems are affected, including the brain (through regulation of appetite, behavior, and reward); organ development (liver, kidney, pancreas, and heart); myocyte, adipocyte, and osteocyte development; and even the immune systems. Although there is variability in the macronutrient content of diets and duration of feeding regimens, studies in animals and nonhuman primates have shown that exposure to maternal overnutrition/obesity consistently results in an offspring metabolic phenotype characterized by higher fat mass and skeletal muscle and adipose tissue IR [126], in addition to decreased mitochondrial function in muscle [133]. Pancreatic development in the subsequent generation may also further be influenced by maternal overnutrition/obesity [134]. The degree to which postnatal obesity itself contributes to these disorders, or whether these metabolic phenotypes are set in play at birth prior to the onset of obesity, is not known.

Animal models, including rodents and nonhuman primates, have shown that multiple metabolic systems are vulnerable to changes in the intrauterine environment, including excess nutrient availability in combination with maternal IR in all three phases of development. These include (1) early gestation during implantation, placentation, and subsequent embryogenesis, when placental nutrient transport patterns and mitochondrial function in the blastocyst [135] and oocyte [136] may be set; (2) mid-gestation, when number, growth, and function of critical organs such as pancreas, brain, kidney, and skeletal muscle develop; and (3) late third trimester, when fetal growth and fat accretion accelerates, and regulatory set points in the brain, pancreas, and neuronal-metabolic feedback loops may be affected.

There is limited knowledge in humans about the impact of environmental exposures on biochemical and molecular processes that govern metabolic risk, particularly in utero on infants born to mothers who are obese. However, there is an emerging and compelling association with maternal obesity and the risk of childhood obesity and metabolic disease, implicating the metabolic milieu of the intrauterine environment as a driving factor for the genesis of adult diabetes and cardiovascular disease [131, 137, 138]. Epidemiologic data in Pima Indian offspring exposed to an intrauterine environment of GDM demonstrate a sevenfold increase in type 2 diabetes in the offspring as young adults compared to offspring (often siblings) in whom the mothers did not develop glucose intolerance until after delivery [139, 140], underscoring the direct influence of the intrauterine environment.

In addition to subcutaneous fat, liver fat may also be increased in the offspring from obese GDM women. Our human studies using magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS) technology have shown that maternal BMI in women with GDM predicts newborn intrahepatocellular lipid storage [141]. Offspring born to obese mothers have increased liver fat at birth [142], and these offspring are at risk later in life, regardless of gender, for progressing to obesity [143], NAFLD [144], and cardiovascular disease [145]. Nonalcoholic fatty liver disease affects ~34% of children with obesity ages 3–18 years in North

America, and half have already progressed to the more severe nonalcoholic steatohepatitis (NASH) at time of diagnosis [146, 147]. In animal models, maternal obesity or high-fat diet exposure increases hepatic oxidative stress and apoptosis in the early third trimester, perhaps priming the liver for later development of NASH [148, 149]. In our nonhuman primate model of maternal obesity, there is innate immune dysfunction and necro-inflammatory changes in fetuses of high-fat diet-fed mother macaques [130, 150]. Importantly, these alterations persist even after weaning these offspring to a normal chow diet [131]. The critical importance of the in utero environment to development of pediatric NAFLD was recently illustrated in a cross-sectional study of 543 children with biopsy-proven NAFLD that showed that those born with high or low birth weight had a twofold increased incidence of NASH and advanced fibrosis [146].

The evidence supporting a fetal programming influence and its contribution to the developmental origins of human disease is one of the most compelling reasons why preventing mothers from entering pregnancy with obesity and pre-existing IR, ingesting a low-quality higher-fat diet, and having excessive GWG are so critical for improving long-term health in both the mother and her offspring [151]. Maternal obesity has also been shown to be a stronger predictor than GDM for offspring risk of metabolic syndrome at ages 6–11 years [152]. Further, maternal obesity is a stronger risk factor than GDM in predicting offspring obesity by dual X-ray absorpitometry (DXA) at 9 years of age [153]. To underscore the impact of modifying pre-pregnancy BMI, studies have found a decreased risk of offspring obesity if the mothers underwent surgical weight loss prior to pregnancy [154, 155].

Maternal diet and GWG are potential interventions and preliminary data from a cohort of mothers who consumed a low- versus high-glycemic index diet during pregnancy demonstrated that, at 1 year of life, the infants of mothers who consumed the low-glycemic index diet showed evidence of improved vascular health (lower aortic intima-medial thickness) [156, 157]. Although pre-pregnancy BMI appears to be a stronger risk factor than GWG [42, 126, 158, 159] for childhood obesity, there are some data to support that GWG is an independent risk factor for increased childhood BMI [111], especially in overweight mothers [127]. Increased GWG has been shown to be associated with increased adiposity not only at birth but also at 6 years of age in the Southampton Women's Study Group [152]. Although pre-pregnancy BMI was shown to increase the risk of childhood metabolic syndrome at ages 6–11 years by nearly twofold, LGA at birth, influenced by both maternal BMI and GWG, increased the risk more than twofold [160].

In addition to increasing childhood metabolic risk, maternal obesity and metabolic disorders accompanied by IR have been associated with a higher risk of attention deficit hyperactivity disorders, autism spectrum disorders, anxiety, depression, schizophrenia, and eating disorders, as well as impairments in offspring cognition [161]. In nonhuman primates, exposure to a maternal high-fat diet independent of maternal obesity can cause persistent changes in offspring behavior with alterations in serotonergic, dopaminergic, and melanocortinergic pathways, in addition to changes in appetite regulatory pathways in the hypothalamus. These changes in brain neurotransmission and behavior persisted in the offspring at 1 year, even after weaning to a normal diet [162]. Elevations in FFA, glucose, insulin, leptin, TNFα(alpha), C-reactive protein (CRP), and interleukin 6 (IL-6)—all biomarkers of underlying maternal insulin resistance—may permanently change neuroendocrine regulation and brain development.

Cellular and Epigenetic Changes Associated with Long-Term Offspring Outcomes

Compelling new evidence has underscored a complex association between maternal obesity, IR, maternal diet, and epigenetic modifications that lead to long-term offspring health outcomes. At the cellular level, animal models demonstrate that obesity during pregnancy can accelerate fetal adipogenesis, affecting lipid storage, and mitochondrial metabolism in both adipocytes and

skeletal myocytes [133]. Skeletal muscle and adipose tissues are both developed from mesenchymal stem cells (MSC). Importantly, MSC also reside in fully developed tissues for repair and maintenance (e.g., adipose stromovascular cells, skeletal muscle satellite cells). Thus, fetal programming of the MSC lineage may not only alter tissue development in utero, but may maintain tissue phenotype throughout life. For example, skeletal muscle satellite cells from adults with established obesity and/or type 2 diabetes exhibit altered lipid partitioning when differentiated to myotubes in vitro [163, 164]. These satellite cell metabolic outcomes often track with the in vivo metabolic phenotype of the donor [163], suggesting that the origins of altered lipid partitioning may be genetically or epigenetically programmed. Our group has shown that umbilical cord mesenchymal stem cells (uMSC) from the newborns of obese women preferentially differentiate into adipocytes compared to the uMSC from newborns of normal-weight women [165]. Furthermore, the differentiated adipocytes have more lipid content, which is correlated to offspring percent fat mass at birth.

In clinical studies, maternal nutrition, GWG, and obesity can alter DNA methylation in infant tissues, such as umbilical cord blood [166], umbilical cord [167], and buccal cells [168]. Interestingly, a study found improved cardiovascular risk profiles and differential methylated patterns of glucose regulatory genes in offspring born before or after maternal bariatric surgery [169] associated with less GWG and maternal obesity. Moreover, animal data suggest that preconception maternal diet and nutritional status may be a key determinant of the fetal epigenome [170–172]. However, there is a paucity of studies investigating maternal nutritional interventions and epigenetic changes, and, in the relatively small number of published studies, examination of the role of maternal nutrition and epigenetics is retrospective. Animal models and epidemiological studies have demonstrated that fetal growth and development are most vulnerable to maternal nutrition in early gestation, specifically during the implantation period followed by rapid fetal development [172–174]. Most nutritional interventions are administered during gestation and postnatal development to improve infant growth and have achieved limited success [175].

There are several animal studies that suggest maternal obesity may be associated with changes in gene methylation in cord blood of newborns [176]. Furthermore, there may be sex specificity as to how insulin-sensitive tissues in the offspring are programmed from maternal obesity, diet, IR, or metabolic influences [126]. Metastable epialleles are loci where DNA methylation occurs stochastically early in embryogenesis and is maintained and propagated through all cell types during differentiation. These epialleles offer a potential mechanism for the epigenotype that is not genetically mediated or cell-type specific, but is influenced by maternal nutrition across every tissue. Marked differences in peri-conception maternal diet within Gambian women due to the rainy season resulted in changes in maternal plasma levels of key methyl-donor pathways (e.g., MET, CHOl, FOL, HCY, B Vits) and the methylation of infant DNA at 2–8 months in lymphocytes and hair follicles [171]. Whether these loci or others are associated with pathways for obesity, as would be expected by changes in maternal nutrition or maternal IR in humans, is being investigated.

Placental Correlates That May Influence Fetal Growth

Although a comprehensive review of placental changes and nutrient transport because of maternal IR and obesity is beyond the scope of this review, there is evidence that placentas affected by obesity and GDM are characterized by upregulation of placental genes involved in lipid transport and metabolism more than glucose metabolism and a striking increase in inflammatory gene expression [42, 177, 178]. Dietary effects on the placenta have been shown in the placentas of Japanese macaque mothers exposed to a high-fat diet. The placentas demonstrated reduced blood flow on the fetal side with increased infarctions and inflammatory cytokines and increased risk of stillbirth [179]. In

humans, lipids are not only an important nutrient source for fetal development and adipose tissue accretion, but have also been shown to stimulate both AA and FA uptake, activating cellular signaling pathways [180, 181]. The placenta has been proposed to act as a nutrient sensor, regulating nutrient transport and subsequently fetal growth in association with changes in maternal IR, fuel supply, and maternal cytokines such as adiponectin [182–184]. Nutrients cross the microvillous membrane from the maternal blood and are processed within the cytoplasm and delivered to the basal membrane on the fetal side before reaching the fetal capillary endothelium. In the human placenta, both FA transport and AA transplacental transfer is regulated by the activity of membrane-bound transporters in the microvillous and basal membrane. It has been demonstrated that both insulin and leptin stimulate the activity of the System *A* amino acid (AA) transporter [185], which is upregulated in the microvillous membrane of pregnancies affected by both gestational diabetes and obesity and is associated with fetal overgrowth [186], and adiponectin decreases it [54, 57, 187]. Fatty acids in maternal blood are present as non-esterified FA bound to albumin, and in VLDL or chylomicrons (CM), which can be hydrolyzed by placental lipoprotein lipase (Fig. 5.1). Our group has recently demonstrated that placental LPL activity correlates with newborn adiposity [33]. Cleaved FAs are transferred to the fetus by either simple diffusion or transfer through FA transport proteins (i.e., FATP2, FATP4, CD36) located on both the microvillous and basal membranes. However, the influence of placental metabolism on fatty acid transfer to the fetus is highly complex, and the placenta incorporates fatty acids into lipid pools that appear to modulate their transfer to the fetus [188]. A recent article suggested that maternal obesity may result in FA accumulation and FA oxidation that is lower than in placentas of normal-weight women, in part due to fewer mitochondria and a decrease in mitochondrial FA oxidation capacity [189].

Interestingly, in the placenta, as opposed to other tissues in which it has an insulin-sensitizing effect, adiponectin appears to cause insulin resistance and appears to prevent insulin-stimulated amino acid uptake in primary human trophoblast cells [54, 57]. This may occur by binding to adiponectin receptor-2, activating PPARα(alpha) and ceramide synthase, which inhibits IRS-1 phosphorylation. This may result in reduced placental insulin responsiveness resulting in limitation of amino acid transport and birth weight [54, 57]. However, in obesity or GDM, reduced adiponectin allows increased amino acid transport and fetal overgrowth [57]. Chronic administration of adiponectin to pregnant mice inhibits placental insulin and mTOR (mammalian target of rapamycin) signaling and downregulates the activity and expression of key placental nutrient transporters. This appears to decrease fetal growth at the level of the placenta in addition to its many other effects on maternal metabolism that reduce glucose and lipid availability [187]. These data suggest that adiponectin, and especially HMW adiponectin, may play an independent and important role in β(beta)-cell adaptation in pregnancy, glucose and lipid metabolism, and placental nutrient transport signaling resulting in an inverse relationship with fetal growth.

Changes in Inflammation During Pregnancy

In pregnancy, profound changes to the immune system occur to prevent rejection of the fetus, and this shift is accomplished by a relative decrease in the maternal adaptive immune response while the innate immune response heightens. As mentioned, recent findings suggested that TNFα(alpha) may be a mediator in the insulin resistance of pregnancy. One investigation reported data showing that the changes in insulin sensitivity between the second and third trimesters of pregnancy were correlated with maternal plasma TNFα(alpha) [9, 22]. Several studies have revealed an association between placental TNFα(alpha) and fetal adiposity [190, 191]. Overall, the placenta is now a target of great interest in understanding the insulin resistance of pregnancy because it is a source and target of cytokines that may impact both mother and infant [192].

Evidence characterizing the role of maternal inflammation in humans correlating with offspring metabolic disease is mixed. However, there is some modest evidence that maternal lipopolysaccharide (LPS) or cytokine exposures may result in increased adiposity, and moreover they have the capacity to be transferred to the fetus and modulate placental transfer of nutrients [193]. Placental inflammation appears to be increased to some extent in obese women, especially IL-6, which could have the capacity to increase FA transport to the fetus [42, 130, 193, 194]. Overall, the degree to which IR induces inflammation in pregnancy given its immune tolerance remains to be elucidated, as does how maternal inflammation impacts offspring outcomes.

The Metabolome in Normal and Obese Pregnancies

Despite the well-established independent risk factor of increased maternal BMI on infant birth weight and adiposity at birth, the metabolic pathways in the infant that contribute to long-term obesity risk remain complex and unresolved. A study that attempted to characterize the cord metabolome of infants at risk for childhood obesity found that the most differentially regulated metabolites in newborns with excessive childhood weight gain were related to food and plant components supporting a strong role for maternal dietary factors influencing the risk of offspring obesity [195]. In a recent study, fasting and 1-hour OGTT samples at 28 weeks were collected from 400 mothers in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) trial, and metabolomics assays were performed in an attempt to correlate metabolites with infant subcutaneous fat by skinfolds. Higher BMI and maternal IR were associated with multiple metabolites, including acylcarnitines, branched-chain amino acids, lipids, and carbohydrates, which were correlated in part with newborn size at birth but none with high specificity [196]. Novel differences in the metabolomics and transcriptomics of stem cells in human infants born to obese women and their correlation with maternal phenotypes and infant adiposity have been carried out by our group [197]. Our overall initial hypothesis that maternal BMI is an important predictor of changes in the umbilical cord mesenchymal stem cells (uMSC) was found to be only partially correct. Percent fat of the infant, which is the summation of all exposures throughout gestation, proved to be a much better correlate of dysregulated metabolism and gene expression, particularly in the offspring from obese mothers. Transcriptionally, there were broad differences between the offspring from normal-weight and obese mothers. Changes in incomplete β(beta)-oxidation, in compensatory amino acid and fatty acid metabolism, and in gene pathways of nutrient sensing and mitochondrial metabolism were related to newborn adiposity in the offspring of obese mothers, and it appeared that maternal obesity has effects on underlying stem cell metabolism, not just on newborn adiposity [197]. Further, we demonstrated that maternal FFA exposure in the second trimester is related to both newborn adiposity and stem cell physiology and energy-related gene expression. The significance of our findings in the human infant MSCs is that the reduced metabolic potential in the infants of obese mothers, with increased adiposity, appears to be maintained in the cells after several passages and after differentiation toward myocytes and adipocytes. Similar incomplete β(beta)-oxidation of lipids was found in fetal skeletal muscle of nonhuman primates exposed to maternal obesity, even in the absence of a Western-style diet [50], suggesting these cells maintain a phenotype as fully differentiated skeletal muscle. If these tissue-specific changes are carried forward in the muscle and adipose tissue of the child, it would favor lipid storage over oxidation upon exposure to an obesogenic environment. Although correlation is not causation, these findings raise the question as to whether cells at birth are partially programmed and can be further programmed postnatally as they are subject to multiple nutritional and postnatal environmental exposures.

Interventions to Attenuate Excess Maternal Insulin Resistance and Improve Pregnancy Outcomes

Although pregnant women tend to be highly motivated to improve their health status for the sake of their unborn child, behavioral changes are difficult. Interventions that may attenuate the increasing maternal insulin resistance and improve maternal and offspring outcomes focus on healthy nutrition patterns and physical activity to minimize excess GWG. Moreover, they hope to prevent development of GDM and decrease the risk of an LGA infant. Given the marked changes in appetite, nausea, fatigue, poor sleeping, musculoskeletal discomfort, dyspnea, and steadily increasing weight gain, starting an exercise/diet program during pregnancy is highly challenging and may be of limited benefit. Over the past 15 years, there have been more than 70 published interventions designed to decrease GWG and LGA, but results have been disappointing [2, 198]. In the past 5 years, there have been at least 15 reviews and meta-analyses summarizing the effects of these interventions to reduce GWG or prevent GDM. The overall reduction in GWG has been modest at best, ranging 0.4–3.5 kg, and dietary changes alone appear to be the most effective in reducing excessive GWG, gestational diabetes, and hypertensive disorders of pregnancy, especially when occurring earlier in pregnancy. Although physical activity may slightly reduce LGA, it is less effective for reducing excess GWG. Increasing data support that interventions to break the intergenerational cycle of offspring obesity and insulin resistance need to start prior to conception in girls of reproductive age [42, 126].

In an attempt to target the microbiome to prevent the development of excess maternal insulin resistance and the development of GDM, a probiotic containing *Lactobacillus rhamnosus GG*/*Bifidobacterium* lactic Bb12 (1010 CFU)/day with intensive diet counseling was studied. This resulted in a 60% reduction in GDM in a study in Finland, but this outcome has not been replicated [199]. Interestingly, there was no statistical difference between groups in GWG. A recent RCT using the *Lactobacillus rhamnosus* given at 14–16 weeks appeared to decrease the development of GDM in women at risk by 34% [200]. A large Australian RCT enrolling 411 women (SPRING) was recently completed using the same probiotic [201] in overweight/obese women with the intent to prevent GDM. Gestational diabetes incidence was similar in the probiotic group versus placebo (18.4% vs. 12.3%) [202]. Myo-inositol has also been investigated as a supplement. An RCT in Italy showed a 60% reduction in gestational diabetes, but interestingly, no difference in GWG when 2 mg/day of myo-inositol with 200 μg folic acid was used [203]. However, a recent trial conducted in Ireland showed no difference in the development of GDM across 240 pregnant women randomized to 1100 mg myo-inositol, 27.6 g D-Chiro inositol, and 400 μg folic acid versus 400 μg folic acid alone as well as no differences in fasting glucoses [204]. Omega-3 fatty acids have also been examined in >35 RCTs and have not been successful in preventing LGA, preeclampsia, or GDM [205]. Although a randomized, double-blinded controlled trial was conducted in overweight/obese pregnant women who were assigned to receive DHA plus EPA (2 g/day) or a placebo twice a day from week 10–16 to term, there was a large drop-out rate. In the 49 analyzed samples, although there was a reduction in adipose tissue and placental inflammatory gene expression biomarkers and reduction in plasma CRP, there were no differences in maternal or infant outcomes [206].

Metformin would be expected to improve IR and prevent GDM through its insulin-sensitizing effects, and, although early observational data suggested that GDM among women with polycystic ovary syndrome (PCOS) was reduced with metformin, the finding was not confirmed in a large RCT from the first trimester [207] or a meta-analysis examining the available RCTs [208]. In the Metformin in Obese Pregnancy trial (MOP), 400 women with a BMI > 35 kg/m^2 received metformin 3 g/day versus placebo in a double-blind, placebo-controlled trial between

12 and 18 weeks of pregnancy. Although the women in the metformin arm gained ~1.7 kg less than the placebo arm and had a lower incidence of preeclampsia, there was no difference in the rate of LGA or GDM [209].

Because metformin is concentrated in the fetal compartment with umbilical artery and vein levels being at least equal to the maternal serum levels [210], there are concerns about its effect on the fetus. Hypothetically, if metformin increases insulin sensitivity in the fetus, it might be possible for excess nutrient flux across the placenta to result in increased fetal adipogenesis. Its potential long-term effect on gluconeogenic enzymes in the fetal liver is unknown [211]. Furthermore, the anticancer effects of metformin that have been shown to decrease rapid cell division may be a concern in a growing fetus [212], and metformin has been recently shown to cause downmodulation of cell proliferation-related proteins through activation of AMP-activated protein kinase (AMPK) [213]. Moreover, metformin has been shown to suppress mitochondrial-dependent biosynthesis [214]. The largest experience with metformin has been in women with gestational diabetes women later in pregnancy from the Metformin in Gestation (MIG) trial [215], in which 751 women were randomized to metformin versus insulin after exclusion of hypertension and fetal growth restriction. Metformin did not appear to increase any adverse outcomes, although it was associated with a slight increase in preterm birth and failed to control glycemia in 46% of the women (who then required the additional of insulin therapy). Interestingly, a greater increase in TG was seen in the metformin group compared to insulin alone, and maternal TG, C-peptide, and maternal BMI were correlated with LGA and anthropometric measures of infant adiposity [216]. The offspring in the MiG trial were followed for 2 years, and it was demonstrated that the children exposed to metformin had increased subcutaneous fat [217, 218]. Another RCT in women with PCOS compared metformin to placebo and showed that, although women randomized to metformin gained less weight during pregnancy, at 1 year postpartum the women who used metformin in pregnancy had less postpartum weight loss and their infants were heavier [219]. Overall, interventions focused on mitigating IR through nutrition, physical activity, and control of GWG show promise for improving maternal and offspring outcomes but are likely going to need to start earlier, possibly target lower gestational weight gain, and need to be more intensively supported.

Key Questions and Future Directions

Although our understanding of IR during pregnancy as both an adaptation and a pathology has increased, many lingering questions remain, particularly with respect to nutritional patterns, the heterogeneity of obesity, and underlying genetic/epigenetic mechanisms that impact the pathways for IR. The impact of paternal phenotype on the offspring metabolic trajectory requires clarification. Moreover, maternal IR may impart a differential influence on the placenta, fetal growth, and fat accretion or programming effects in male versus female offspring [220]. Although only modestly effective to date, lifestyle interventions incorporating nutrition, physical activity, and control of GWG may reveal long-term benefits to maternal and offspring health, especially if they can implemented pre-pregnancy or early in pregnancy. Moreover, it may be possible to use targeted nutrition therapy or supplements to target specific nutrients (glucose, TG, FFA, AA) and/or explore the use of microbes that might favorably modify intrauterine conditions to optimize both maternal outcomes and fetal growth patterns. Furthermore, targeting sleep disorders with interventional strategies to improve sleep quality could improve IR and the associated disorders in glucose and lipid metabolism. The importance of interventions in the pre-conception period, or during oocyte development, has yet to be clarified. Whether there is a causative role for epigenetic modifications influenced by the intrauterine environment or in the changes in the early microbiome and their metabolic relevance is

largely unknown. Future research focused on how to separate the postnatal influences of nutrition and breastfeeding from pregnancy exposures, given the plasticity of offspring tissues, is critical to the design of new interventions to promote long-term offspring health.

References

1. Devlieger R, Benhalima K, Damm P, Van Assche A, Mathieu C, Mahmood T, et al. Maternal obesity in Europe: where do we stand and how to move forward?: a scientific paper commissioned by the European Board and College of Obstetrics and Gynaecology (EBCOG). Eur J Obstet Gynecol Reprod Biol. 2016;201:203–8.
2. Nicklas JM, Barbour LA. Optimizing weight for maternal and infant health – tenable, or too late? Expert Rev Endocrinol Metab. 2015;10(2):227–42.
3. Gynecologists Acooa. Obesity in pregnancy committee opinion. Obstet Gynecol. 2013;121(1):210–2.
4. Davies GA, Maxwell C, Mcleod L, Gagnon R, Basso M, Bos H, et al. SOGC clinical practice guidelines: obesity in pregnancy. No. 239, February 2010. Int J Gynaecol Obstet. 2010;110(2):167–73.
5. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. JAMA. 2014;311(8):806–14.
6. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among us adults, 1999–2010. JAMA. 2012;307(5):491–7.
7. Ogden CL, Carroll MD, Curtin LR, Mcdowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999–2004. JAMA. 2006;295(13):1549–55.
8. Handwerger S, Freemark M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab. 2000;13(4):343–56.
9. Barbour LA, Mccurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care. 2007;30(Supplement 2):S1–8.
10. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Backhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012;150(3):470–80.
11. O'Keeffe M, St-Onge MP. Sleep duration and disorders in pregnancy: implications for glucose metabolism and pregnancy outcomes. Int J Obes. 2013;37(6):765–70.
12. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Del Prato S. Intermediate metabolism in normal pregnancy and in gestational diabetes. Diabetes Metab Res Rev. 2003;19(4):259–70.
13. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr. 2000;71(5 Suppl):1256s–61s.
14. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. Am J Obstet Gynecol. 1999;180(4):903–16.
15. Holme AM, Roland MC, Lorentzen B, Michelsen TM, Henriksen T. Placental glucose transfer: a human in vivo study. PLoS One. 2015;10(2):E0117084.
16. Illsley NP. Placental glucose transport in diabetic pregnancy. Clin Obstet Gynecol. 2000;43(1):116–26.
17. De Veciana M. Diabetes ketoacidosis in pregnancy. Semin Perinatol. 2013;37(4):267–73.
18. Lain KY, Catalano PM. Metabolic changes in pregnancy. Clin Obstet Gynecol. 2007;50(4):938–48.
19. Diderholm B, Stridsberg M, Ewald U, Lindeberg-Norden S, Gustafsson J. Increased lipolysis in non-obese pregnant women studied in the third trimester. BJOG. 2005;112(6):713–8.
20. Freinkel N. Banting Lecture 1980. Of pregnancy and progeny. Diabetes. 1980;29(12):1023–35.
21. Mcintyre HD, Zeck W, Russell A. Placental growth hormone, fetal growth and the IGF axis in normal and diabetic pregnancy. Curr Diabetes Rev. 2009;5(3):185–9.
22. Kirwan JP, Hauguel-De MS, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, et al. TNF-alpha is a predictor of insulin resistance in human pregnancy. Diabetes. 2002;51(7):2207–13.
23. Barbour LA, Shao J, Qiao L, Pulawa LK, Jensen DR, Bartke A, et al. Human placental growth hormone causes severe insulin resistance in transgenic mice. Am J Obstet Gynecol. 2002;186(3):512–7.
24. Mesiano S. The endocrinology of human pregnancy and fetoplacental development. In: Barbieri JFSRL, editor. Yes and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical Management. Philadelphia: Saunders Elsevier; 2009. p. 249–82.
25. Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, et al. Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. Endocrinology. 1993;132(2):879–87.
26. Baeyens L, Hindi S, Sorenson RL, German MS. Beta-cell adaptation in pregnancy. Diabetes Obes Metab. 2016;18(Suppl 1):63–70.
27. Iliodromiti S, Sassarini J, Kelsey TW, Lindsay RS, Sattar N, Nelson SM. Accuracy of circulating adiponectin for predicting gestational diabetes: a

systematic review and meta-analysis. Diabetologia. 2016;59(4):692–9.
28. Lacroix M, Battista MC, Doyon M, Menard J, Ardilouze JL, Perron P, et al. Lower adiponectin levels at first trimester of pregnancy are associated with increased insulin resistance and higher risk of developing gestational diabetes mellitus. Diabetes Care. 2013;36(6):1577–83.
29. Retnakaran R, Qi Y, Connelly PW, Sermer M, Hanley AJ, Zinman B. Low adiponectin concentration during pregnancy predicts postpartum insulin resistance, beta cell dysfunction and fasting glycaemia. Diabetologia. 2010;53(2):268–76.
30. Retnakaran R. Adiponectin and beta-cell adaptation in pregnancy. Diabetes. 2017;66(5):1121–2.
31. Harmon KA, Gerard L, Jensen DR, Kealey EH, Hernandez TL, Reece MS, et al. Continuous glucose profiles in obese and normal-weight pregnant women on a controlled diet: metabolic determinants of fetal growth. Diabetes Care. 2011;34(10):2198–204.
32. Sivan E, Homko CJ, Whittaker PG, Reece EA, Chen X, Boden G. Free fatty acids and insulin resistance during pregnancy. J Clin Endocrinol Metab. 1998;83(7):2338–42.
33. Heerwagen MJ, De La Houssaye BE, Krause MA, Kramer A, Fisher B, Reece MS, et al. Human placental LPL activity is strongly associated with infant adiposity at birth. Diabetes. 2012;61(Suppl 1):A52.
34. Barbour LA, Hernandez TL, Hirsch N, Reece MS, Chartier-Logan C, Friedman JE, et al. Lipid predictors of infant adiposity in early and late pregnancy, fasting and fed. Diabetes. 2017;66(Suppl 1):A55.
35. Whyte K, Kelly H, O'Dwyer V, Gibbs M, O'Higgins A, Turner MJ. Offspring birth weight and maternal fasting lipids in women screened for gestational diabetes mellitus (GDM). Eur J Obstet Gynecol Reprod Biol. 2013;170(1):67–70.
36. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care. 2008;31(9):1858–63.
37. Hernandez TL, Friedman JE, Van Pelt RE, Barbour LA. Patterns of glycemia in normal pregnancy: should the current therapeutic targets be challenged? Diabetes Care. 2011;34(7):1660–8.
38. Siegmund T, Rad N, Ritterath C, Siebert G, Henrich W, Buhling KJ. Longitudinal changes in the continuous glucose profile measured by the CGMS in healthy pregnant women and determination of cut-off values. Eur J Obstet Gynecol Reprod Biol. 2008;139(1):46–52.
39. Yogev Y, Ben Haroush A, Chen R, Rosenn B, Hod M, Langer O. Diurnal glycemic profile in obese and normal weight nondiabetic pregnant women. Am J Obstet Gynecol. 2004;191(3):949–53.
40. Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest. 2005;115(3):485–91.
41. Barrett HL, Dekker NM, Mcintyre HD, Callaway LK. Normalizing metabolism in diabetic pregnancy: is it time to target lipids? Diabetes Care. 2014;37(5):1484–93.
42. Catalano PM, Shankar K. Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. BMJ. 2017;356:J1.
43. Olmos PR, Rigotti A, Busso D, Berkowitz L, Santos JL, Borzone GR, et al. Maternal hypertriglyceridemia: a link between maternal overweight-obesity and macrosomia in gestational diabetes. Obesity (Silver Spring). 2014;22(10):2156–63.
44. Barbour LA, Hernandez TL, Reece MS, Kahn B, Horton T, Galan HL, et al. Change in fasting triglycerides from early to late gestation are highly predictive of neonatal adiposity independent of maternal BMI. Diabetes. 2009;58(Suppl 1):A84.
45. Metzger BE, Phelps RL, Freinkel N, Navickas IA. Effects of gestational diabetes on diurnal profiles of plasma glucose, lipids, and individual amino acids. Diabetes Care. 1980;3(3):402–9.
46. Phelps RL, Metzger BE, Freinkel N. Carbohydrate metabolism in pregnancy. XVII. Diurnal profiles of plasma glucose, insulin, free fatty acids, triglycerides, cholesterol, and individual amino acids in late normal pregnancy. Am J Obstet Gynecol. 1981;140(7):730–6.
47. Ryckman KK, Donovan BM, Fleener DK, Bedell B, Borowski KS. Pregnancy-related changes of amino acid and acylcarnitine concentrations: the impact of obesity. AJP Rep. 2016;6(3):E329–36.
48. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab. 2012;15(5):606–14.
49. Catalano PM, Nizielski SE, Shao J, Preston L, Qiao L, Friedman JE. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. Am J Physiol Endocrinol Metab. 2002;282(3):E522–E33.
50. Zeghari N, Vidal H, Younsi M, Ziegler O, Drouin P, Donner M. Adipocyte membrane phospholipids and Ppar-gamma expression in obese women: relationship to hyperinsulinemia. Am J Physiol Endocrinol Metab. 2000;279(4):E736–E43.
51. Lappas M. Effect of pre-existing maternal obesity, gestational diabetes and adipokines on the expression of genes involved in lipid metabolism in adipose tissue. Metabolism. 2014;63(2):250–62.
52. Catalano PM, Hoegh M, Minium J, Huston-Presley L, Bernard S, Kalhan S, et al. Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. Diabetologia. 2006;49(7):1677–85.
53. Lowe LP, Metzger BE, Lowe WL Jr, Dyer AR, Mcdade TW, Mcintyre HD, et al. Inflammatory mediators and glucose in pregnancy: results from a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. J Clin Endocrinol Metab. 2010;95(12):5427–34.

54. Aye IL, Powell TL, Jansson T. Review: adiponectin–the missing link between maternal adiposity, placental transport and fetal growth? Placenta. 2013;34(Suppl):S40–5.
55. Combs TP, Marliss EB. Adiponectin signaling in the liver. Rev Endocr Metab Disord. 2014;15(2):137–47.
56. Qiao L, Wattez JS, Lee S, Nguyen A, Schaack J, Hay WW Jr, et al. Adiponectin deficiency impairs maternal metabolic adaptation to pregnancy in mice. Diabetes. 2017;66(5):1126–35.
57. Howell KR, Powell TL. Effects of maternal obesity on placental function and fetal development. Reproduction. 2017;153(3):R97–R108.
58. Xiang A, Kawakubo M, Trigo E, Kjos SL, Buchanan TA. Declining beta-cell compensation for insulin resistance in hispanic women with recent gestational diabetes mellitus: association with changes in weight, adiponectin, and C-reactive protein. Diabetes Care. 2010;33(2):396–401.
59. Christou GA, Kiortsis DN. Adiponectin and lipoprotein metabolism. Obes Rev. 2013;14(12):939–49.
60. Ong GK, Hamilton JK, Sermer M, Connelly PW, Maguire G, Zinman B, et al. Maternal serum adiponectin and infant birthweight: the role of adiponectin isoform distribution. Clin Endocrinol. 2007;67(1):108–14.
61. Lekva T, Roland MCP, Michelsen AE, Friis CM, Aukrust P, Bollerslev J, et al. Large reduction in adiponectin during pregnancy is associated with large-for-gestational-age newborns. J Clin Endocrinol Metab. 2017;102(7):2552–9.
62. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr. 2008;88(4):894–9.
63. Basu S, Haghiac M, Surace P, Challier JC, Guerre-Millo M, Singh K, et al. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. Obesity (Silver Spring). 2011;19(3):476–82.
64. Soderborg TK, Borengasser SJ, Barbour LA, Friedman JE. Microbial transmission from mothers with obesity or diabetes to infants: an innovative opportunity to interrupt a vicious cycle. Diabetologia. 2016;59(5):895–906.
65. Ma J, Prince AL, Bader D, Hu M, Ganu R, Baquero K, et al. High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. Nat Commun. 2014;5:3889.
66. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep. 2012;13(5):440–7.
67. Prince AL, Antony KM, Chu DM, Aagaard KM. The microbiome, parturition, and timing of birth: more questions than answers. J Reprod Immunol. 2014;104–105:12–9.
68. Mueller NT, Shin H, Pizoni A, Werlang IC, Matte U, Goldani MZ, et al. Birth mode-dependent association between pre-pregnancy maternal weight status and the neonatal intestinal microbiome. Sci Rep. 2016;6:23133.
69. Chu DM, Antony KM, Ma J, Prince AL, Showalter L, Moller M, et al. The early infant gut microbiome varies in association with a maternal high-fat diet. Genome Med. 2016;8(1):77.
70. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, et al. Dietary intervention impact on gut microbial gene richness. Nature. 2013;500(7464):585–8.
71. Lemas DJ, Yee S, Cacho N, Miller D, Cardel M, Gurka M, et al. Exploring the contribution of maternal antibiotics and breastfeeding to development of the infant microbiome and pediatric obesity. Semin Fetal Neonatal Med. 2016;21(6):406–9.
72. Young BE, Patinkin ZW, Palmer C, De La Houssaye B, Barbour LA, Hernandez TL, et al. Human milk insulin is related to maternal plasma insulin and BMI–but other components of human milk do not differ by BMI. Eur J Clin Nutr. 2017;71(9):1094–100.
73. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. Cell. 2014;159(4):789–99.
74. Yogev Y, Catalano PM. Pregnancy and obesity. Obstet Gynecol Clin North Am. 2009;36(2):285–300, viii.
75. Beard JH, Bell RL, Duffy AJ. Reproductive considerations and pregnancy after bariatric surgery: current evidence and recommendations. Obes Surg. 2008;18(8):1023–7.
76. Guelinckx I, Devlieger R, Vansant G. Reproductive outcome after bariatric surgery: a critical review. Hum Reprod Update. 2009;15(2):189–201.
77. Hackmon R, James R, O'Reilly Green C, Ferber A, Barnhard Y, Divon M. The impact of maternal age, body mass index and maternal weight gain on the glucose challenge test in pregnancy. J Matern Fetal Neonatal Med. 2007;20(3):253–7.
78. Saldana TM, Siega-Riz AM, Adair LS, Suchindran C. The relationship between pregnancy weight gain and glucose tolerance status among black and white women in central North Carolina. Am J Obstet Gynecol. 2006;195(6):1629–35.
79. Gibson KS, Waters TP, Catalano PM. Maternal weight gain in women who develop gestational diabetes mellitus. Obstet Gynecol. 2012;119(3):560–5.
80. Witter FR, Caulfield LE, Stoltzfus RJ. Influence of maternal anthropometric status and birth weight on the risk of cesarean delivery. Obstet Gynecol. 1995;85(6):947–51.
81. Young TK, Woodmansee B. Factors that are associated with cesarean delivery in a large private practice: the importance of prepregnancy body mass index and weight gain. Am J Obstet Gynecol. 2002;187(2):312–8; Discussion 8–20.
82. Cheng YW, Chung JH, Kurbisch-Block I, Inturrisi M, Shafer S, Caughey AB. Gestational weight gain and gestational diabetes mellitus: perinatal outcomes. Obstet Gynecol. 2008;112(5):1015–22.

83. Devader SR, Neeley HL, Myles TD, Leet TL. Evaluation of gestational weight gain guidelines for women with normal prepregnancy body mass index. Obstet Gynecol. 2007;110(4):745–51.
84. Kiel DW, Dodson EA, Artal R, Boehmer TK, Leet TL. Gestational weight gain and pregnancy outcomes in obese women: how much is enough? Obstet Gynecol. 2007;110(4):752–8.
85. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ. 2005;331(7522):929.
86. Watkins ML, Rasmussen SA, Honein MA, Botto LD, Moore CA. Maternal obesity and risk for birth defects. Pediatrics. 2003;111(5 Pt 2):1152–8.
87. Mojtabai R. Body mass index and serum folate in childbearing age women. Eur J Epidemiol. 2004;19(11):1029–36.
88. Laraia BA, Bodnar LM, Siega-Riz AM. Pregravid body mass index is negatively associated with diet quality during pregnancy. Public Health Nutr. 2007;10(9):920–6.
89. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991–2002.
90. Catalano PM, Mcintyre HD, Cruickshank JK, Mccance DR, Dyer AR, Metzger BE, et al. The hyperglycemia and adverse pregnancy outcome study: associations of GDM and obesity with pregnancy outcomes. Diabetes Care. 2012;35(4):780–6.
91. Ryan EA. Diagnosing gestational diabetes. Diabetologia. 2011;54(3):480–6.
92. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Phenotype of infants of mothers with gestational diabetes. Diabetes Care. 2007;30(Suppl 2):S156–S60.
93. Defronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Phys. 1979;237(3):E214–E23.
94. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest. 1981;68(6):1456–67.
95. Bergman RN, Hope ID, Yang YJ, Watanabe RM, Meador MA, Youn JH, et al. Assessment of insulin sensitivity in vivo: a critical review. Diabetes Metab Rev. 1989;5(5):411–29.
96. Bergman RN. Lilly Lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes. 1989;38(12):1512–27.
97. Wallace TM, Matthews DR. The assessment of insulin resistance in man. Diabet Med. 2002;19(7):527–34.
98. Wallace TM, Levy JC, Matthews DR. Use and abuse of homa modeling. Diabetes Care. 2004;27(6):1487–95.
99. Crume TL, Shapiro AL, Brinton JT, Glueck DH, Martinez M, Kohn M, et al. Maternal fuels and metabolic measures during pregnancy and neonatal body composition: the Healthy Start study. J Clin Endocrinol Metab. 2015;100(4):1672–80.
100. Matsuda M, Defronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22(9):1462–70.
101. Kirwan JP, Huston-Presley L, Kalhan SC, Catalano PM. Clinically useful estimates of insulin sensitivity during pregnancy: validation studies in women with normal glucose tolerance and gestational diabetes mellitus. Diabetes Care. 2001;24(9):1602–7.
102. Abdul-Ghani MA, Matsuda M, Balas B, Defronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. Diabetes Care. 2007;30(1):89–94.
103. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J, Carr DB, Boyko EJ, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-H glucose levels. Diabetes Care. 2009;32(2):335–41.
104. Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, et al. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. Diabetes. 2002;51(9):2796–803.
105. Hamilton JK, Odrobina E, Yin J, Hanley AJ, Zinman B, Retnakaran R. Maternal insulin sensitivity during pregnancy predicts infant weight gain and adiposity at 1 year of age. Obesity (Silver Spring). 2010;18(2):340–6.
106. Shapiro AL, Schmiege SJ, Brinton JT, Glueck D, Crume TL, Friedman JE, et al. Testing the fuel-mediated hypothesis: maternal insulin resistance and glucose mediate the association between maternal and neonatal adiposity, the Healthy Start study. Diabetologia. 2015;58(5):937–41.
107. Isganaitis E, Woo M, Ma H, Chen M, Kong W, Lytras A, et al. Developmental programming by maternal insulin resistance: hyperinsulinemia, glucose intolerance, and dysregulated lipid metabolism in male offspring of insulin-resistant mice. Diabetes. 2014;63(2):688–700.
108. Friedman JE. Obesity and gestational diabetes mellitus pathways for programming in mouse, monkey, and man-where do we go next? The 2014 Norbert Freinkel Award lecture. Diabetes Care. 2015;38(8):1402–11.
109. Margerison Zilko CE, Rehkopf D, Abrams B. Association of maternal gestational weight gain with short- and long-term maternal and child health outcomes. Am J Obstet Gynecol. 2010;202(6):574 E1–8.
110. Jedrychowski W, Maugeri U, Kaim I, Budzyn-Mrozek D, Flak E, Mroz E, et al. Impact of excessive gestational weight gain in non-smoking mothers on body fatness in infancy and early childhood. Prospective prebirth cohort study in Cracow. J Physiol Pharmacol. 2011;62(1):55–64.

111. Crozier SR, Inskip HM, Godfrey KM, Cooper C, Harvey NC, Cole ZA, et al. Weight gain in pregnancy and childhood body composition: findings from the southampton women's survey. Am J Clin Nutr. 2010;91(6):1745–51.
112. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Increased fetal adiposity: a very sensitive marker of abnormal in utero development. Am J Obstet Gynecol. 2003;189(6):1698–704.
113. Catalano PM, Roman NM, Tyzbir ED, Merritt AO, Driscoll P, Amini SB. Weight gain in women with gestational diabetes. Obstet Gynecol. 1993;81(4):523–8.
114. Starling AP, Brinton JT, Glueck DH, Shapiro AL, Harrod CS, Lynch AM, et al. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. Am J Clin Nutr. 2015;101(2):302–9.
115. Hernandez TL, Anderson MA, Chartier-Logan C, Friedman JE, Barbour LA. Strategies in the nutritional management of gestational diabetes. Clin Obstet Gynecol. 2013;56(4):803–15.
116. Hernandez TL. Higher-complex carbohydrate diets in gestational diabetes. In: Rajendram R, Preedy VR, Patel V, editors. Nutrition and diet in maternal diabetes: an evidence-based approach. New York: Springer; 2018.
117. Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis. 2000;150(2):227–43.
118. Zhang C, Schulze MB, Solomon CG, Hu FB. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. Diabetologia. 2006;49(11):2604–13.
119. Zhang C, Liu S, Solomon CG, Hu FB. Dietary fiber intake, dietary glycemic load, and the risk for gestational diabetes mellitus. Diabetes Care. 2006;29(10):2223–30.
120. Mcgowan CA, Walsh JM, Byrne J, Curran S, Mcauliffe FM. The influence of a low glycemic index dietary intervention on maternal dietary intake, glycemic index and gestational weight gain during pregnancy: a randomized controlled trial. Nutr J. 2013;12(1):140.
121. Hernandez TL. Nutrition therapy in gestational diabetes: the case for complex carbohydrates. Diabetes Spectr. 2016;29(2):82–8.
122. Hernandez TL, Van Pelt RE, Anderson MA, Daniels LJ, West NA, Donahoo WT, et al. A higher-complex carbohydrate diet in gestational diabetes mellitus achieves glucose targets and lowers postprandial lipids: a randomized crossover study. Diabetes Care. 2014;37(5):1254–62.
123. Hernandez TL, Van Pelt RE, Anderson MA, Reece MS, Reynolds RM, De La Houssaye BA, et al. Women with gestational diabetes mellitus randomized to a higher-complex carbohydrate/low-fat diet manifest lower adipose tissue insulin resistance, inflammation, glucose, and free fatty acids: a pilot study. Diabetes Care. 2016;39(1):39–42.
124. Cunningham SA, Kramer MR, Narayan KM. Incidence of childhood obesity in the United States. N Engl J Med. 2014;370(5):403–11.
125. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the global burden of disease study 2013. Lancet. 2014;384(9945):766–81.
126. Nicholas LM, Morrison JL, Rattanatray L, Zhang S, Ozanne SE, Mcmillen IC. The early origins of obesity and insulin resistance: timing, programming and mechanisms. Int J Obes. 2016;40(2):229–38.
127. Hull HR, Thornton JC, Ji Y, Paley C, Rosenn B, Mathews P, et al. Higher infant body fat with excessive gestational weight gain in overweight women. Am J Obstet Gynecol. 2011;205(3):211.E1–7.
128. Andersen CS, Gamborg M, Sorensen TI, Nohr EA. Weight gain in different periods of pregnancy and offspring's body mass index at 7 years of age. Int J Pediatr Obes. 2011;6(2–2):E179–86.
129. Wu LL, Russell DL, Wong SL, Chen M, Tsai TS, St John JC, et al. Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. Development. 2015;142(4):681–91.
130. Mccurdy CE, Bishop JM, Williams SM, Grayson BE, Smith MS, Friedman JE, et al. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. J Clin Invest. 2009;119(2):323–35.
131. Thorn SR, Baquero KC, Newsom SA, El Kasmi KC, Bergman BC, Shulman GI, et al. Early life exposure to maternal insulin resistance has persistent effects on hepatic NAFLD in juvenile nonhuman primates. Diabetes. 2014;63(8):2702–13.
132. Kubo A, Ferrara A, Windham GC, Greenspan LC, Deardorff J, Hiatt RA, et al. Maternal hyperglycemia during pregnancy predicts adiposity of the offspring. Diabetes Care. 2014;37(11):2996–3002.
133. Mccurdy CE, Schenk S, Hetrick B, Houck J, Drew BG, Kaye S, et al. Maternal obesity reduces oxidative capacity in fetal skeletal muscle of Japanese macaques. JCI Insight. 2016;1(16):E86612.
134. Graus-Nunes F, Dalla Corte Frantz E, Lannes WR, Da Silva Menezes MC, Mandarim-De-Lacerda CA, Souza-Mello V. Pregestational maternal obesity impairs endocrine pancreas in male F1 and F2 progeny. Nutrition. 2015;31(2):380–7.
135. Saben JL, Boudoures AL, Asghar Z, Thompson A, Drury A, Zhang W, et al. Maternal metabolic syndrome programs mitochondrial dysfunction via germline changes across three generations. Cell Rep. 2016;16(1):1–8.
136. Seidler EA, Moley KH. Metabolic determinants of mitochondrial function in oocytes. Semin Reprod Med. 2015;33(6):396–400.
137. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988–1994 to 2007–2010. J Pediatr. 2013;162(3):496–500 E1.

138. Alisi A, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. J Pediatr. 2009;155(4):469–74.
139. Dabelea D, Knowler WC, Pettitt DJ. Effect of diabetes in pregnancy on offspring: follow-up research in the Pima Indians. J Matern Fetal Med. 2000;9(1):83–8.
140. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. Diabetes. 2000;49(12):2208–11.
141. Brumbaugh DE, Tearse P, Cree-Green M, Fenton LZ, Brown M, Scherzinger A, et al. Intrahepatic fat is increased in the neonatal offspring of obese women with gestational diabetes. J Pediatr. 2013;162(5):930–6.e1.
142. Modi N, Murgasova D, Ruager-Martin R, Thomas EL, Hyde MJ, Gale C, et al. The influence of maternal body mass index on infant adiposity and hepatic lipid content. Pediatr Res. 2011;70(3):287–91.
143. Faupel-Badger JM, Mcelrath TF, Lauria M, Houghton LC, Lim KH, Parry S, et al. Maternal circulating angiogenic factors in twin and singleton pregnancies. Am J Obstet Gynecol. 2015;212(5):636 E1–8.
144. Patel S, Lawlor DA, Callaway M, Macdonald-Wallis C, Sattar N, Fraser A. Association of maternal diabetes/glycosuria and pre-pregnancy body mass index with offspring indicators of non-alcoholic fatty liver disease. BMC Pediatr. 2016;16:47.
145. Gaillard R, Welten M, Oddy WH, Beilin LJ, Mori TA, Jaddoe VW, et al. Associations of maternal pre-pregnancy body mass index and gestational weight gain with cardio-metabolic risk factors in adolescent offspring: a prospective cohort study. BJOG. 2016;123(2):207–16.
146. Newton KP, Feldman HS, Chambers CD, Wilson L, Behling C, Clark JM, et al. Low and high birth weights are risk factors for nonalcoholic fatty liver disease in children. J Pediatr. 2017;187:141–146.e1.
147. Goyal NP, Schwimmer JB. The progression and natural history of pediatric nonalcoholic fatty liver disease. Clin Liver Dis. 2016;20(2):325–38.
148. Heerwagen MJ, Stewart MS, De La Houssaye BA, Janssen RC, Friedman JE. Transgenic increase in N-3/N-6 fatty acid ratio reduces maternal obesity-associated inflammation and limits adverse developmental programming in mice. PLoS One. 2013;8(6):E67791.
149. Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC, et al. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology. 2009;50(6):1796–808.
150. Grant WF, Nicol LE, Thorn SR, Grove KL, Friedman JE, Marks DL. Perinatal exposure to a high-fat diet is associated with reduced hepatic sympathetic innervation in one-year old male Japanese macaques. PLoS One. 2012;7(10):E48119.
151. Whitaker RC. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. Pediatrics. 2004;114(1):E29–36.
152. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics. 2005;115(3):E290–E6.
153. Catalano PM, Farrell K, Thomas A, Huston-Presley L, Mencin P, De Mouzon SH, et al. Perinatal risk factors for childhood obesity and metabolic dysregulation. Am J Clin Nutr. 2009;90(5):1303–13.
154. Barisione M, Carlini F, Gradaschi R, Camerini G, Adami GF. Body weight at developmental age in siblings born to mothers before and after surgically induced weight loss. Surg Obes Relat Dis. 2012;8(4):387–91.
155. Kral JG, Biron S, Simard S, Hould FS, Lebel S, Marceau S, et al. Large maternal weight loss from obesity surgery prevents transmission of obesity to children who were followed for 2 to 18 years. Pediatrics. 2006;118(6):E1644–9.
156. Kizirian NV, Kong Y, Muirhead R, Brodie S, Garnett SP, Petocz P, et al. Effects of a low-glycemic index diet during pregnancy on offspring growth, body composition, and vascular health: a pilot randomized controlled trial. Am J Clin Nutr. 2016;103(4):1073–82.
157. Markovic TP, Muirhead R, Overs S, Ross GP, Louie JC, Kizirian N, et al. Randomized controlled trial investigating the effects of a low-glycemic index diet on pregnancy outcomes in women at high risk of gestational diabetes mellitus: the GI baby 3 study. Diabetes Care. 2016;39(1):31–8.
158. Barbour LA. Changing perspectives in pre-existing diabetes and obesity in pregnancy: maternal and infant short- and long-term outcomes. Curr Opin Endocrinol Diabetes Obes. 2014;21(4):257–63.
159. Jharap VV, Santos S, Steegers EA, Jaddoe VW, Gaillard R. Associations of maternal obesity and excessive weight gain during pregnancy with subcutaneous fat mass in infancy. Early Hum Dev. 2017;108:23–8.
160. Rasmussen KM, Catalano PM, Yaktine AL. New guidelines for weight gain during pregnancy: what obstetrician/gynecologists should know. Curr Opin Obstet Gynecol. 2009;21(6):521–6.
161. Rivera HM, Christiansen KJ, Sullivan EL. The role of maternal obesity in the risk of neuropsychiatric disorders. Front Neurosci. 2015;9:194.
162. Sullivan EL, Rivera HM, True CA, Franco JG, Baquero K, Dean TA, et al. Maternal and postnatal high-fat diet consumption programs energy balance and hypothalamic melanocortin signaling in nonhuman primate offspring. Am J Physiol Regul Integr Comp Physiol. 2017;313(2):R169–79.
163. Bajpeyi S, Myrland CK, Covington JD, Obanda D, Cefalu WT, Smith SR, et al. Lipid in skeletal muscle myotubes is associated to the donors' insulin

sensitivity and physical activity phenotypes. Obesity (Silver Spring). 2014;22(2):426–34.
164. Boyle KE, Zheng D, Anderson EJ, Neufer PD, Houmard JA. Mitochondrial lipid oxidation is impaired in cultured myotubes from obese humans. Int J Obes. 2012;36(8):1025–31.
165. Boyle KE, Patinkin ZW, Shapiro AL, Baker PR 2nd, Dabelea D, Friedman JE. Mesenchymal stem cells from infants born to obese mothers exhibit greater potential for adipogenesis: the healthy start baby-bump project. Diabetes. 2016;65(3):647–59.
166. Teh AL, Pan H, Chen L, Ong ML, Dogra S, Wong J, et al. The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. Genome Res. 2014;24(7):1064–74.
167. Ollikainen M, Smith KR, Joo EJ, Ng HK, Andronikos R, Novakovic B, et al. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. Hum Mol Genet. 2010;19(21):4176–88.
168. Cooper WN, Khulan B, Owens S, Elks CE, Seidel V, Prentice AM, et al. DNA methylation profiling at imprinted loci after periconceptional micronutrient supplementation in humans: results of a pilot randomized controlled trial. FASEB J. 2012;26(5):1782–90.
169. Guenard F, Deshaies Y, Cianflone K, Kral JG, Marceau P, Vohl MC. Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. Proc Natl Acad Sci U S A. 2013;110(28):11439–44.
170. Khulan B, Cooper WN, Skinner BM, Bauer J, Owens S, Prentice AM, et al. Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the gambia. Hum Mol Genet. 2012;21(9):2086–101.
171. Dominguez-Salas P, Moore SE, Baker MS, Bergen AW, Cox SE, Dyer RA, et al. Maternal nutrition at conception modulates DNA methylation of human metastable epialleles. Nat Commun. 2014;5:3746.
172. Waterland RA, Kellermayer R, Laritsky E, Rayco-Solon P, Harris RA, Travisano M, et al. Season of conception in rural Gambia affects DNA methylation at putative human metastable epialleles. PLoS Genet. 2010;6(12):E1001252.
173. Ganu RS, Harris RA, Collins K, Aagaard KM. Maternal diet: a modulator for epigenomic regulation during development in nonhuman primates and humans. Int J Obes Suppl. 2012;2(Suppl 2):S14–S8.
174. Fu Q, Yu X, Callaway CW, Lane RH, Mcknight RA. Epigenetics: intrauterine growth retardation (IUGR) modifies the histone code along the rat hepatic IGF-1 gene. FASEB J. 2009;23(8):2438–49.
175. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet. 2013;382(9890):452–77.
176. Godfrey KM, Sheppard A, Gluckman PD, Lillycrop KA, Burdge GC, Mclean C, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. Diabetes. 2011;60(5):1528–34.
177. Radaelli T, Lepercq J, Varastehpour A, Basu S, Catalano PM, Hauguel-De MS. Differential regulation of genes for fetoplacental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. Am J Obstet Gynecol. 2009;201(2):209.
178. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta. 2015;36(7):709–15.
179. Frias AE, Morgan TK, Evans AE, Rasanen J, Oh KY, Thornburg KL, et al. Maternal high-fat diet disturbs uteroplacental hemodynamics and increases the frequency of stillbirth in a nonhuman primate model of excess nutrition. Endocrinology. 2011;152(6):2456–64.
180. Acharya G, Albrecht C, Benton SJ, Cotechini T, Dechend R, Dilworth MR, et al. IFPA meeting 2011 workshop report I: placenta: predicting future health; roles of lipids in the growth and development of fetoplacental unit; placental nutrient sensing; placental research to solve clinical problems–a translational approach. Placenta. 2012;33(Suppl):S4–8.
181. Lager S, Gaccioli F, Ramirez VI, Jones HN, Jansson T, Powell TL. Oleic acid stimulates system a amino acid transport in primary human trophoblast cells mediated by toll-like receptor 4. J Lipid Res. 2013;54(3):725–33.
182. Ategbo JM, Grissa O, Yessoufou A, Hichami A, Dramane KL, Moutairou K, et al. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. J Clin Endocrinol Metab. 2006;91(10):4137–43.
183. Jones HN, Jansson T, Powell TL. Full-length adiponectin attenuates insulin signaling and inhibits insulin-stimulated amino acid transport in human primary trophoblast cells. Diabetes. 2010;59(5):1161–70.
184. Aye IL, Waddell BJ, Mark PJ, Keelan JA. Oxysterols exert proinflammatory effects in placental trophoblasts via TLR4-dependent, cholesterol-sensitive activation of NF-kappab. Mol Hum Reprod. 2012;18(7):341–53.
185. Jansson N, Greenwood SL, Johansson BR, Powell TL, Jansson T. Leptin stimulates the activity of the system a amino acid transporter in human placental villous fragments. J Clin Endocrinol Metab. 2003;88(3):1205–11.
186. Jansson T, Ekstrand Y, Bjorn C, Wennergren M, Powell TL. Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. Diabetes. 2002;51(7):2214–9.
187. Jansson N, Rosario FJ, Gaccioli F, Lager S, Jones HN, Roos S, et al. Activation of placental mTOR signaling and amino acid transporters in obese women

giving birth to large babies. J Clin Endocrinol Metab. 2013;98(1):105–13.
188. Perazzolo S, Hirschmugl B, Wadsack C, Desoye G, Lewis RM, Sengers BG. The influence of placental metabolism on fatty acid transfer to the fetus. J Lipid Res. 2017;58(2):443–54.
189. Calabuig-Navarro V, Haghiac M, Minium J, Glazebrook P, Ranasinghe GC, Hoppel C, et al. Effect of maternal obesity on placental lipid metabolism. Endocrinology. 2017;158(8):2543–55.
190. Radaelli T, Varastehpour A, Catalano P, Hauguel-De Mouzon S. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. Diabetes. 2003;52(12):2951–8.
191. Perichart-Perera O, Munoz-Manrique C, Reyes-Lopez A, Tolentino-Dolores M, Espino YSS, Ramirez-Gonzalez MC. Metabolic markers during pregnancy and their association with maternal and newborn weight status. PLoS One. 2017;12(7):E0180874.
192. Desoye G, Hauguel-De MS. The human placenta in gestational diabetes mellitus. The insulin and cytokine network. Diabetes Care. 2007;30(Suppl 2):S120–S6.
193. Ingvorsen C, Brix S, Ozanne SE, Hellgren LI. The effect of maternal inflammation on foetal programming of metabolic disease. Acta Physiol (Oxf). 2015;214(4):440–9.
194. Brumbaugh DE, Friedman JE. Developmental origins of nonalcoholic fatty liver disease. Pediatr Res. 2014;75(1–2):140–7.
195. Isganaitis E, Rifas-Shiman SL, Oken E, Dreyfuss JM, Gall W, Gillman MW, et al. Associations of cord blood metabolites with early childhood obesity risk. Int J Obes. 2015;39(7):1041–8.
196. Sandler V, Reisetter AC, Bain JR, Muehlbauer MJ, Nodzenski M, Stevens RD, et al. Associations of maternal BMI and insulin resistance with the maternal metabolome and newborn outcomes. Diabetologia. 2017;60(3):518–30.
197. Baker PR 2nd, Patinkin ZW, Shapiro S, De La Houssaye B, Boyle K, Vanderlinden L, et al. Maternal obesity and increased neonatal adiposity are associated with compromised infant mesenchymal stem cell metabolism and energy sensing. JCI Insight. 2017;2(21). pii: 94200. https://doi.org/10.1172/jci.insight.94200.
198. Simmons D. Prevention of gestational diabetes mellitus: where are we now? Diabetes Obes Metab. 2015;17(9):824–34.
199. Luoto R, Laitinen K, Nermes M, Isolauri E. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. Br J Nutr. 2010;103(12):1792–9.
200. Wickens KL, Barthow CA, Murphy R, Abels PR, Maude RM, Stone PR, et al. Early pregnancy probiotic supplementation with Lactobacillus rhamnosus HN001 may reduce the prevalence of gestational diabetes mellitus: a randomised controlled trial. Br J Nutr. 2017;117(6):804–13.
201. Nitert MD, Barrett HL, Foxcroft K, Tremellen A, Wilkinson S, Lingwood B, et al. Spring: an RCT study of probiotics in the prevention of gestational diabetes mellitus in overweight and obese women. BMC Pregnancy Childbirth. 2013;13:50.
202. Callaway LK, Mcintyre HD, Barrett HL, Foxcroft K, Tremellen A, Lingwood BE, et al. Probiotics for the prevention of gestational diabetes mellitus in overweight and obese women: findings from the spring double-blind randomized controlled trial. Diabetes Care. 2019;42(3):364–71.
203. D'Anna R, Scilipoti A, Giordano D, Caruso C, Cannata ML, Interdonato ML, et al. myo-Inositol supplementation and onset of gestational diabetes mellitus in pregnant women with a family history of type 2 diabetes: a prospective, randomized, placebo-controlled study. Diabetes Care. 2013;36(4):854–7.
204. Farren M, Daly N, Mckeating A, Kinsley B, Turner MJ, Daly S. The prevention of gestational diabetes mellitus with antenatal oral inositol supplementation: a randomized controlled trial. Diabetes Care. 2017;40(6):759–63.
205. Saccone G, Saccone I, Berghella V. Omega-3 long-chain polyunsaturated fatty acids and fish oil supplementation during pregnancy: which evidence? J Matern Fetal Neonatal Med. 2016;29(15):2389–97.
206. Haghiac M, Yang X, Presley L, Smith S, Dettelback S, Minium J, et al. Dietary omega-3 fatty acid supplementation reduces inflammation in obese pregnant women: a randomized double-blind controlled clinical trial. PLoS One. 2015;10(9):E0137309.
207. Vanky E, Stridsklev S, Heimstad R, Romundstad P, Skogoy K, Kleggetveit O, et al. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. J Clin Endocrinol Metab. 2010;95(12):E448–55.
208. Zhuo Z, Wang A, Yu H. Effect of metformin intervention during pregnancy on the gestational diabetes mellitus in women with polycystic ovary syndrome: a systematic review and meta-analysis. J Diabetes Res. 2014;2014:381231.
209. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, et al. Metformin versus placebo in obese pregnant women without diabetes mellitus. N Engl J Med. 2016;374(5):434–43.
210. Balsells M, Garcia-Patterson A, Sola I, Roque M, Gich I, Corcoy R. Glibenclamide, metformin, and insulin for the treatment of gestational diabetes: a systematic review and meta-analysis. BMJ. 2015;350:H102.
211. Barbour LA, Scifres C, Valent AM, Friedman JE, Buchanan TA, Coustan D, et al. A cautionary response to SMFM statement: pharmacological treatment of gestational diabetes. Am J Obstet Gynecol. 2018;219(4):367 E1–7.
212. Barbour LA. Unresolved controversies in gestational diabetes: implications on maternal and infant health. Curr Opin Endocrinol Diabetes Obes. 2014;21(4):264–70.

213. Sacco F, Calderone A, Castagnoli L, Cesareni G. The cell-autonomous mechanisms underlying the activity of metformin as an anticancer drug. Br J Cancer. 2016;115(12):1451–6.
214. Griss T, Vincent EE, Egnatchik R, Chen J, Ma EH, Faubert B, et al. Metformin antagonizes cancer cell proliferation by suppressing mitochondrial-dependent biosynthesis. PLoS Biol. 2015;13(12):E1002309.
215. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, Mi GTI. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008;358(19):2003–15.
216. Barrett HL, Dekker NM, Jones L, O'Rourke P, Lust K, Gatford KL, et al. Determinants of maternal triglycerides in women with gestational diabetes mellitus in the Metformin in Gestational Diabetes (MiG) study. Diabetes Care. 2013;36(7):1941–6.
217. Rowan JA, Rush EC, Obolonkin V, Battin M, Wouldes T, Hague WM. Metformin in Gestational diabetes: The Offspring Follow-Up (MiG TOFU): body composition at 2 years of age. Diabetes Care. 2011;34(10):2279–84.
218. Barbour LA, Van Pelt RE, Brumbaugh DE, Hernandez TL, Friedman JE. Comment on: Rowan et al. Metformin in Gestational diabetes: The Offspring Follow-Up (MiG TOFU): body composition at 2 years of age. Diabetes Care. 2011;34:2279–2284. Diabetes Care. 2012;35(3):E28.
219. Carlsen SM, Martinussen MP, Vanky E. Metformin's effect on first-year weight gain: a follow-up study. Pediatrics. 2012;130(5):E1222–6.
220. Eriksson JG, Kajantie E, Osmond C, Thornburg K, Barker DJ. Boys live dangerously in the womb. Am J Hum Biol. 2010;22(3):330–5.

6 Maternal-Fetal Contributors to Insulin Resistance Syndrome in Youth

Jill Landsbaugh Kaar and Dana Dabelea

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, as marked by low birth weight or thinness at birth, with an increased risk for the metabolic syndrome (MetS) [2, 3], insulin resistance (IR) [4–6], poor glucose tolerance and/or type 2 diabetes mellitus (T2DM) [7, 8], and indicators of early cardiovascular disease (CVD) [9–14] in adulthood. 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 [15]. At the other end of the birth weight distribution, macrosomic infants of women with diabetes during pregnancy are a group exposed to overnutrition during the intrauterine life. They have also been shown to have an increased risk for obesity and T2DM as adults [16, 17].

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Metabolic syndrome is a complex disorder comprised of a set of clinical abnormalities, including obesity, abnormal glucose tolerance, insulin resistance, dyslipidemia, and hypertension [18]. Individuals with MetS are at significant risk for future CVD and T2DM. Interest in the MetS in the pediatric population has been driven by increasing rates of overweight and obesity, particularly among youth [19]. To date, the diagnosis of MetS in a pediatric population has not been standardized, and multiple definitions are currently in use [20]. However, 1 in 10 US adolescents are estimated to have the insulin resistance syndrome (IR syndrome)—a cluster of abnormalities similar to those used to define metabolic syndrome in adults [21]. 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 [22, 23]. For many years considered a disease found only in adults, T2DM is now present and increasing in prevalence much earlier in life [24, 25] in close temporal association with the epidemic of obesity affecting all ages [19, 26]. 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.

Figure 6.1 summarizes recent concepts and hypotheses pertinent to the perinatal road to later chronic diseases. This chapter summarizes the

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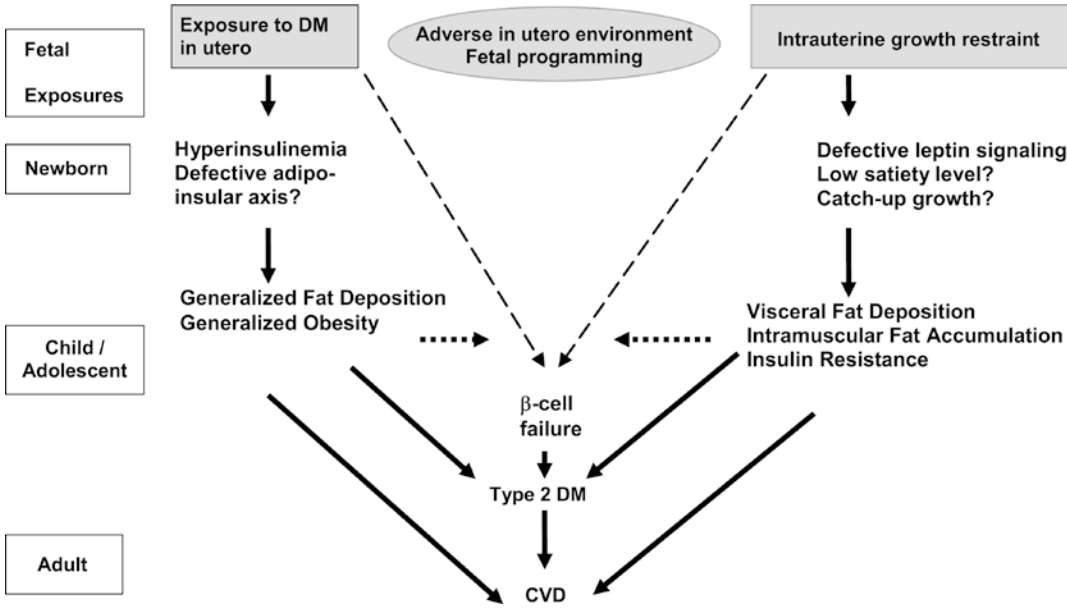

Fig. 6.1 The perinatal road to future chronic diseases. DM diabetes mellitus, CVD cardiovascular disease

available literature on the effects of two main fetal exposures on components of MetS in youth: (1) exposure to diabetes in utero and (2) intrauterine growth restraint (IUGR). 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 [27] proposes that intrauterine exposure to an excess of fuel (e.g., glucose) causes permanent fetal changes. In pregnancies complicated by diabetes, this would lead to malformations, greater birth weight, and an increased risk of developing both obesity and T2DM in later life. These complications have been confirmed in animal studies, as offspring of mothers with gestational diabetes (GDM) have been shown to have an increased risk for diabetes, obesity, and CVD [28–30].

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 [27]. 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.

The role of exposure to diabetes in utero on childhood growth and later obesity has been prospectively examined in large cohort studies, including the Pima Indian Study, Northwestern University Diabetes in Pregnancy Study, the Exploring Perinatal Outcomes in Children (EPOCH) Study in Colorado, and the Project Viva cohort in Massachusetts. The offspring of Pima Indian women with preexisting T2DM and gestational diabetes (GDM) were larger for gestational age at birth and, at every age, heavier for height than the offspring of prediabetic (women who did not have diabetes before or during the index pregnancy but developed diabetes later) or nondiabetic women [16, 31, 32]. Relative weight in the 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 that in turn leads to the childhood obesity. However, in offspring of diabetic pregnancies with normal birth weight, childhood obesity was

still more common than among offspring of nondiabetic pregnancies [33]. Even the normal birth weight offspring of the Pima Indian diabetic women were heavier by age 5–9 years than the offspring of nondiabetic and prediabetic women.

The Diabetes in Pregnancy Center at Northwestern University in Chicago has conducted the only other longitudinal study that reported excessive growth in non-Hispanic white (NHW) offspring of women with diabetes during pregnancy—mostly GDM and insulin-treated DM [34]. Children included in this study were examined at birth, at age 6 months, and annually to age 8 years. The symmetry index (an obesity index), which was normal at 1 year of age, increased during follow-up so that by age 8, the mean symmetry index was almost 1.3; i.e., the children were, on average, 30% heavier than expected for their height.

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 [35, 36]. Using total body electrical conductivity (TOBEC) estimates of body composition, Catalano et al. [36] 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.

The EPOCH cohort, based in Colorado, examined the effects of exposure to diabetes in utero from birth through childhood [37]. Body mass index (BMI) growth trajectories from children exposed or not exposed to diabetes in utero were similar from birth to 27 months; however, from 27 months to 13 years, offspring exposed to diabetes had a significantly greater BMI growth velocity compared to offspring not exposed to diabetes. The greatest difference in growth velocity was found when the children were 10–13 years. These offspring also had significantly more centralized fat distribution (assessed via magnetic resonance imagine [MRI]), although this finding was attenuated after adjustment for maternal prepregnancy BMI [38]. Project Viva has found similar differences in adiposity during childhood in children exposed to diabetes in utero. Specifically, children exposed to diabetes were shown to have a significantly greater skinfold thickness during childhood compared to children not exposed to diabetes in utero [35].

In general, evidence suggests that offspring of diabetic pregnancies are larger [35, 38–40] and have more adiposity (fat mass) [41] than offspring of nondiabetic pregnancies. These long-term effects of the diabetic intrauterine environment on the body size of the offspring also seem to be similar regardless of whether the mother has T1DM, T2DM, or GDM [41].

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 nondiabetic women [16, 42]. Second, obesity in the Pima Indian offspring of diabetic women cannot be accounted for by maternal obesity [33]. Third, the excessive growth seen in the offspring of diabetic mothers is not found in offspring of diabetic fathers [43]. Fourth, within Pima Indian families with nondiabetic offspring, BMI was significantly higher (+2.6 kg/m^2) in the 62 siblings exposed to their mothers' type 2 DM during pregnancy (the diabetic intrauterine environment) than in the 121 siblings born before maternal diabetes was diagnosed [44]. In contrast, there was no significant difference between siblings born before or after their father was diagnosed with type 2 DM (mean BMI difference: 0.4 kg/m^2) [44]. 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- to 14-year-old non-Hispanic white children in the Growing Up Today Study [45] 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 Type 2 Diabetes

Exposure to diabetes in utero has also been shown to have long-lasting effects on glucose-insulin homeostasis. In the SEARCH Case-Control Study, youth (aged 10–22 years) exposed to maternal diabetes in utero were more likely to have type 2 diabetes [46]. Almost half (47.2%) of T2DM in SEARCH youth was attributed to intrauterine exposure to maternal diabetes and obesity. The Diabetes in Pregnancy Center at Northwestern University enrolled offspring of women with preexisting 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 [34]. 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%); 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 preexisting insulin-dependent or non-insulin-dependent diabetes [31].

More than 400 children were followed prospectively in the Generation 1 Australian cohort [47] to examine the independent contribution of gestational diabetes to subsequent offspring insulin resistance at age 9–10 years. Children exposed to GDM in utero were found to have a significantly higher homeostatic model assessment of insulin resistance (HOMA-IR) compared to children not exposed. This result was not attenuated after adjustment for the child's birth weight or current BMI.

A significant correlation between the 2-hour post-load plasma glucose in 15- to 24-year-old Pima women and their mothers' 2-hour glucose during pregnancy has been described [48]. By ages 5–9 and 10–14 years, T2DM was almost exclusively present among the offspring of diabetic Pima Indian women [49]. In all age groups, there was significantly more diabetes in the offspring of diabetic women than in those of prediabetic and nondiabetic women, and there were much smaller differences in diabetes prevalence between offspring of prediabetic and nondiabetic 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 prediabetic 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, 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 exposed offspring. Moreover, within the same family, siblings born after the mother's diagnosis of diabetes were three 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 the same risk of inheriting susceptibility genes, the different observed outcomes reflect the effect of intrauterine exposure to hyperglycemia [44].

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 cut-points used to diagnose GDM is still important. A 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 nondiabetic pregnancies [50]. 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, child's weight, or estimated insulin resistance at 8 years [51].

In Pima Indian children aged 5–19 years, the prevalence of T2DM has increased two- to threefold over the last 30 years [49]. The percent of children who have been exposed to diabetes in utero has also increased significantly over the same time period, which was associated with a doubling of the amount of diabetes in children attributed to this exposure (from 18.1% in 1967–1976 to 35.4% in 1987–1996). The "epidemic" of type 2 diabetes 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 type 2 diabetes in Pima Indian youth (odds ratio 10.4, $p < 0.0001$) [49]. The effects of maternal diabetes on the child may, thus, be viewed as a cross-generational vicious cycle [52]. 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 [53] and 11% annually between 1994 and 2002 in Colorado [54]. 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 US 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 Indian 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 Goto-Kakizaki (GK) rats [55], in whom diabetes is induced by streptozotocin 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 [56]. Several studies performed in newborns of diabetic mothers have shown an enhanced insulin secretion to a glycemic stimulus in these neonates [57], and consistent with these findings, Van Assche [58] and Heding [59] described hyperplasia of pancreatic β (beta) 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 to glucose was 40% lower in individuals whose mothers had DM during pregnancy than in those whose mothers developed DM after the birth of the subject [60]. Based on 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 [33, 61, 62], they appear to be similar regardless of maternal diabetes type [63, 64], and they may not be entirely explained by the development of obesity in exposed offspring [31, 65].

Effects on the Adipoinsular Axis

As shown in Fig. 6.1, 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 [66]. In the Diabetes in Pregnancy study, amniotic fluid insulin was collected at 32–38 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 with 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 [67]. 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 (adipoinsular axis). An inverse relationship between cord blood leptin and BMI growth in the first year of life has been found; however, exposure to overnutrition in utero (maternal diabetes or obesity) did not significantly modify this association [68].

Elevated cord blood leptin concentrations were found in both infants of T1DM (24.7 ng/ml) and GDM mothers (29.3 ng/ml), compared to controls (7.9 ng/ml) [69]. Offspring of mothers with preexisting 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 [70] reflecting the influence of early 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 [71, 72], 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 [71]. Fetal overnutrition may therefore result in a resetting of the adipoinsular axis leading to adiposity during childhood—a hypothesis that requires further testing.

Other Effects

Recent epidemiological evidence suggests fetal life influences the risk of cardiovascular disease later in life [73, 74]. Animal studies have shown that maternal diabetes can induce cardiovascular dysfunction in adult offspring [75]. 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 nondiabetic pregnancies [34]. 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 nondiabetic pregnancies, independent of current body mass index [74]. Recently, the Project Viva cohort has shown that children exposed to GDM had significantly higher systolic blood pressure at age 3 years; however, this association was attenuated by further adjustment for the child's adiposity assessed via skinfold thickness [35]. In contrast, the Pima Indian investigators have previously shown that, independent of adiposity, 7- to 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 [76].

These data suggest that in utero exposure to diabetes confers risks for the development of cardiovascular disease later in life that are independent of adiposity and may be additive to genetic predisposition to diabetes or cardiovascular disease [74].

Intrauterine Growth Restraint

Human epidemiological studies over the last 20 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 [17, 77–79]. Subsequently, more than 30 studies worldwide have confirmed different aspects of this research [9, 15]. The association has been explained as representing long-term effects of nutritional deprivation in utero on fetal growth, development of the endocrine pancreas, and future risk for IR and T2DM (the "thrifty phenotype hypothesis") [15], or as following from pleiotropic effects of genes influencing both fetal growth and susceptibility to IR/T2DM (the "thrifty fetal genotype hypothesis") [80]. 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 toward 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 utero in the last trimester of pregnancy [81]. 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 [82].

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 size at birth [83]. 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 [84]. Moreover, the association between birth weight and components of the IR syndrome in adult life was greatly enhanced by the presence of adult obesity [85], 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 [83]. 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) [83], non-Hispanic white infants who were smaller and thinner at birth but who showed "catch-up growth" during the first 1–2 years of life were larger and had more fat mass 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 0 and 2 years greater than the width of any percentile band on standard growth charts (greater than 0.67 SD scores). Similar results were found in a multiethnic cohort of healthy children from Colorado; infants (birth to 12 months of age) exposed to IUGR experienced a higher growth velocity (i.e., "catch-up growth") compared to unexposed children [6]. However, these differences were not detected beyond this first year of life.

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 body mass index (BMI) [86]. 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 [87, 88], 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 later develop the metabolic syndrome.

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 has examined the relationship between IR and birth size among children is given in Table 6.1 [3, 4, 6, 8, 87, 89–94]. 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 6.1). However, in 9- to 12-year-old British children, birth weight was negatively associated with 30-minute blood glucose, independent of gestation or subsequent growth [95]. In contrast, plasma insulin concentrations were more strongly associated with the pattern of childhood

Table 6.1 Studies relating measures of insulin resistance to size at birth

Population	N	Age (years)	Outcome	Measurement	Relationship
Salisbury children [89]	250	7	Fasting insulin	Ponderal index	Inverse association
Pune children [90, 91]	379	4	30-minute insulin	Birth weight	Inverse association
		8	Fasting, 30-minute, 2-hour insulin	Birth weight	Inverse association with fasting and 30-minute insulin.[a] No association with 2-hour insulin
UK children [92]	1138	10–11	Fasting, 30-minute insulin	Birth weight	Inverse association[a]
NHW and AA US adolescents [93]	296	15	Fasting insulin, IR (euglycemic clamp)	Birth weight	Inverse association with fasting insulin.[a] No association with insulin sensitivity
AA US youth [94]	53	4–14	Fasting insulin, visceral fat, IR (FSIGT)	Birth weight	Inverse association
US Pima Indians [87]	2272	5–29	Fasting, 2-hour insulin, HOMA-IR	Birth weight	Inverse association[a]
Chinese adults [4]	975	41–52	Metabolic syndrome	Ponderal index	Inverse association
Young adults in 5 low–middle-income countries [8]	6511	15–30	Fasting glucose	Birth weight	Inverse association
Contemporary US cohort [6]	506	9–13	Fasting insulin and fasting glucose	IUGR	Inverse association[a]
Young Italian women [3]	85	20–22	Fasting, 30 minutes glucose, fasting insulin, fasting, 20 minutes insulin	Birth weight	Inverse association

IR insulin resistance, *FSIGT* frequently sampled intravenous glucose tolerance test, *IUGR* intrauterine growth restriction

[a]Adjusted for body mass index

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 2 years of life [96]. Among 4-year-old Asian Indian children, low birth weight was associated with increased glucose and insulin concentrations [91] and with most of the IR syndrome components in otherwise healthy 8-year-olds [90]. In the United States, among Pima Indian children and young adults, birth weight was inversely associated with fasting and 2-hour insulin concentrations and with IR estimated from the homeostasis model, when adjusted for current weight and height [87]. Another US study examined 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 [94]. 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. In the EPOCH Colorado study, children (mean age 10.6 years) exposed to IUGR did have significantly higher subcutaneous abdominal adipose tissue as well as increased insulin resistance biomarkers including insulin, higher HOMA-IR, and lower adiponectin [6].

Effects on Other Components of the Insulin Resistance Syndrome

Among adult diseases associated with fetal growth restriction, 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 [97]. In youth, although the weight of the evidence seems to favor an inverse relationship, the data are not uniform [10, 11]. As with previously discussed outcomes, the vast majority of the data come from studies in Europeans and Asians. Among 17-year-old Israeli youth, no correlation was found between birth weight and blood pressure, but a positive correlation between blood pressure and body mass index at age 17 existed [98]. 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 [98, 99]. Data also suggested that race or gender may modify the association between birth weight and childhood blood pressure [100]. Very strong evidence for an association between intrauterine growth restraint and blood pressure in childhood comes from the ALSPAC study [101], 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 non-Hispanic white 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. Similarly, a cohort of children (more than 90% non-Hispanic white) aged 5 years found that children with fetal growth restriction had different cardiac shape, stroke volume was reduced significantly, and higher blood pressure was found compared to normal controls [11]. However, a group of 39 all non-Hispanic white 8-year-olds found that IUGR had no influence on blood pressure [10]. Hence the influence of IUGR on blood pressure during childhood is still not fully supported as the relationship is in large, adult cohorts [12].

The association of IUGR 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 [102]. Increased BMI, central adiposity, IR, hyperten-

sion, and dyslipidemia in childhood were shown to track during adulthood and predict later type 2 DM and CVD [103].

Intrauterine Growth Restraint and Insulin-Like Growth Factors

The mechanism(s) 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 restriction in response to poor placental nutrition, and it may represent a mechanism to optimize fetal survival [104]. IR may be exacerbated by postnatal catch-up growth, with its associated increased central fat deposition [83]. 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 [105]. The regulation of fetal IGF-1 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 [106]. It has also been shown that intrauterine growth restriction is associated with later resistance to insulin, IGF-1, and growth hormone [81]. 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 [107], while in the ALSPAC population-based study, leptin levels at birth were inversely related to rates of infant growth [108].

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 is needed to help 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.

References

1. Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. Int J Epidemiol. 2002;31(2):285–93.
2. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia. 1993;36(1):62–7.
3. Pandolfi C, Zugaro A, Lattanzio F, Necozione S, Barbonetti A, Colangeli MS, et al. Low birth weight and later development of insulin resistance and biochemical/clinical features of polycystic ovary syndrome. Metab Clin Exp. 2008;57(7):999–1004.
4. Mi J, Cheng H, Zhao XY, Hou DQ, Chen FF, Zhang KL. Developmental origin of metabolic syndrome: interaction of thinness at birth and overweight during adult life in Chinese population. Obes Rev. 2008;9(Suppl 1):91–4.
5. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. Diabetologia. 1994;37(2):150–4.
6. Crume TL, Scherzinger A, Stamm E, McDuffie R, Bischoff KJ, Hamman RF, et al. The long-term impact of intrauterine growth restriction in a diverse U.S. cohort of children: the EPOCH study. Obesity. 2014;22(2):608–15.
7. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ. 1991;303(6809):1019–22.
8. Norris SA, Osmond C, Gigante D, Kuzawa CW, Ramakrishnan L, Lee NR, et al. Size at birth, weight gain in infancy and childhood, and adult diabetes risk in five low- or middle-income country birth cohorts. Diabetes Care. 2012;35(1):72–9.
9. Barker DJ, Fall CH. Fetal and infant origins of cardiovascular disease. Arch Dis Child. 1993;68(6):797–9.

10. Bilge I, Poyrazoglu S, Bas F, Emre S, Sirin A, Gokalp S, et al. Ambulatory blood pressure monitoring and renal functions in term small-for-gestational age children. Pediatr Nephrol. 2011;26(1):119–26.
11. Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, Le Noble F, et al. Fetal growth restriction results in remodeled and less efficient hearts in children. Circulation. 2010;121(22):2427–36.
12. Li L, Law C, Power C. Body mass index throughout the life-course and blood pressure in mid-adult life: a birth cohort study. J Hypertens. 2007;25(6):1215–23.
13. Salonen M, Tenhola S, Laitinen T, Lyyra-Laitinen T, Romppanen J, Jaaskelainen J, et al. Tracking serum lipid levels and the association of cholesterol concentrations, blood pressure and cigarette smoking with carotid artery intima-media thickness in young adults born small for gestational age. Circ J. 2010;74(11):2419–25.
14. Stanner SA, Bulmer K, Andres C, Lantseva OE, Borodina V, Poteen VV, et al. Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. BMJ. 1997;315(7119):1342–8.
15. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5–20.
16. Pettitt DJ, Baird HR, Aleck KA, Bennett PH, Knowler WC. Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. N Engl J Med. 1983;308(5):242–5.
17. McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? BMJ. 1994;308(6934):942–5.
18. National Institutes of Health. The third report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). Bethesda; 2001. Contract No.: NIH Publication 01-3670.
19. Ogden CL, Carroll MD, Lawman HG, Fryar CD, Kruszon-Moran D, Kit BK, et al. Trends in obesity prevalence among children and adolescents in the United States, 1988–1994 through 2013–2014. JAMA. 2016;315(21):2292–9.
20. Magge SN, Goodman E, Armstrong SC. The metabolic syndrome in children and adolescents: shifting the focus to cardiometabolic risk factor clustering. Pediatrics. 2017;140(2):e20171603.
21. Miller JM, Kaylor MB, Johannsson M, Bay C, Churilla JR. Prevalence of metabolic syndrome and individual criterion in US adolescents: 2001–2010 National Health and Nutrition Examination Survey. Metab Syndr Relat Disord. 2014;12(10):527–32.
22. Hannon TS, Rao G, Arslanian SA. Childhood obesity and type 2 diabetes mellitus. Pediatrics. 2005;116(2):473–80.
23. Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, et al. A potential decline in life expectancy in the United States in the 21st century. N Engl J Med. 2005;352(11):1138–45.
24. Dabelea D, Pettitt DJ, Jones KL, Arslanian SA. Type 2 diabetes mellitus in minority children and adolescents. An emerging problem. Endocrinol Metab Clin N Am. 1999;28(4):709–29, viii.
25. Fagot-Campagna A. Emergence of type 2 diabetes mellitus in children: epidemiological evidence. J Pediatr Endocrinol Metab. 2000;13(Suppl 6):1395–402.
26. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in obesity among adults in the United States, 2005 to 2014. JAMA. 2016;315(21):2284–91.
27. Freinkel N. Banting Lecture 1980. Of pregnancy and progeny. Diabetes. 1980;29(12):1023–35.
28. Aerts L, Van Assche FA. Animal evidence for the transgenerational development of diabetes mellitus. Int J Biochem Cell Biol. 2006;38(5–6):894–903.
29. Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen TD. Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. Diabetologia. 2016;59(7):1396–9.
30. Harder T, Aerts L, Franke K, Van Bree R, Van Assche FA, Plagemann A. Pancreatic islet transplantation in diabetic pregnant rats prevents acquired malformation of the ventromedial hypothalamic nucleus in their offspring. Neurosci Lett. 2001;299(1–2):85–8.
31. Pettitt DJ, Nelson RG, Saad MF, Bennett PH, Knowler WC. Diabetes and obesity in the offspring of Pima Indian women with diabetes during pregnancy. Diabetes Care. 1993;16(1):310–4.
32. Petitt DJ, Bennett PH, Knowler WC, Baird HR, Aleck KA. Gestational diabetes mellitus and impaired glucose tolerance during pregnancy. Long-term effects on obesity and glucose tolerance in the offspring. Diabetes. 1985;34(Suppl 2):119–22.
33. Pettitt DJ, Knowler WC, Bennett PH, Aleck KA, Baird HR. Obesity in offspring of diabetic Pima Indian women despite normal birth weight. Diabetes Care. 1987;10(1):76–80.
34. Silverman BL, Rizzo T, Green OC, Cho NH, Winter RJ, Ogata ES, et al. Long-term prospective evaluation of offspring of diabetic mothers. Diabetes. 1991;40(Suppl 2):121–5.
35. Wright CS, Rifas-Shiman SL, Rich-Edwards JW, Taveras EM, Gillman MW, Oken E. Intrauterine exposure to gestational diabetes, child adiposity, and blood pressure. Am J Hypertens. 2009;22(2):215–20.
36. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Increased fetal adiposity: a very sensitive marker of abnormal in utero development. Am J Obstet Gynecol. 2003;189(6):1698–704.
37. Crume TL, Ogden L, Daniels S, Hamman RF, Norris JM, Dabelea D. The impact of in utero exposure to diabetes on childhood body mass index

growth trajectories: the EPOCH study. J Pediatr. 2011;158(6):941–6.
38. Crume TL, Ogden L, West NA, Vehik KS, Scherzinger A, Daniels S, et al. Association of exposure to diabetes in utero with adiposity and fat distribution in a multiethnic population of youth: the Exploring Perinatal Outcomes among Children (EPOCH) Study. Diabetologia. 2011;54(1):87–92.
39. Page KA, Romero A, Buchanan TA, Xiang AH. Gestational diabetes mellitus, maternal obesity, and adiposity in offspring. J Pediatr. 2014;164(4):807–10.
40. Nilsson C, Carlsson A, Landin-Olsson M. Increased risk for overweight among Swedish children born to mothers with gestational diabetes mellitus. Pediatr Diabetes. 2014;15(1):57–66.
41. Logan KM, Gale C, Hyde MJ, Santhakumaran S, Modi N. Diabetes in pregnancy and infant adiposity: systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2017;102(1):F65–f72.
42. White P. Childhood diabetes. Its course, and influence on the second and third generations. Diabetes. 1960;9:345–55.
43. Pettitt DJ, Knowler WC. Long-term effects of the intrauterine environment, birth weight, and breast-feeding in Pima Indians. Diabetes Care. 1998;21(Suppl 2):B138–41.
44. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. Diabetes. 2000;49(12):2208–11.
45. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal gestational diabetes, birth weight, and adolescent obesity. Pediatrics. 2003;111(3):e221–6.
46. Dabelea D, Mayer-Davis EJ, Lamichhane AP, D'Agostino RB Jr, Liese AD, Vehik KS, et al. Association of intrauterine exposure to maternal diabetes and obesity with type 2 diabetes in youth: the SEARCH Case-Control Study. Diabetes Care. 2008;31(7):1422–6.
47. Maftei O, Whitrow MJ, Davies MJ, Giles LC, Owens JA, Moore VM. Maternal body size prior to pregnancy, gestational diabetes and weight gain: associations with insulin resistance in children at 9–10 years. Diabet Med. 2015;32(2):174–80.
48. Pettitt DJ, Aleck KA, Baird HR, Carraher MJ, Bennett PH, Knowler WC. Congenital susceptibility to NIDDM. Role of intrauterine environment. Diabetes. 1988;37(5):622–8.
49. Dabelea D, Hanson RL, Bennett PH, Roumain J, Knowler WC, Pettitt DJ. Increasing prevalence of type II diabetes in American Indian children. Diabetologia. 1998;41(8):904–10.
50. Franks PW, Looker HC, Kobes S, Touger L, Tataranni PA, Hanson RL, et al. Gestational glucose tolerance and risk of type 2 diabetes in young Pima Indian offspring. Diabetes. 2006;55(2):460–5.
51. Jeffery AN, Metcalf BS, Hosking J, Murphy MJ, Voss LD, Wilkin TJ. Little evidence for early programming of weight and insulin resistance for contemporary children: EarlyBird Diabetes Study report 19. Pediatrics. 2006;118(3):1118–23.
52. Pettitt DJ, Knowler WC. Diabetes and obesity in the Pima Indians: a cross-generational vicious cycle. J Obes Weight Regul. 1988;7:61–5.
53. Ferrara A, Kahn HS, Quesenberry CP, Riley C, Hedderson MM. An increase in the incidence of gestational diabetes mellitus: Northern California, 1991–2000. Obstet Gynecol. 2004;103(3):526–33.
54. Dabelea D, Snell-Bergeon JK, Hartsfield CL, Bischoff KJ, Hamman RF, McDuffie RS. Increasing prevalence of gestational diabetes mellitus (GDM) over time and by birth cohort: Kaiser Permanente of Colorado GDM Screening Program. Diabetes Care. 2005;28(3):579–84.
55. Gauguier D, Nelson I, Bernard C, Parent V, Marsac C, Cohen D, et al. Higher maternal than paternal inheritance of diabetes in GK rats. Diabetes. 1994;43(2):220–4.
56. Gauguier D, Bihoreau MT, Ktorza A, Berthault MF, Picon L. Inheritance of diabetes mellitus as consequence of gestational hyperglycemia in rats. Diabetes. 1990;39(6):734–9.
57. Pildes RS, Hart RJ, Warrner R, Cornblath M. Plasma insulin response during oral glucose tolerance tests in newborns of normal and gestational diabetic mothers. Pediatrics. 1969;44(1):76–83.
58. Van Assche FA, Gepts W. The cytological composition of the foetal endocrine pancreas in normal and pathological conditions. Diabetologia. 1971;7(6):434–44.
59. Heding LG, Persson B, Stangenberg M. B-cell function in newborn infants of diabetic mothers. Diabetologia. 1980;19(5):427–32.
60. Gautier JF, Wilson C, Weyer C, Mott D, Knowler WC, Cavaghan M, et al. Low acute insulin secretory responses in adult offspring of people with early onset type 2 diabetes. Diabetes. 2001;50(8):1828–33.
61. Harder T, Kohlhoff R, Dorner G, Rohde W, Plagemann A. Perinatal 'programming' of insulin resistance in childhood: critical impact of neonatal insulin and low birth weight in a risk population. Diabet Med. 2001;18(8):634–9.
62. Catalano PM, Kirwan JP, Haugel-de Mouzon S, King J. Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. J Nutr. 2003;133(5 Suppl 2):1674s–83s.
63. Weiss PA, Scholz HS, Haas J, Tamussino KF, Seissler J, Borkenstein MH. Long-term follow-up of infants of mothers with type 1 diabetes: evidence for hereditary and nonhereditary transmission of diabetes and precursors. Diabetes Care. 2000;23(7):905–11.
64. Silverman BL, Metzger BE, Cho NH, Loeb CA. Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. Diabetes Care. 1995;18(5):611–7.

65. Innes KE, Byers TE, Marshall JA, Baron A, Orleans M, Hamman RF. Association of a woman's own birth weight with subsequent risk for gestational diabetes. JAMA. 2002;287(19):2534–41.
66. Metzger BE, Silverman BL, Freinkel N, Dooley SL, Ogata ES, Green OC. Amniotic fluid insulin concentration as a predictor of obesity. Arch Dis Child. 1990;65(10 Spec No):1050–2.
67. Koistinen HA, Koivisto VA, Andersson S, Karonen SL, Kontula K, Oksanen L, et al. Leptin concentration in cord blood correlates with intrauterine growth. J Clin Endocrinol Metab. 1997;82(10):3328–30.
68. Kaar JL, Brinton JT, Crume T, Hamman RF, Glueck DH, Dabelea D. Leptin levels at birth and infant growth: the EPOCH study. J Dev Orig Health Dis. 2014;5(3):214–8.
69. Persson B, Westgren M, Celsi G, Nord E, Ortqvist E. Leptin concentrations in cord blood in normal newborn infants and offspring of diabetic mothers. Horm Metab Res. 1999;31(8):467–71.
70. Hieronimus S, Bastard S, Gillet JY, Giudicelli J, Brucker-Davis F, Berthier F, et al. Significance of cord-blood leptin in newborns of diabetic mothers. Diabetes Care. 2002;25(10):1886–7.
71. Simmons D, Breier BH. Fetal overnutrition in polynesian pregnancies and in gestational diabetes may lead to dysregulation of the adipoinsular axis in offspring. Diabetes Care. 2002;25(9):1539–44.
72. Wolf HJ, Ebenbichler CF, Huter O, Bodner J, Lechleitner M, Foger B, et al. Fetal leptin and insulin levels only correlate in large-for-gestational age infants. Eur J Endocrinol. 2000;142(6):623–9.
73. Barker DJ. The intrauterine origins of cardiovascular disease. Acta Paediatr Suppl. 1993;82(Suppl 391):93–9; discussion 100.
74. Manderson JG, Mullan B, Patterson CC, Hadden DR, Traub AI, McCance DR. Cardiovascular and metabolic abnormalities in the offspring of diabetic pregnancy. Diabetologia. 2002;45(7):991–6.
75. Holemans K, Gerber RT, Meurrens K, De Clerck F, Poston L, Van Assche FA. Streptozotocin diabetes in the pregnant rat induces cardiovascular dysfunction in adult offspring. Diabetologia. 1999;42(1):81–9.
76. Bunt JC, Tataranni PA, Salbe AD. Intrauterine exposure to diabetes is a determinant of hemoglobin A(1)c and systolic blood pressure in pima Indian children. J Clin Endocrinol Metab. 2005;90(6):3225–9.
77. Rolfe Ede L, Loos RJ, Druet C, Stolk RP, Ekelund U, Griffin SJ, et al. Association between birth weight and visceral fat in adults. Am J Clin Nutr. 2010;92(2):347–52.
78. Phipps K, Barker DJ, Hales CN, Fall CH, Osmond C, Clark PM. Fetal growth and impaired glucose tolerance in men and women. Diabetologia. 1993;36(3):225–8.
79. Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP. Birthweight and adult health outcomes in a biethnic population in the USA. Diabetologia. 1994;37(6):624–31.
80. McCarthy M. Weighing in on diabetes risk. Nat Genet. 1998;19(3):209–10.
81. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to famine. Lancet. 1998;351(9097):173–7.
82. Montgomery SM, Ekbom A. Smoking during pregnancy and diabetes mellitus in a British longitudinal birth cohort. BMJ. 2002;324(7328):26–7.
83. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967–71.
84. Harding JE. The nutritional basis of the fetal origins of adult disease. Int J Epidemiol. 2001;30(1):15–23.
85. Hales CN, Ozanne SE. For debate: fetal and early postnatal growth restriction lead to diabetes, the metabolic syndrome and renal failure. Diabetologia. 2003;46(7):1013–9.
86. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Size at birth, childhood growth and obesity in adult life. Int J Obes Relat Metab Disord. 2001;25(5):735–40.
87. Dabelea D, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC. Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. Diabetes Care. 1999;22(6):944–50.
88. Parsons TJ, Power C, Logan S, Summerbell CD. Childhood predictors of adult obesity: a systematic review. Int J Obes Relat Metab Disord. 1999;23(Suppl 8):S1–107.
89. Law CM, Gordon GS, Shiell AW, Barker DJ, Hales CN. Thinness at birth and glucose tolerance in seven-year-old children. Diabet Med. 1995;12(1):24–9.
90. Bavdekar A, Yajnik CS, Fall CH, Bapat S, Pandit AN, Deshpande V, et al. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? Diabetes. 1999;48(12):2422–9.
91. Yajnik CS, Fall CH, Vaidya U, Pandit AN, Bavdekar A, Bhat DS, et al. Fetal growth and glucose and insulin metabolism in four-year-old Indian children. Diabet Med. 1995;12(4):330–6.
92. Whincup PH, Cook DG, Adshead F, Taylor SJ, Walker M, Papacosta O, et al. Childhood size is more strongly related than size at birth to glucose and insulin levels in 10–11-year-old children. Diabetologia. 1997;40(3):319–26.
93. Murtaugh MA, Jacobs DR Jr, Moran A, Steinberger J, Sinaiko AR. Relation of birth weight to fasting insulin, insulin resistance, and body size in adolescence. Diabetes Care. 2003;26(1):187–92.
94. Li C, Johnson MS, Goran MI. Effects of low birth weight on insulin resistance syndrome in caucasian and African-American children. Diabetes Care. 2001;24(12):2035–42.
95. Fewtrell MS, Doherty C, Cole TJ, Stafford M, Hales CN, Lucas A. Effects of size at birth, gestational age and early growth in preterm infants on glucose and insulin concentrations at 9–12 years. Diabetologia. 2000;43(6):714–7.

96. Ong KK, Petry CJ, Emmett PM, Sandhu MS, Kiess W, Hales CN, et al. Insulin sensitivity and secretion in normal children related to size at birth, postnatal growth, and plasma insulin-like growth factor-I levels. Diabetologia. 2004;47(6):1064–70.
97. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens. 2000;18(7):815–31.
98. Taylor SJ, Whincup PH, Cook DG, Papacosta O, Walker M. Size at birth and blood pressure: cross sectional study in 8–11 year old children. BMJ. 1997;314(7079):475–80.
99. Laor A, Stevenson DK, Shemer J, Gale R, Seidman DS. Size at birth, maternal nutritional status in pregnancy, and blood pressure at age 17: population based analysis. BMJ. 1997;315(7106):449–53.
100. Donker GA, Labarthe DR, Harrist RB, Selwyn BJ, Wattigney W, Berenson GS. Low birth weight and blood pressure at age 7–11 years in a biracial sample. Am J Epidemiol. 1997;145(5):387–97.
101. Whincup PH, Bredow M, Payne F, Sadler S, Golding J. Size at birth and blood pressure at 3 years of age. The Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC). Am J Epidemiol. 1999;149(8):730–9.
102. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001;86(5):1930–5.
103. Guo SS, Roche AF, Chumlea WC, Gardner JD, Siervogel RM. The predictive value of childhood body mass index values for overweight at age 35 y. Am J Clin Nutr. 1994;59(4):810–9.
104. Phillips DI. Insulin resistance as a programmed response to fetal undernutrition. Diabetologia. 1996;39(9):1119–22.
105. Vatten LJ, Nilsen ST, Odegard RA, Romundstad PR, Austgulen R. Insulin-like growth factor I and leptin in umbilical cord plasma and infant birth size at term. Pediatrics. 2002;109(6):1131–5.
106. Langford K, Blum W, Nicolaides K, Jones J, McGregor A, Miell J. The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by undernutrition? Eur J Clin Invest. 1994;24(12):851–6.
107. Ounsted M, Sleigh G. The infant's self-regulation of food intake and weight gain. Difference in metabolic balance after growth constraint or acceleration in utero. Lancet. 1975;1(7922):1393–7.
108. Ong KK, Ahmed ML, Sherriff A, Woods KA, Watts A, Golding J, et al. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. J Clin Endocrinol Metab. 1999;84(3):1145–8.

7 Early Childhood Contributions to Insulin Resistance

David B. Dunger, Burak Salgin, and Ken K. Ong

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 3 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.

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

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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 2 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 firstborn babies appears to be restrained, as they are smaller at birth, and then show rapid postnatal catchup weight gain [32]. In these firstborns, birth weight correlations with maternal and grandmaternal 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 firstborn 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 catchup weight gain (they crossed centiles upwards over the first 6–12 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 "catchup" 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].

Catchup 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]. Catchup in height also occurs in these infants, but this is generally completed by the age of 6–12 months, and growth then continues along a centile appropriate for midparental height [42]. In contrast, the rapid weight gain may continue and, in the ALSPAC cohort, the early catchup 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, catchup 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 catchup 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 United States 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 catchup 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, 1988–1994 [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 2–4 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

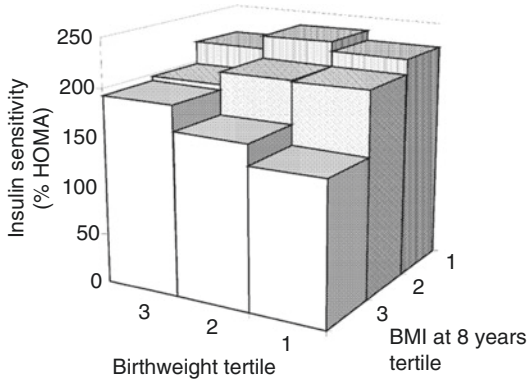

Fig. 7.1 Fasting insulin sensitivity (HOMA) at 8 years of age by tertiles of birth weight and current BMI. There was interaction between birth weight and current BMI on insulin sensitivity at 8 years (p-interaction < 0.05), such that lower birth weight was related to lower insulin sensitivity only among children with the highest BMI at age 8 years (front row, p-trend = 0.006). (Reproduced with permission from Ong et al. [10])

et al. observed that catchup 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. 7.1).

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 catchup weight gain, these SGA infants 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 postoral 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 catchup 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 has depended on surrogate end points. Nevertheless, there are data to indicate that low birth weight followed by postnatal catchup 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

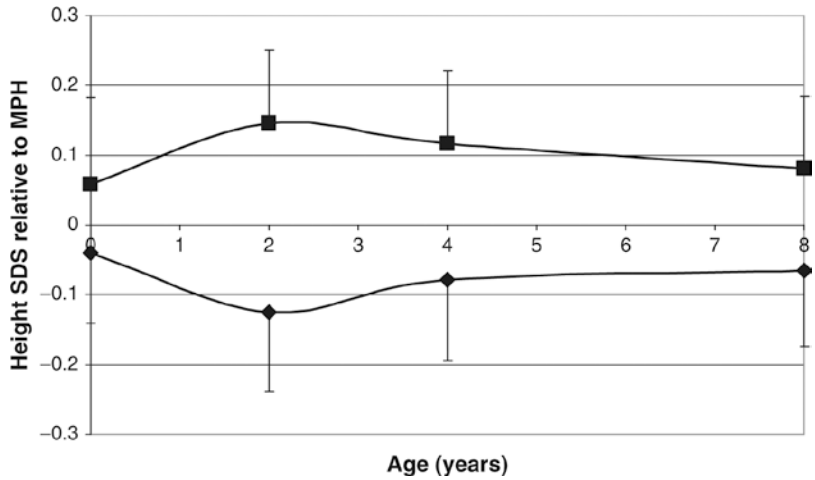

Fig. 7.2 Height SDS from birth to age 8 years by extreme tertiles of insulin secretion adjusted for insulin sensitivity (disposition index). Data are mean ±1 SE. □ = lowest tertile; ■ = highest tertile. Repeated measures analysis showed significant differences in height SDS over time ($p = 0.03$)

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 minutes, 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, midparental 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. 7.2) and reduced levels of IGF-I between ages 6 months and 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, catchup 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, 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.

References

1. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet. 1989;2(8663):577–80.
2. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35(7):595–601.
3. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ. 1991;303(6809):1019–22.
4. Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease-the hypothesis revisited. BMJ. 1999;319(7204):245–9.
5. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967–71.
6. Bavdekar A, Yajnik CS, Fall CH, Bapat S, Pandit AN, Deshpande V, et al. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? Diabetes. 1999;48(12):2422–99.
7. Crowther NJ, Cameron N, Trusler J, Gray IP. Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. Diabetologia. 1998;41(10):1163–7.
8. Whincup PH, Cook DG, Adshead F, Taylor SJ, Walker M, Papacosta O, et al. Childhood size is more strongly related than size at birth to glucose and insulin levels in 10–11-year-old children. Diabetologia. 1997;40(3):319–26.
9. Yajnik CS, Fall CH, Vaidya U, Pandit AN, Bavdekar A, Bhat DS, et al. Fetal growth and glucose and insulin metabolism in four-year-old Indian children. Diabet Med. 1995;12(4):330–6.
10. Ong KK, Petry CJ, Emmett PM, Sandhu MS, Kiess W, Hales CN, et al. Insulin sensitivity and secretion in normal children related to size at birth, postnatal growth, and plasma insulin-like growth factor-I levels. Diabetologia. 2004;47(6):1064–70.
11. Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002;31(6):1235–9.
12. Lawlor DA, Ronalds G, Clark H, Smith GD, Leon DA. Birth weight is inversely associated with incident coronary heart disease and stroke among individuals born in the 1950s: findings from the Aberdeen Children of the 1950s prospective cohort study. Circulation. 2005;112(10):1414–8.
13. Leon DA, Lithell HO, Vâgerö D, Koupilová I, Mohsen R, Berglund L, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915–1929. BMJ. 1998;317(7153):241–5.
14. Rich-Edwards JW, Kleinman K, Michels KB, Stampfer MJ, Manson JE, Rexrode KM, et al. Longitudinal study of birth weight and adult body mass index in predicting risk of coronary heart disease and stroke in women. BMJ. 2005;330(7500):1115.
15. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. BMJ. 1990;301(6746):259–62.
16. Curhan GC, Chertow GM, Willett WC, Spiegelman D, Colditz GA, Manson JE, et al. Birth weight and adult hypertension and obesity in women. Circulation. 1996;94(6):1310–5.
17. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation. 1996;94(12):3246–50.
18. Lundgren EM, Cnattingius S, Jonsson B, Tuvemo T. Intellectual and psychological performance in males born small for gestational age with and without catch-up growth. Pediatr Res. 2001;50(1):91–6.
19. Martyn CN, Barker DJ, Osmond C. Mothers' pelvic size, fetal growth, and death from stroke and coronary heart disease in men in the UK. Lancet. 1996;348(9037):1264–8.
20. Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Gillman MW, Hennekens CH, et al. Birthweight and the risk for type 2 diabetes mellitus in adult women. Ann Intern Med. 1999;130(4 Pt 1):278–84.

21. Zhao M, Shu XO, Jin F, Yang G, Li HL, Liu DK, et al. Birthweight, childhood growth and hypertension in adulthood. Int J Epidemiol. 2002;31(5):1043–51.
22. Lindsay RS, Dabelea D, Roumain J, Hanson RL, Bennett PH, Knowler WC. Type 2 diabetes and low birth weight: the role of paternal inheritance in the association of low birth weight and diabetes. Diabetes. 2000;49(3):445–9.
23. McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? BMJ. 1994;308(6934):942–5.
24. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. Diabetes. 2000;49(12):2208–11.
25. Eriksson JG, Forsen TJ, Osmond C, Barker DJ. Pathways of infant and childhood growth that lead to type 2 diabetes. Diabetes Care. 2003;26(11):3006–10.
26. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med. 1976;295(7):349–53.
27. Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C. Intrauterine programming of adult body composition. J Clin Endocrinol Metab. 2001;86(1):267–72.
28. McKeigue PM, Lithell HO, Leon DA. Glucose tolerance and resistance to insulin-stimulated glucose uptake in men aged 70 years in relation to size at birth. Diabetologia. 1998;41(10):1133–8.
29. Bundred P, Kitchiner D, Buchan I. Prevalence of overweight and obese children between 1989 and 1998: population based series of cross sectional studies. BMJ. 2001;322(7282):326–8.
30. Reilly JJ, Dorosty AR, Emmett PM. Prevalence of overweight and obesity in British children: cohort study. BMJ. 1999;319(7216):1039.
31. Widdowson EM, McCance RA. A review: new thoughts on growth. Pediatr Res. 1975;9(3):154–6.
32. Ong KK, Preece MA, Emmett PM, Ahmed ML, Dunger DB. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: longitudinal birth cohort study and analysis. Pediatr Res. 2002;52(6):863–7.
33. Ounsted M, Scott A, Moar VA. Constrained and unconstrained fetal growth: associations with some biological and pathological factors. Ann Hum Biol. 1988;15(2):119–29.
34. Ounsted M, Scott A, Ounsted C. Transmission through the female line of a mechanism constraining human fetal growth. Ann Hum Biol. 1986;13(2):143–51.
35. Casteels K, Ong K, Phillips D, Bendall H, Pembrey M. Mitochondrial 16189 variant, thinness at birth, and type-2 diabetes. ALSPAC study team. Avon longitudinal study of pregnancy and childhood. Lancet. 1999;353(9163):1499–500.
36. Petry CJ, Ong KK, Barratt BJ, Wingate D, Cordell HJ, Ring SM, et al. Common polymorphism in H19 associated with birth-weight and cord blood IGF-II levels in humans. BMC Genet. 2005;6(1):22.
37. Engel N, West AG, Felsenfeld G, Bartolomei MS. Antagonism between DNA hypermethylation and enhancer-blocking activity at the H19 DMD is uncovered by CpG mutations. Nat Genet. 2004;36(8):883–8.
38. Seghieri G, Anichini R, Bellis A, De Alviggi L, Franconi F, Breschi MC. Relationship between gestational diabetes mellitus and low maternal birth weight. Diabetes Care. 2002;25(10):1761–5.
39. Gohlke BC, Huber A, Hecher K, Fimmers R, Bartmann P, Roth CL. Fetal insulin-like growth factor (IGF)-I, IGF-II, and ghrelin in association with birth weight and postnatal growth in monozygotic twins with discordant growth. J Clin Endocrinol Metab. 2005;90(4):2270–4.
40. Ong KK, Ahmed ML, Sherriff A, Woods KA, Watts A, Golding J, et al. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC Study Team. Avon longitudinal study of pregnancy and childhood. J Clin Endocrinol Metab. 1999;84(3):1145–8.
41. Ong KK, Emmett PM, Noble S, Ness A, Dunger DB. Dietary energy intake at the age of 4 months predicts postnatal weight gain and childhood body mass index. Pediatrics. 2006;117(3):e503–8.
42. Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, et al. Association of the INS VNTR with size at birth. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Nat Genet. 1998;19(1):98–100.
43. Stettler N, Bovet P, Shamlaye H, Zemel BS, Stallings VA, Paccaud F. Prevalence and risk factors for overweight and obesity in children from Seychelles, a country in rapid transition: the importance of early growth. Int J Obes Relat Metab Disord. 2002;26(2):214–9.
44. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics. 2002;109(2):194–9.
45. Ekelund U, Ong K, Linné Y, Neovius M, Brage S, Dunger DB, et al. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). Am J Clin Nutr. 2006;83(2):324–30.
46. Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. J Epidemiol Community Health. 1992;46(3):184–6.
47. Loos RJ, Beunen G, Fagard R, Derom C, Vlietinck R. Birth weight and body composition in young adult men – a prospective twin study. Int J Obes Relat Metab Disord. 2001;25(10):1537–45.
48. Loos RJ, Beunen G, Fagard R, Derom C, Vlietinck R. Birth weight and body composition in young women: a prospective twin study. Am J Clin Nutr. 2002;75(4):676–82.
49. Yajnik C. Interactions of perturbations in intrauterine growth and growth during childhood on

the risk of adult-onset disease. Proc Nutr Soc. 2000;59(2):257–65.
50. Hediger ML, Overpeck MD, Maurer KR, Kuczmarski RJ, McGlynn A, Davis WW. Growth of infants and young children born small or large for gestational age: findings from the Third National Health and Nutrition Examination Survey. Arch Pediatr Adolesc Med. 1998;152(12):1225–31.
51. Garnett SP, Cowell CT, Baur LA, Fay RA, Lee J, Coakley J, et al. Abdominal fat and birth size in healthy prepubertal children. Int J Obes Relat Metab Disord. 2001;25(11):1667–73.
52. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet. 2001;357(9254):413–9.
53. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet. 2003;361(9363):1089–97.
54. Ibanez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91(6):2153–8.
55. Bazaes RA, Salazar TE, Pittaluga E, Peña V, Alegría A, Iñiguez G, et al. Glucose and lipid metabolism in small for gestational age infants at 48 hours of age. Pediatrics. 2003;111(4 Pt 1):804–9.
56. Hales CN, Desai M, Ozanne SE. The thrifty phenotype hypothesis: how does it look after 5 years? Diabet Med. 1997;14(3):189–95.
57. Mericq V, Ong KK, Bazaes R, Peña V, Avila A, Salazar T, et al. Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. Diabetologia. 2005;48(12):2609–14.
58. Soto N, Bazaes RA, Peña V, Salazar T, Avila A, Iñiguez G, et al. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. J Clin Endocrinol Metab. 2003;88(8):3645–50.
59. Leger J, Levy-Marchal C, Bloch J, Pinet A, Chevenne D, Porquet D, et al. Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. BMJ. 1997;315(7104):341–7.
60. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C. Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab. 2000;85(4):1401–6.
61. Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, Robinson EM, et al. Premature birth and later insulin resistance. N Engl J Med. 2004;351(21):2179–86.
62. Hovi P, Andersson S, Eriksson JG, Järvenpää AL, Strang-Karlsson S, Mäkitie O, et al. Glucose regulation in young adults with very low birth weight. N Engl J Med. 2007;356(20):2053–63.
63. Davies AA, Smith GD, Ben-Shlomo Y, Litchfield P. Low birth weight is associated with higher adult total cholesterol concentration in men: findings from an occupational cohort of 25,843 employees. Circulation. 2004;110(10):1258–62.
64. Fagerberg B, Bondjers L, Nilsson P. Low birth weight in combination with catch-up growth predicts the occurrence of the metabolic syndrome in men at late middle age: the Atherosclerosis and Insulin Resistance study. J Intern Med. 2004;256(3):254–9.
65. Ibanez L, Ferrer A, Ong K, Amin R, Dunger D de Zegher F. Insulin sensitization early after menarche prevents progression from precocious pubarche to polycystic ovary syndrome. J Pediatr. 2004;144(1):23–9.
66. Leeson CP, Kattenhorn M, Morley R, Lucas A, Deanfield JE. Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. Circulation. 2001;103(9):1264–8.
67. Liese AD, Mayer-Davis EJ, Haffner SM. Development of the multiple metabolic syndrome: an epidemiologic perspective. Epidemiol Rev. 1998;20(2):157–72.
68. Stumvoll M, Tataranni PA, Bogardus C. The hyperbolic law – a 25-year perspective. Diabetologia. 2005;48(2):207–9.
69. Phillips DI, Clark PM, Hales CN, Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med. 1994;11(3):286–92.
70. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
71. Iñiguez G, Ong K, Bazaes R, Avila A, Salazar T, Dunger D, et al. Longitudinal changes in insulin-like growth factor-I, insulin sensitivity, and secretion from birth to age three years in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91(11):4645–9.
72. Low LC, Tam SY, Kwan EY, Tsang AM, Karlberg J. Onset of significant GH dependence of serum IGF-I and IGF-binding protein 3 concentrations in early life. Pediatr Res. 2001;50(6):737–42.
73. Silbergeld A, Lazar L, Erster B, Keret R, Tepper R, Laron Z. Serum growth hormone binding protein activity in healthy neonates, children and young adults: correlation with age, height and weight. Clin Endocrinol (Oxf). 1989;31(3):295–303.
74. Williams DR, Wareham NJ, Brown DC, Byrne CD, Clark PM, Cox BD, et al. Undiagnosed glucose intolerance in the community: the Isle of Ely Diabetes Project. Diabet Med. 1995;12(1):30–5.
75. Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ. Circulating concentra-

tions of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. Lancet. 2002;359(9319):1740–5.
76. Consultation WE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363(9403):157–63.
77. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. Lancet. 1991;337(8738):382–6.
78. Godfrey KM, Barker DJ, Robinson S, Osmond C. Maternal birthweight and diet in pregnancy in relation to the infant's thinness at birth. Br J Obstet Gynaecol. 1997;104(6):663–7.
79. Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. BMJ. 1999;319(7206):339–43.
80. Rogers I, Emmett P, Baker D, Golding J. Financial difficulties, smoking habits, composition of the diet and birthweight in a population of pregnant women in the South West of England. ALSPAC Study Team. Avon longitudinal study of pregnancy and childhood. Eur J Clin Nutr. 1998;52(4):251–60.
81. Hesse V, Voigt M, Sälzler A, Steinberg S, Friese K, Keller E, et al. Alterations in height, weight, and body mass index of newborns, children, and young adults in eastern Germany after German reunification. J Pediatr. 2003;142(3):259–62.
82. Franks PW, Looker HC, Kobes S, Touger L, Tataranni PA, Hanson RL, et al. Gestational glucose tolerance and risk of type 2 diabetes in young Pima Indian offspring. Diabetes. 2006;55(2):460–5.
83. Haig D. Altercation of generations: genetic conflicts of pregnancy. Am J Reprod Immunol. 1996;35(3):226–32.
84. Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet. 1962;14:353–62.

Insulin Resistance in Puberty

8

Jennifer E. Sprague, Rachelle Gandica, and Megan Moriarty Kelsey

Introduction

Puberty is a critical period of physiologic and neurobehavioral development, hallmarked by the transition to full reproductive potential. During this period, there is also a physiologic decrease in insulin sensitivity that recovers by early adulthood. This chapter will explore what is known about this pubertal change in insulin sensitivity and its potential impact on future health and disease.

Introduction to Puberty

Puberty Stages, Ages of Puberty

Puberty represents a period of increased linear growth, development of secondary sex characteristics, and achievement of reproductive capacity. Pubertal development is commonly described using the stages of Tanner and Marshall who characterized sexual maturation in white, institutionalized, British children in the 1950s [1, 2]. For both sexes, development progresses from prepubertal stage 1 (Tanner 1 [T1]) to adult stage 5 (T5). The onset of puberty at T2 is identified by breast bud development (thelarche) in girls, typically between ages 8 and 13 years, with progression to menarche over 2–3 years [3–5]. In boys, genital and testicular enlargement is the first sign of puberty, typically between 9.5 and 14 years of age, with progression to adult development over the course of 3–5 years [6–8]. Development of pubic hair (pubarche) is similarly staged but should not be used to assess pubertal initiation, as it develops in response to increasing adrenal androgen secretion (adrenarche) [9].

It is well accepted that undernutrition results in growth and pubertal delay and that improving general nutrition has resulted in earlier pubertal onset in the mid-twentieth century compared to the nineteenth century [10, 11]. Recent studies support a continued trend toward earlier pubertal onset and menarche in girls over the last several decades. Data from the National Health and Nutrition Examination Survey III (NHANES III, 1988–1994) demonstrate mean onset of breast development of 10.3 years in non-Hispanic white girls [12], while data from the Pediatric Research in Office Settings study (PROS, 1992–1993), which was specifically designed to assess

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pubertal development, suggests a slightly earlier onset of T2 breast development at 9.9 years [3]. The mean age for menarche in non-Hispanic white girls in NHANES was 12.6 years. Studies also demonstrate earlier onset of breast development, pubic hair, and menarche (by approximately 6 months) in non-Hispanic black and Hispanic girls [3, 12]. This trend may, in part, be explained by the increasing prevalence of obesity, as higher body mass index (BMI) is thought to lead to earlier onset of puberty in girls [13].

Trends toward earlier puberty are not as well established in boys. In NHANES III, pubertal onset occurred at a mean age of 10.1 years for white, 9.5 years for black, and 10.4 years for Latino boys [8]. PROS (2005–2010) reported the average age of T2 genital development as 9.9 years for non-Hispanic white, 9.7 for non-Hispanic black, and 9.6 years for Hispanic boys [14]. Similar to the study in girls, the PROS study included younger boys than in other studies. At age 7, 15% of non-Hispanic white, 17% of non-Hispanic black, and 3% of Hispanic boys were reported to have T2 genital development.

Normal Growth Patterns/Growth Hormone During Puberty

Prepubertal children grow at an average of 5–6 cm/year. During puberty, height velocity increases, such that girls achieve peak height velocity in early puberty, though boys do not reach peak height velocity until later [15, 16]. Linear growth occurs in response to growth hormone and its downstream effector, insulin-like growth factor 1 (IGF-1). Growth hormone secretion is pulsatile, with the highest amplitude pulses occurring in the early night in prepubertal children. Pulse amplitude increases with onset of puberty and peaks at T3 breast development in girls and at T4 in boys, coinciding with the pubertal growth spurt in each sex [17, 18]. IGF-1 levels increase during puberty in both sexes with peak levels at T3 in girls and T4 in boys [19]. Sex steroids increase growth hormone secretion and promote longitudinal bone growth, while estradiol is responsible for closure of the epiphyseal growth plates [20, 21].

Reproductive Hormone Changes

During childhood, the hypothalamic-pituitary-gonadal (HPG) axis is actively inhibited. The onset of puberty is marked by increasing pulsatile gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus, resulting in increased pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary [22]. Initially LH pulses occur primarily during sleep, with clear diurnal rhythm [23, 24]. LH acts on the ovarian theca cells in girls and testicular Leydig cells in boys, resulting in production of androstenedione and testosterone. FSH stimulates aromatase in ovarian granulosa cells, leading to conversion of androgens to estradiol. In the testes, FSH stimulates gonadal growth and gametogenesis. Peak estradiol levels occur midday in girls, but peak testosterone levels are found in the early morning in boys. As puberty progresses, pulsatile LH secretion expands into the day, such that diurnal variation is lost in girls after menarche. With continued maturation, girls begin to have regular menstrual cycles. During the follicular phase, estradiol concentrations rise until reaching a threshold for positive feedback, resulting in the mid-cycle LH surge and ovulation [25]. Estradiol and progesterone levels are high during the luteal phase but fall with involution of the corpus luteum, resulting in endometrial sloughing and menstrual flow.

As recognized since the 1970s, nutritional factors influence pubertal onset and normal function of the HPG axis, with need for girls to maintain a threshold weight for maintenance of regular menstrual cycles [26, 27]. Leptin, which is secreted by adipocytes, acts as a measure of energy (fat) reserve, increases with increasing adiposity [28], and permits HPG activation. Mutations in leptin or the leptin receptor result in pubertal delay due to hypogonadotropic hypogonadism and treatment of patients with leptin deficiency and hypogonadotropic hypogonadism results in development of secondary sex characteristics and onset of menses [29–31].

Changes in Fat Distribution During Puberty

Body composition is similar in boys and girls at birth and fat increases during the first year of life [32]. During childhood, sexual dimorphism in body fat begins to emerge, with boys maintaining a stable percent body fat while girls increase theirs [33, 34]. With the onset of puberty, body composition and fat distribution further diverge; both sexes gain lean body mass but this is more pronounced in boys, who gain more muscle mass in the upper body [35]. However, girls gain more fat mass, resulting in higher percent body fat compared to boys. Fat accumulation in girls is largely subcutaneous and results in decreasing waist-to-hip ratios and gynecoid body shape [36]. By adulthood, men have greater intra-abdominal visceral fat than women [37].

Patterns of Change in Insulin Sensitivity During Puberty

Timing

A pubertal state of insulin resistance was first reported in 1986 in a cross-sectional study exploring reasons for increased insulin requirements during adolescence in youth with type 1 diabetes [38]. In this study, 16 normal weight youth without diabetes who were prepubertal (T1) or pubertal (T2–5) were studied by high-dose hyperinsulinemic-euglycemic clamp, and those in puberty were found to have 36% lower insulin sensitivity than prepubertal youth (Fig. 8.1). An adult comparison group from a previous study had insulin sensitivity similar to prepubertal youth. Since then, multiple cross-sectional [39–46] and a small number of longitudinal studies [47–50] have confirmed a transient reduction in insulin sensitivity in normal-weight youth without diabetes during puberty. The largest study to date involved 357 youth (80% non-Hispanic white, 20% non-Hispanic black) across stages of puberty who had measurement of insulin sensitivity by hyperinsulinemic-euglycemic clamp [42]. Results suggest a nadir in insulin sensitivity in mid-puberty and improvement in insulin sensitivity by T5. This trajectory of insulin sensitivity was confirmed by a relatively large (n = 92) semi-longitudinal study of non-Hispanic black (n = 46) and non-Hispanic white (n = 46) US youth using intravenous glucose tolerance testing (IVGTT), in which insulin sensitivity reached a nadir in T3 and then increased again in both T4 and T5 [47]. Of note, while the mean body mass index in this study was in the normal weight range, the study

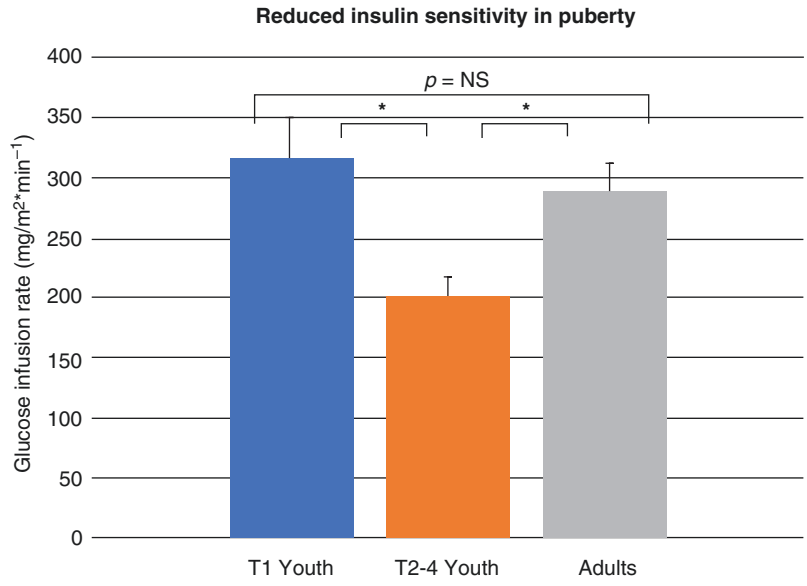

Fig. 8.1 Results from the original Amiel et al. study demonstrating reduced insulin sensitivity, measured by hyperinsulinemic-euglycemic clamp, in normal-weight youth during puberty compared to prepubertal youth and normal-weight adults ($*p < 0.01$ [38])

did include obese participants. However, the effect of puberty remained even after adjustment for body fat. Only one study using a rigorous method for estimation of insulin sensitivity (IVGTT) has failed to find an effect of puberty on insulin sensitivity [51]. Little is known about when the full recovery to adult insulin sensitivity occurs; however, follow-up of the large study from Minnesota suggests that insulin sensitivity may not fully recover until several years after puberty has completed [52]. This finding was confirmed by the EarlyBird study, a large longitudinal cohort study of puberty in the United Kingdom [50]. Though EarlyBird uses a less rigorous estimate of insulin sensitivity (homeostatic model assessment IR [HOMA-IR]), it includes data from 270 youth who were followed from age 9 until 16 years and suggests that insulin sensitivity does not yet recover by 16 years, despite the fact that the majority of girls and a proportion of boys have reached final height by that age.

Sex Differences

Multiple studies suggest that insulin sensitivity is lower in girls than in boys during adolescence; however, the data are inconsistent, and other studies have found no sex differences. Results from the EarlyBird study suggest that insulin sensitivity begins to decline in both sexes prior to puberty but that girls have lower insulin sensitivity even in the early prepubertal years [53]. Longitudinal data from EarlyBird and the Minnesota study [54] suggest that the sex differences in insulin sensitivity may reverse later in adolescence, with boys showing a continuing decline in insulin sensitivity and girls starting to recover. These sex differences persist even after adjustment for sex differences in pubertal timing. Little is known about underlying reasons for these sex differences, although it is known that girls accumulate more fat mass and less lean mass during puberty and that physical activity declines in girls more than in boys during puberty (discussed later). There is also a possible role for sex steroids, but this has not yet been studied in relation to puberty.

Racial and Ethnic Differences

Little is known about racial and ethnic variation in insulin sensitivity during puberty. Several of the larger cohort studies have included non-Hispanic black youths [42, 47], but Hispanic representation is primarily limited to studies of obese youths only. In studies involving non-Hispanic black youths, it appears that pubertal insulin sensitivity is lower than in non-Hispanic whites, independent of BMI. The SOLAR study included longitudinal follow-up with measurement of insulin sensitivity by IVGTT and suggests that reduced insulin sensitivity in non-Hispanic black youths is compensated for by increased insulin secretion and/or reduced insulin clearance. While studies using hyperinsulinemic-euglycemic clamps confirm lower insulin sensitivity in non-Hispanic black than in non-Hispanic white adolescents [55–57], one small study comparing prepubertal and pubertal non-Hispanic black youths (n = 26) demonstrated that these youths failed to compensate for reduced insulin sensitivity with increased insulin secretion during puberty [57]. However, while on average the youths studied appear to be normal weight, BMI percentiles or standard deviation scores were not included in the results, and it is unclear whether overweight or obesity was an exclusion criterion for the study; the presence of overweight/obese youth could significantly confound the results. Given the significantly increased risk of youth-onset type 2 diabetes in non-Hispanic black adolescents, further studies are needed.

Alterations in Fuel Metabolism During Puberty

Two groups have used sophisticated techniques, including graded-dose hyperinsulinemic-euglycemic clamps, stable isotope tracers, indirect calorimetry, and hyperglycemic clamps, to compare basal and insulin-stimulated fuel metabolism between small numbers of pubertal and prepubertal youth and adults. These studies uniformly demonstrate that non-oxidative glucose disposal is decreased in response to

high-dose insulin during puberty [40, 58, 59] and that skeletal muscle insulin resistance is the strongest contributor to pubertal insulin resistance. Studies are conflicting, however, regarding the effects of puberty on glucose oxidation. It does not appear that hepatic glucose output is altered in the fasted state in pubertal youth when compared with adults [40, 60]. Multiple groups have found that high-dose insulin-stimulated glucose disposal (insulin sensitivity) is inversely correlated with IGF-1 and/or 24-hour growth hormone concentrations during puberty [41, 49, 61, 62], suggesting that peripheral insulin resistance is mediated by the characteristic pubertal increases in growth hormone. These findings fit with the observation that growth hormone increases transiently during puberty and is known to result in decreased insulin sensitivity. In addition, hyperglycemic clamp studies have demonstrated that both first- and second-phase insulin response is increased during puberty; thus, adolescents are exposed to higher concentrations of insulin [49, 61]. Furthermore, Caprio et al. have demonstrated that insulin secretion is directly related to IGF-1 and that free IGF-1 is inversely related to IGF-binding protein 1 (IGFBP1) [61]. Thus, suppression of IGF-BP1 by insulin may further mobilize free IGF-1 to facilitate the rapid growth seen in puberty.

Using stable leucine tracer infusion, hyperinsulinemic-euglycemic clamp, and indirect calorimetry, Caprio et al. also demonstrated that protein turnover is similar in normal-weight adolescents as in adults in both the basal and insulin-stimulated states [58]. However, the overall higher insulin concentrations seen in adolescents favor anabolism.

There are conflicting data regarding lipid metabolism during puberty. Using glycerol tracers, Robinson et al. showed that pubertal adolescents (n = 7, age = 13.1 ± 0.4 years) had similar basal rates of lipolysis when compared to normal-weight adults (n = 9, age = 23.8 ± 1.2), both expressed for whole body and adjusted for fat mass [60]. The same group also demonstrated that suppression of lipolysis, as measured by glycerol turnover, was equally sensitive to insulin in normal-weight pubertal adolescents as in adults [60]. Hannon et al., in a study of 9 youths in prepuberty and during puberty, also found that fat mass-adjusted basal rates of lipolysis were not elevated in pubertal youth, but insulin suppression of lipolysis was not reported in this study [49]. In a cross-sectional study of prepubertal (n = 9, age = 10.6 ± 0.5 years) and pubertal (n = 9, age = 13.8 ± 0.4 years) youth and adults (n = 5, age = 21.9 ± 0.6 years), the same group demonstrated that, although insulin suppression of lipolysis was similar in prepubertal and pubertal youth, insulin failed to suppress lipolysis as well during puberty when compared with adults [40]. There are multiple explanations for the contrasting findings regarding pubertal insulin suppression of lipolysis, including the cross-sectional nature of the studies, small participant numbers, and the lack of correction for fat mass in the latter study. In terms of fat oxidation (as measured by indirect calorimetry), it does not appear to be affected during puberty when compared with prepubertal youth [40], but is higher in pubertal youth than in adults when expressed per kg body weight [40] or per lean body mass [60]. Finally, data suggest that insulin suppression of fat oxidation is impaired in pubertal youth when compared with adults [40, 60]. Arslanian et al. propose that increased fat oxidation is one of the potential mechanisms for pubertal insulin resistance, particularly given that IGF-1 is associated with lipolysis and fat oxidation. However, larger longitudinal studies are needed to fully understand how puberty alters fuel metabolism.

Puberty and Pregnancy: Parallel Conditions?

There are interesting parallels between puberty and pregnancy (see Chap. 5): Both are periods of increased nutrient demand, hormonal change, including increased growth hormone and sex steroids, and enhanced compensatory insulin response to decreases in insulin sensitivity. Both are times of increases in total cholesterol and increased risk for development of diabetes, although the risk for type 2 diabetes during puberty is limited to youth with obesity.

Physiologic studies are challenging to perform during puberty due to the fact that age limits invasiveness of procedures that can be performed, and, while procedures such as clamps, IV glucose tolerance testing, and magnetic resonance imaging (MRI) are acceptable in youth, there are significant challenges to doing studies such as muscle and adipose biopsies, which allow more detailed analysis of metabolic changes at a tissue and cellular level. Moreover, animal models of puberty are lacking due to a lack of a parallel phenotype and due to an extremely shortened adolescence in most animals, in comparison with humans. However, parallels with pregnancy may lead to enhanced understanding of underlying physiology behind insulin resistance in puberty and may direct future study design.

Both puberty and pregnancy are associated with increases in growth hormone. In puberty, growth hormone is secreted by the pituitary and has direct physiologic effects in addition to causing increased IGF-1. In pregnancy, the placenta provides the primary source of increased growth hormone in the form of human placental growth hormone (hPGH). hPGH is similar in structure to human pituitary growth hormone and has been shown to increase insulin resistance in animal models, partly due to disruption of the phosphoinositol-3 kinase pathway in skeletal muscle tissue, leading to decreased translocation of the GLUT-4 receptor to the plasma membrane. A similar mechanism may cause the decreased peripheral insulin sensitivity in puberty, since disruption of the metabolic PI3-kinase insulin signaling pathway during puberty may promote upregulation of the mitogenic insulin signaling pathway through MAP-kinase during a period of rapid physiologic growth (Fig. 8.2a, b). Further studies are needed, either in a parallel primate model or using newer less invasive techniques, to further understand underlying cellular pathways contributing to peripheral pubertal insulin resistance.

As mentioned previously, puberty does not appear to be associated with increased basal lipolysis but may be associated with decreased ability of insulin to suppress lipolysis. Pregnancy is also associated with increased lipolysis and

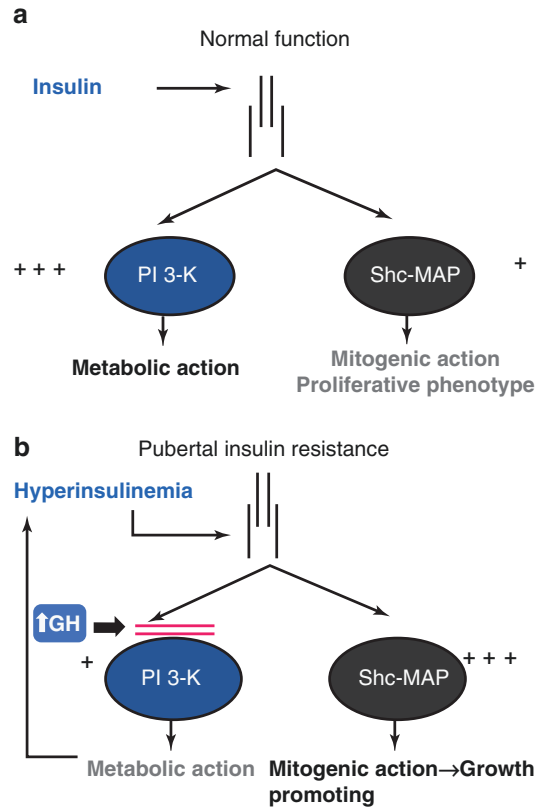

Fig. 8.2 Proposed mechanism and advantage for insulin resistance in puberty. This simplified insulin signaling pathway demonstrates the metabolic actions of insulin though the PI3-kinase pathway, and mitogenic actions through the MAP-kinase pathway (see Chaps. 4 and 11 for detailed pathways). (**a**) In normal physiology, the PI3-kinase signaling pathway is favored, promoting the metabolic actions of insulin. (**b**) During puberty, potentially due to known direct effects of growth hormone (GH) to attenuate PI3-kinase signaling and decreased metabolic effects of insulin. This promotes increased insulin secretion through the mitogenic pathway and, thus, potentiates growth-promoting effects of insulin

decreased insulin suppression of lipolysis. In pregnancy, the increase in lipolysis appears to be due to depletion of glycogen stores, mediated by increases in human placental lactogen. The lack of a parallel hormone of puberty may explain the lack of alteration in basal lipolysis. The increased lipogenesis in pregnancy during the first trimester is thought to be due to mild increases in insulin sensitivity, but parallel transient increases in insulin sensitivity are not seen during early puberty. Therefore, further research

is needed to understand these changes during puberty, particularly given that they may differ in girls and boys.

Alterations in Cardiovascular Metabolic Markers During Puberty

Cardiovascular markers linked to insulin sensitivity have been shown to be affected by puberty. We will review the impact of puberty in both girls and boys on blood pressure, lipids, leptin, and adiponectin.

High blood pressure is a well-known cardiovascular risk factor. Pediatric providers diagnose hypertension using blood pressure percentiles stratified by sex, age, and height. Blood pressure increases in both boys and girls as they progress through childhood, including during puberty [63]. In 2007, a longitudinal study of 507 schoolchildren ages 11–19 years was conducted to evaluate the effect of puberty on several cardiovascular risk factors [52]. Blood pressure was similar in both sexes at the onset of the study (age 11). Systolic blood pressure increased in both girls and boys during adolescence, but at a more accelerated rate in boys. Diastolic blood pressure also increased in both sexes, but there was no sex difference noted [52].

Pubertal effects on serum lipid levels also differ between sexes. These differences were first described in non-Hispanic white boys and girls enrolled in the Princeton Maturation Study in the late 1970s [64]. In this study, boys had reductions in total cholesterol (TC) and low-density lipoprotein (LDL) in Tanner stages 1 through 4, ending with a rise in both at Tanner stage 5. High-density lipoprotein (HDL) dropped in boys during mid-puberty (between Tanner stages 3 and 4). In girls, TC tended to fall during Tanner stages 3 and 4, but then rise at the end of puberty. LDL cholesterol rose between Tanner stages 3 and 4, whereas HDL showed a downward trend throughout puberty [64]. Since sexual development appears to have a greater impact on cholesterol than does chronological age, it is recommended that Tanner staging, not age, be used to assess lipid trends and determine lipid-related cardiovascular risk [65–67]. Table 8.1 shows cholesterol parameter trends in boys and girls in four publications over the past few decades [52, 64, 65, 68]. Unfortunately, most of the results shown are from cross-sectional analyses. Not all studies performed Tanner staging, instead opting to evaluate changes over time by age. HDL has been shown to fall faster in non-Hispanic black youth during puberty compared to their non-Hispanic white peers [52], but more studies are needed to evaluate the race/ethnicity trends of all lipid parameters.

Leptin is a hormone made in adipocytes that regulates appetite and body weight [69] through

Table 8.1 Lipid parameter changes during puberty

Lipid measure	Morrison, 1979 [64]	Porkka, 1994 [68]	Moran, 2008 [52]	Altwaijri, 2009 [65]
TC girls	↓ TS 3–4, ↑ TS 5	↓ TS 2–4, ↑ TS 5	↓ then ↑ between ages 11–19	↓ TS 2–4, then↑ TS 5
TC boys	↓ TS 2–4, ↑ TS 5	↓ TS 2–4, ↑ TS 5	↓ then ↑ between ages 11–19	↓ TS 2–4, then ↑ TS 5
LDL girls	↑ TS 3–4	↓ TS 2–4, ↑ TS 5	↓ then ↑ between ages 11–19	Not examined
LDL boys	↓ TS 1–4, ↑TS 5	↓ TS 2–4, ↑ TS 5	↓ then ↑ between ages 11–19	
HDL girls	↓ TS 1–5	No change	↑ between ages 11–19	Not examined
HDL boys	↓ TS 3–4	↓ TS 1–5	↓ between ages 11–19	
TG girls	↑ TS 1–5, not 4	No change	↓ between ages 11–19	Not examined
TG boys	↑ TS 1–5, not 4	↑TS 1–5	↑ between ages 11–19	

TC total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TG* triglycerides, *TS* Tanner stage

effects on the hypothalamus to stimulate energy expenditure and suppress food intake [70]. Prior to puberty, serum leptin is similar in boys and girls, but differs between sexes during puberty [70–72]. The first study to establish normal leptin concentrations in healthy children and adolescents was published in 1997 [70]. Serum leptin in girls was overall higher than boys. In girls, leptin increases with the progression of puberty from Tanner stage 1 to 5. BMI was the main factor regulating serum leptin in girls [70]. In boys, leptin decreases with age, particularly from Tanner stage 3 through 5 (despite an increase in BMI during that time). Leptin in boys was affected by age, BMI, testosterone, and IGF-binding protein 2 (IGFBP2) [70]. In both sexes, leptin is directly related to fat mass and fasting insulin, but not to insulin sensitivity or resting energy expenditure [72]. However, other studies suggest that leptin is inversely associated with insulin sensitivity during puberty [43, 51, 72]. High leptin is also associated with abnormal vascular function and atherosclerotic risk. This correlation appears to be independent of the typical metabolic and inflammatory markers of obesity (fat mass, blood pressure, C-reactive protein [CRP], fasting insulin, and LDL cholesterol) [69].

Adiponectin is another hormone synthesized in adipocytes that has been shown to have protective cardiovascular effect, in addition to insulin-sensitizing and anti-inflammatory actions [73, 74]. Unlike leptin, adiponectin does not increase with body fat mass in obese adults [74]. Normal adiponectin patterns in lean and obese children and adolescents were first described in 2004. Across both sexes, adiponectin concentrations negatively correlated with BMI, age, height, and IGF-1 [74]. Normal-weight prepubertal boys and girls had similar serum adiponectin. As puberty progressed, adiponectin in lean girls did not change, but fell in lean boys, particularly after Tanner stage 2 [74]. Testosterone has a negative effect on adiponectin in boys and this association proved even stronger than other factors, including BMI and age [73, 74]. Adiponectin concentrations in lean children and adolescents were significantly higher than their obese counterparts, especially during and after puberty [73, 74]. The high molecular weight (HMW) isoform of adiponectin, which has been shown to correlate well with insulin sensitivity, has different effects during puberty between sexes. In males, HMW adiponectin was noted to be lower in T1 than in T5. There was no difference in females [73]. In girls, plasma adiponectin was inversely related to fasting insulin and HOMA-IR in mid-puberty [63]. In boys, negative correlations were found only in late puberty. No correlations were found between adiponectin and β(beta)-cell function [63].

Changes in Physical Activity During Puberty

While most youth experience a decline in physical activity during puberty, that decline is exaggerated in girls, particularly in minority girls. This decline in physical activity is particularly relevant in relation to type 2 diabetes, as obese minority girls are at the highest risk for development of diabetes during adolescence. The potential role of sedentary lifestyle in development of diabetes is demonstrated by a study comparing physical activity, as measured by gold standard accelerometry, in youth from the Treatment Options for Adolescents and Youth (TODAY) study with similarly obese adolescents in the National Health and Nutrition Examination Survey (NHANES) cohort. In this study, while moderate and vigorous activity was similarly low in both cohorts, the TODAY cohort was sedentary for a significantly greater proportion of time than obese adolescents in NHANES.

There are many potential psychosocial and biological factors contributing to the sex differences in decline in physical activity during puberty, including sex steroids, sex differences in fat accumulation, and neuromuscular adaptations. While prepubertal girls and boys have

similar neuromuscular performance, there is a relative increase in neuromuscular function in boys during development that contributes to significant sex differences in athletic ability as adults. In a cross-sectional study of healthy girls and boys (n = 72) in prepuberty and early puberty, we found that girls were already less active than boys by T2, demonstrating that changes in physical activity in girls appear early in the pubertal transition. Furthermore, measures of muscle strength, including knee flexor and extensor strength, were predictive of physical activity (steps/day).

There is also literature suggesting that puberty may have differential effects on cardiorespiratory fitness in girls and boys, with girls showing greater declines in VO_{2max}, an important component of fitness, during puberty. It is well established that low physical activity results in impairment of insulin sensitivity. Furthermore, training programs have been demonstrated to improve insulin sensitivity, in youth. However, recent paradigm-shifting research also suggests that reductions in insulin sensitivity may *result in* impaired VO_{2max}. In one study of both obese adolescents with and without diabetes and normal-weight controls of similar fat-free mass and physical activity, and controlling for BMI and physical activity, insulin sensitivity was the strongest predictor of VO_{2max}. Further support of the hypothesis that insulin resistance negatively affects fitness comes from a placebo-controlled study of rosiglitazone, an insulin sensitizer, in adults with type 2 diabetes. In this study, 4 months of treatment with rosiglitazone resulted in significantly improved VO_{2max} when compared with the control group. Furthermore, this improvement in cardiorespiratory fitness was correlated with insulin sensitivity as measured by both HOMA-IR ($r = -0.89$, $p < 0.001$) and hyperinsulinemic-euglycemic clamp ($r = 0.68$, $p < 0.05$). It is possible that reductions in insulin sensitivity during puberty in youth who are already obese and sedentary may also result in further reduction in cardiorespiratory fitness, adding to the risk of obesity-associated comorbidities.

Relationship of Puberty with Youth-Onset Type 2 Diabetes, Potential Impact of Obesity on Metabolic Change in Puberty

As might be expected, obese youth are more insulin resistant than lean youth prior to pubertal onset, and insulin sensitivity is lower in obese youth than in lean youth in all pubertal stages [42, 75–78]. Several studies suggest that obese youth do not recover insulin sensitivity at the end of puberty [75, 76, 78, 79], which may have a negative impact on β(beta)-cell function during this time [78]. Furthermore, there is evidence that puberty negatively impacts general metabolic health in obese youth [80–82]. This is best described in a 1-year longitudinal follow-up study of more than 1000 obese youth that shows that puberty onset and further weight gain are the two strongest predictors of transition from metabolically healthy to metabolically unhealthy obesity [81]. Understanding the relationships among obesity, insulin sensitivity, β(beta)-cell function, and forms of metabolic dysfunction during puberty is critical, as the incidence of youth-onset type 2 diabetes is associated with rapid β(beta)-cell decline [83–85] and its timing is tightly linked with puberty.

Other Considerations

As noted, there are many challenges to studying physiology during puberty, including limited ability to perform invasive measurements in pediatric participants, the variability in timing and duration of puberty among youth, the subjective nature of puberty staging, and the lack of a good parallel animal model. Many studies evaluating normal physiology of changes in insulin sensitivity in puberty and how this change may impact pathophysiology of various diseases lack precise staging of central puberty, particularly in boys; use less rigorous estimate of insulin sensitivity, such as the HOMA-IR; and are cross-sectional in nature. This leads to difficulty interpreting the data that are currently available.

Areas for Future Study

Due to the challenges noted previously, better models for studying puberty are needed. These include the use of accelerated longitudinal designs, in which youth are recruited in various stages of puberty and followed, and then the data are put together in an overlapping fashion to get a semi-longitudinal picture. Also, less invasive measures of metabolism, such as MR spectroscopy, are increasingly available and are ideally suited for the pediatric population. Finally, more rigorous methods for staging puberty are needed, perhaps including overnight urine measurement of sex steroids and gonadotropins, which have been demonstrated to be sensitive markers of central precocious puberty [86] and of normal menstrual cycle variations in youth [87].

Conclusion

In summary, puberty is a time of complex metabolic and hormonal changes, which include a transient reduction in insulin sensitivity accompanied by compensatory insulin secretion. While these changes in insulin sensitivity likely play a critical role in the rapid linear growth and the transition to adult physiology, they may also potentiate risk for cardiometabolic disease in those youth who are at higher risk going into puberty.

References

1. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291–303.
2. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45(239):13–23.
3. Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the pediatric research in office settings network. Pediatrics. 1997;99(4):505–12.
4. Reynolds EL, Wines JV. Individual differences in physical changes associated with adolescence in girls. Am J Dis Child (1911). 1948 Mar;75(3):329–50.
5. Foster TA, Voors AW, Webber LS, Frerichs RR, Berenson GS. Anthropometric and maturation measurements of children, ages 5 to 14 years, in a biracial community–the Bogalusa heart study. Am J Clin Nutr. 1977;30(4):582–91.
6. Lee PA. Normal ages of pubertal events among American males and females. J Adolesc Health Care. 1980 Sep;1(1):26–9.
7. Reynolds EL, Wines JV. Physical changes associated with adolescence in boys. AMA Am J Dis Child. 1951;82(5):529–47.
8. Herman-Giddens ME, Wang L, Koch G. Secondary sexual characteristics in boys: estimates from the national health and nutrition examination survey iii, 1988–1994. Arch Pediatr Adolesc Med. 2001;155(9):1022–8.
9. Kempná P, Marti N, Udhane S, Flück CE. Regulation of androgen biosynthesis – a short review and preliminary results from the hyperandrogenic starvation NCI-H295R cell model. Mol Cell Endocrinol. 2015;408:124–32.
10. Kulin HE, Bwibo N, Mutie D, Santner SJ. The effect of chronic childhood malnutrition on pubertal growth and development. Am J Clin Nutr. 1982;36(3):527–36.
11. Wyshak G, Frisch RE. Evidence for a secular trend in age of menarche. N Engl J Med. 1982;306(17):1033–5.
12. Wu T, Mendola P, Buck GM. Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the third National Health and nutrition examination survey, 1988–1994. Pediatrics. 2002;110(4):752–7.
13. Kaplowitz PB. Link between body fat and the timing of puberty. Pediatrics. 2008;121(Suppl 3):S208–17.
14. Herman-Giddens ME, Steffes J, Harris D, Slora E, Hussey M, Dowshen SA, et al. Secondary sexual characteristics in boys: data from the pediatric research in office settings network. Pediatrics. 2012;130(5):e1058–e68.
15. Frisch RE, Revelle R. The height and weight of girls and boys at the time of initiation of the adolescent growth spurt in height and weight and the relationship to menarche. Hum Biol. 1971;43(1):140–59.
16. Tanner JM, Whitehouse RH, Marubini E, Resele LF. The adolescent growth spurt of boys and girls of the Harpenden growth study. Ann Hum Biol. 1976;3(2):109–26.
17. Rose SR, Municchi G, Barnes KM, Kamp GA, Uriarte MM, Ross JL, et al. Spontaneous growth hormone secretion increases during puberty in normal girls and boys∗. J Clin Endocrinol Metabol. 1991;73(2):428–35.
18. Albertsson-Wikland K, Rosberg S, Karlberg J, Groth T. Analysis of 24-hour growth hormone profiles in healthy boys and girls of normal stature: relation to puberty. J Clin Endocrinol Metabol. 1994;78(5):1195–201.
19. Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J, et al. Reference inter-

vals for Insulin-like Growth Factor-1 (IGF-I) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. J Clin Endocrinol Metabol. 2014;99(5):1712–21.
20. Karimian E, Savendahl L. Estrogen signaling in growth plate cartilage. Endocr Dev. 2011;21:42–51.
21. Veldhuis JD, Metzger DL, Martha PM Jr, Mauras N, Kerrigan JR, Keenan B, et al. Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab. 1997;82(10):3414–20.
22. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4(3):254–64.
23. Boyar RM, Wu RH, Roffwarg H, Kapen S, Weitzman ED, Hellman L, et al. Human puberty: 24-hour estradiol in pubertal girls. J Clin Endocrinol Metab. 1976;43(6):1418–21.
24. Judd HL, Parker DC, Siler TM, Yen SS. The nocturnal rise of plasma testosterone in pubertal boys. J Clin Endocrinol Metab. 1974;38(4):710–3.
25. Messinis IE. From menarche to regular menstruation: endocrinological background. Ann N Y Acad Sci. 2006;1092:49–56.
26. Frisch RE, McArthur JW. Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. Science (New York, NY). 1974;185(4155):949–51.
27. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science (New York, NY). 1970;169(3943):397–9.
28. Tena-Sempere M. Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology. 2007;86(3):229–41.
29. von Schnurbein J, Moss A, Nagel SA, Muehleder H, Debatin KM, Farooqi IS, et al. Leptin substitution results in the induction of menstrual cycles in an adolescent with leptin deficiency and hypogonadotropic hypogonadism. Horm Res Paediatr. 2012;77(2):127–33.
30. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 1998;18(3):213–5.
31. Farooqi IS, O'Rahilly S. 20 years of leptin: human disorders of leptin action. J Endocrinol. 2014;223(1):T63–70.
32. Hager A, Sjostrm L, Arvidsson B, Bjorntorp P, Smith U. Body fat and adipose tissue cellularity in infants: a longitudinal study. Metab Clin Exp. 1977;26(6):607–14.
33. Arfai K, Pitukcheewanont PD, Goran MI, Tavare CJ, Heller L, Gilsanz V. Bone, muscle, and fat: sex-related differences in prepubertal children. Radiology. 2002;224(2):338–44.
34. van der Sluis IM, de Ridder MA, Boot AM, Krenning EP, de Muinck Keizer-Schrama SM. Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults. Arch Dis Child. 2002;87(4):341–7; discussion −7.
35. Maynard LM, Wisemandle W, Roche AF, Chumlea WC, Guo SS, Siervogel RM. Childhood body composition in relation to body mass index. Pediatrics. 2001;107(2):344–50.
36. de Ridder CM, de Boer RW, Seidell JC, Nieuwenhoff CM, Jeneson JA, Bakker CJ, et al. Body fat distribution in pubertal girls quantified by magnetic resonance imaging. Int J Obes Relat Metab Disord. 1992;16(6):443–9.
37. Weits T, van der Beek EJ, Wedel M, Ter Haar Romeny BM. Computed tomography measurement of abdominal fat deposition in relation to anthropometry. Int J Obes. 1988;12(3):217–25.
38. Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV. Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. N Engl J Med. 1986;315(4):215–9.
39. Amiel SA, Caprio S, Sherwin RS, Plewe G, Haymond MW, Tamborlane WV. Insulin resistance of puberty: a defect restricted to peripheral glucose metabolism. J Clin Endocrinol Metab. [Research Support, Non--U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1991;72(2):277–82.
40. Arslanian SA, Kalhan SC. Correlations between fatty acid and glucose metabolism. Potential explanation of insulin resistance of puberty. Diabetes. [Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1994;43(7):908–14.
41. Bloch CA, Clemons P, Sperling MA. Puberty decreases insulin sensitivity. J Pediatr. 1987;110(3):481–7.
42. Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes. [Research Support, U.S. Gov't, P.H.S.]. 1999;48(10):2039–44.
43. Roemmich JN, Clark PA, Lusk M, Friel A, Weltman A, Epstein LH, et al. Pubertal alterations in growth and body composition. VI. Pubertal insulin resistance: relation to adiposity, body fat distribution and hormone release. Int J Obes Relat Metab Disord. 2002;26(5):701–9.
44. Travers SH, Labarta JI, Gargosky SE, Rosenfeld RG, Jeffers BW, Eckel RH. Insulin-like growth factor binding protein-I levels are strongly associated with insulin sensitivity and obesity in early pubertal children. J Clin Endocrinol Metab. 1998;83(6):1935–9.
45. Guercio G, Rivarola MA, Chaler E, Maceiras M, Belgorosky A. Relationship between the growth hormone/insulin-like growth factor-I axis, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal girls. J Clin Endocrinol Metab. 2003;88(3):1389–93.

46. Guercio G, Rivarola MA, Chaler E, Maceiras M, Belgorosky A. Relationship between the GH/IGF-I axis, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal boys. J Clin Endocrinol Metab. 2002;87(3):1162–9.
47. Ball GD, Huang TT, Gower BA, Cruz ML, Shaibi GQ, Weigensberg MJ, et al. Longitudinal changes in insulin sensitivity, insulin secretion, and beta-cell function during puberty. J Pediatr. 2006;148(1):16–22.
48. Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes. [Research Support, U.S. Gov't, P.H.S.]. 2001;50(11):2444–50.
49. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. Pediatr Res. [Evaluation Studies Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2006;60(6):759–63.
50. Jeffery SC, Hosking J, Jeffery AN, Murphy MJ, Voss LD, Wilkin TJ, et al. Insulin resistance is higher in prepubertal girls but switches to become higher in boys at age 16: a cohort study (EarlyBird 57). Pediatr Diabetes. 2018;19(2):223–30.
51. Hoffman RP, Vicini P, Sivitz WI, Cobelli C. Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. Pediatr Res. [Research Support, Non-U.S. Gov't]. 2000;48(3):384–8.
52. Moran A, Jacobs DR Jr, Steinberger J, Steffen LM, Pankow JS, Hong CP, et al. Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. Circulation. [Comparative Study Research Support, N.I.H., Extramural]. 2008;117(18):2361–8.
53. Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). Diabetes Care. 2012;35(3):536–41.
54. Sinaiko AR, Steinberger J, Moran A, Hong CP, Prineas RJ, Jacobs DR Jr. Influence of insulin resistance and body mass index at age 13 on systolic blood pressure, triglycerides, and high-density lipoprotein cholesterol at age 19. Hypertension. [Research Support, N.I.H., Extramural]. 2006;48(4):730–6.
55. Arslanian S. Insulin secretion and sensitivity in healthy African-American vs American white children. Clin Pediatr. [Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 1998;37(2):81–8.
56. Arslanian S, Suprasongsin C. Differences in the in vivo insulin secretion and sensitivity of healthy black versus white adolescents. J Pediatr. 1996;129(3):440–3.
57. Saad RJ, Danadian K, Lewy V, Arslanian SA. Insulin resistance of puberty in African-American children: lack of a compensatory increase in insulin secretion. Pediatr Diabetes. 2002;3(1):4–9.
58. Caprio S, Cline G, Boulware S, Permanente C, Shulman GI, Sherwin RS, et al. Effects of puberty and diabetes on metabolism of insulin-sensitive fuels. Am J Phys. 1994;266(6 Pt 1):E885–91.
59. Caprio S, Hyman LD, Limb C, McCarthy S, Lange R, Sherwin RS, et al. Central adiposity and its metabolic correlates in obese adolescent girls. Am J Phys. 1995;269(1 Pt 1):E118–26.
60. Robinson C, Tamborlane WV, Maggs DG, Enoksson S, Sherwin RS, Silver D, et al. Effect of insulin on glycerol production in obese adolescents. Am J Physiol. [Research Support, U.S. Gov't, P.H.S.]. 1998;274(4 Pt 1):E737–43.
61. Caprio S, Plewe G, Diamond MP, Simonson DC, Boulware SD, Sherwin RS, et al. Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. J Pediatr. 1989;114(6):963–7.
62. Moran A, Jacobs DR Jr, Steinberger J, Cohen P, Hong CP, Prineas R, et al. Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. J Clin Endocrinol Metab. 2002;87(10):4817–20.
63. Tsou PL, Jiang YD, Chang CC, Wei JN, Sung FC, Lin CC, et al. Sex-related differences between adiponectin and insulin resistance in schoolchildren. Diabetes Care. [Research Support, Non-U.S. Gov't]. 2004;27(2):308–13.
64. Morrison JA, Laskarzewski PM, Rauh JL, Brookman R, Mellies M, Frazer M, et al. Lipids, lipoproteins, and sexual maturation during adolescence: the Princeton maturation study. Metabolism. [Research Support, U.S. Gov't, P.H.S.]. 1979;28(6):641–9.
65. Altwaijri YA, Day RS, Harrist RB, Dwyer JT, Ausman LM, Labarthe DR. Sexual maturation affects diet-blood total cholesterol association in children: Project HeartBeat! Am J Prev Med. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. 2009;37(1 Suppl):S65–70.
66. Kwiterovich PO Jr, Barton BA, McMahon RP, Obarzanek E, Hunsberger S, Simons-Morton D, et al. Effects of diet and sexual maturation on low-density lipoprotein cholesterol during puberty: the Dietary Intervention Study in Children (DISC). Circulation. 1997;96(8):2526–33.
67. Tell GS, Mittelmark MB, Vellar OD. Cholesterol, high density lipoprotein cholesterol and triglycerides during puberty: the Oslo Youth Study. Am J Epidemiol. [Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1985;122(5):750–61.
68. Porkka KV, Viikari JS, Ronnemaa T, Marniemi J, Akerblom HK. Age and gender specific serum lipid and apolipoprotein fractiles of Finnish children and young adults. The Cardiovascular Risk in Young Finns Study. Acta Paediatr. [Research Support, Non--U.S. Gov't]. 1994;83(8):838–48.
69. Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, et al. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? Circulation. [Research Support, Non-U.S. Gov't]. 2002;106(15):1919–24.

70. Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab. 1997;82(9):2904–10.
71. Ahmed ML, Ong KK, Morrell DJ, Cox L, Drayer N, Perry L, et al. Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. J Clin Endocrinol Metab. [Research Support, Non-U.S. Gov't]. 1999;84(3):899–905.
72. Arslanian S, Suprasongsin C, Kalhan SC, Drash AL, Brna R, Janosky JE. Plasma leptin in children: relationship to puberty, gender, body composition, insulin sensitivity, and energy expenditure. Metab Clin Exp. 1998;47(3):309–12.
73. Andersen KK, Frystyk J, Wolthers OD, Heuck C, Flyvbjerg A. Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. J Clin Endocrinol Metab. 2007;92(5):1857–62.
74. Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E, et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab. 2004;89(8):4053–61.
75. Ball GD, Weigensberg MJ, Cruz ML, Shaibi GQ, Kobaissi HA, Goran MI. Insulin sensitivity, insulin secretion and beta-cell function during puberty in overweight Hispanic children with a family history of type 2 diabetes. Int J Obes. 2005;29(12):1471–7.
76. Pinhas-Hamiel O, Lerner-Geva L, Copperman NM, Jacobson MS. Lipid and insulin levels in obese children: changes with age and puberty. Obesity (Silver Spring, Md). 2007;15(11):2825–31.
77. Goran MI, Bergman RN, Avila Q, Watkins M, Ball GD, Shaibi GQ, et al. Impaired glucose tolerance and reduced beta-cell function in overweight Latino children with a positive family history for type 2 diabetes. J Clin Endocrinol Metab. 2004;89(1):207–12.
78. Kelly LA, Lane CJ, Weigensberg MJ, Toledo-Corral CM, Goran MI. Pubertal changes of insulin sensitivity, acute insulin response, and beta-cell function in overweight Latino youth. J Pediatr. 2011;158(3):442–6.
79. Guzzaloni G, Grugni G, Mazzilli G, Moro D, Morabito F. Comparison between beta-cell function and insulin resistance indexes in prepubertal and pubertal obese children. Metabolism. 2002;51(8):1011–6.
80. Reinehr T, Toschke AM. Onset of puberty and cardiovascular risk factors in untreated obese children and adolescents: a 1-year follow-up study. Arch Pediatr Adolesc Med. 2009;163(8):709–15.
81. Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong impact of pubertal status on metabolic health in obese children: a longitudinal study. J Clin Endocrinol Metab. 2015;100(1):301–8.
82. Agirbasli M, Agaoglu NB, Orak N, Caglioz H, Ocek T, Poci N, et al. Sex hormones and metabolic syndrome in children and adolescents. Metabolism. [Research Support, Non-U.S. Gov't]. 2009;58(9):1256–62.
83. Burns SF, Bacha F, Lee SJ, Tfayli H, Gungor N, Arslanian SA. Declining beta-cell function relative to insulin sensitivity with escalating OGTT 2-h glucose concentrations in the nondiabetic through the diabetic range in overweight youth. Diabetes Care. [Research Support, N.I.H., Extramural Research Support, Non--U.S. Gov't]. 2011;34(9):2033–40.
84. Giannini C, Weiss R, Cali A, Bonadonna R, Santoro N, Pierpont B, et al. Evidence for early defects in insulin sensitivity and secretion before the onset of glucose dysregulation in obese youths: a longitudinal study. Diabetes. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2012;61(3):606–14.
85. Sjaarda LG, Bacha F, Lee S, Tfayli H, Andreatta E, Arslanian S. Oral disposition index in obese youth from normal to prediabetes to diabetes: relationship to clamp disposition index. J Pediatr. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non--P.H.S.]. 2012;161(1):51–7.
86. Demir A, Voutilainen R, Stenman UH, Dunkel L, Albertsson-Wikland K, Norjavaara E. First morning voided urinary gonadotropin measurements as an alternative to the GnRH test. Horm Res Paediatr. 2016;85(5):301–8.
87. Zhang K, Pollack S, Ghods A, Dicken C, Isaac B, Adel G, et al. Onset of ovulation after menarche in girls: a longitudinal study. J Clin Endocrinol Metab. [Research Support, N.I.H., Extramural]. 2008;93(4):1186–94.

9 Adiposity Is the Enemy: Body Composition and Insulin Sensitivity

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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 a 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 1990s, 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 fueled 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 mechanisms behind the detrimental effect of body fat on insulin sensitivity. Finally, we are faced with the "chicken and the 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 fatness on insulin sensitivity through the human lifespan—from childhood precursors to adult disease. The focus will be on recent literature, with the data on childhood and adolescence taking prominence, as other texts have comprehensively reviewed the adult literature in the past. Hopefully, the data presented in this chapter will convince you that adiposity is a major contributor to insulin resistance throughout the lifespan.

Assessment of Body Composition

Numerous methods are available to quantify adiposity by assessing body composition, each having a dedicated purpose and its own usefulness in different settings (Table 9.1). All of these methods rely on models with assumptions of body composition that vary with age, race, and sex. The four-compartment model is the most rigorous but also the most intensive, measuring three fat-free mass components, including total body water, protein content, and mineral content, and

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Table 9.1 Summary of tools to measure body composition in pediatric cohorts

Method	Pediatric ages	Benefits	Drawbacks
Dual-energy X-ray absorptiometry (DEXA)	0–18 years	Three-compartment model; norms available; fairly accessible	Cost; availability; radiation exposure (minimal)
Air displacement plethysmography	0–6 months; ~2–18 years	Very safe; excellent for following change in infants	Less feasible and accurate in toddlers; cost/availability
Skinfold thickness	0–18 years[a]	Transportable; low cost; good for large studies; greater accuracy than BIA	Inter-observer variability; lower accuracy in obesity
Bioelectrical impedance analysis (BIA)	0–18 years[a]	Transportable; low cost; good for large studies	Not reliable in conditions with water shifts (weight loss, dehydration, edema; pregnancy)
Computerized tomography (CT)	0–18 years	Assessment of visceral and organ fat with high accuracy	Radiation exposure (particularly in pediatrics); cost; availability; variable methods
Magnetic resonance imaging (MRI)	0–18 years	Assessment of visceral and organ fat with high accuracy; also can do total body composition	Time-consuming; cost; availability; variable methods. May require sedation for infants and young children

[a]Less reliable in infants

subtracting from the total mass to obtain fat mass. The first of these components, total body water, can be measured by the deuterium dilution technique using an oral tracer and serial blood, urine, or serum analysis. Protein mass is very difficult to measure directly; however, an estimation of total body potassium can be obtained through ^{40}K counting followed by equations accounting for the known nitrogen-to-potassium ratio and nitrogen content of protein. Finally, bone mineral content can be measured by DXA (dual-energy X-ray absorptiometry). Fat mass is then determined by total body mass minus the three components that make up fat-free mass. Not surprisingly, four-compartment modeling is not feasible for the great majority of research studies and certainly not in clinical practice. Therefore, alternative methods are routinely used to estimate body composition.

Dual-Energy X-Ray Absorptiometry (DXA)

DXA is currently recognized as the "gold standard" for assessment of body composition. DXA uses photons of two different energy levels that attenuate at different rates when passing through different tissue types, allowing for visualization and separate analysis of bone, muscle, and fat, facilitating estimation of total body fat, bone mass, and other nonfat mass (generally referred to as fat-free mass) using a three-compartment model [1]. This technique was originally time-consuming and relatively expensive but has become faster, cheaper, and widely available. Current generation DXA machines take less than 5 minutes to conduct a whole-body scan. Thus, this technique is now suitable for children and other participants who have difficulty lying still for extended periods of time. There are methods to quantify location of adipose tissue as well, although this is less sensitive than other modalities. DXA technology does use ionizing radiation to capture data and, although the radiation exposure is regarded as minimal (much less than one chest X-ray), this is a consideration when using this method in populations who already receive a high radiation dose or in a study design requiring multiple, repeated measures.

Underwater Weighing (UWW)

Underwater weighing (UWW) is one of the oldest methods for calculating body composition. UWW works by measuring body mass and volume via water displacement to estimate a person's

total density and then deriving fat-free and fat mass through equations. This assumes that the fat-free mass components (water, protein, bone mineral content) are relatively constant for every person, which has been shown to not be true in many populations including children and certain health conditions. This technique requires a large sealed tank of water that must be maintained and is extremely stressful for the subject who must repeatedly hold their breath underwater for long periods of time. Therefore, UWW is too strenuous to be utilized in many populations, including children or anyone with a physical impairment or breathing abnormality. Due to these limitations, UWW is rarely used.

Air Displacement Plethysmography

Air displacement plethysmography relies on the same principles as UWW for estimating a person's total density, but instead of water displacement, it measures air displacement from an enclosed chamber [2]. Outcome measures include fat mass, fat-free mass, and calculated percent body fat. There is no accommodation for localization of fat. The assessment is fast, noninvasive, and does not expose subjects to radiation. There are now air displacement plethysmography systems to accommodate infants, children, and adults, and it is one of the best methods for assessing body composition in infants [3]. However, it is important to be aware that there are age gaps posed by using different machines at different ages, particularly between ~6 months and 2 years of age, limiting prospective assessments. Also, the expense and maintenance do limit the availability of these systems largely to research centers. In addition, the overall size of the unit is problematic for those who fear confined spaces and can limit the size of the person measured; very tall or obese people are difficult to accommodate in the plethysmography chamber. While DXA and air displacement plethysmography are systematically different and not interchangeable, the measures correlate with one another and track changes in body composition over time similarly [4].

Bioelectrical Impedance (BIA)

Bioelectrical impedance (BIA) is an alternative method that uses the electrical resistance and reactance between two sets of electrodes to calculate body composition. BIA directly measures differences in body water, which are extrapolated to reflect differences in body composition. This tool has limited utility in situations that significantly alter total body water, such as acute weight loss or pregnancy, limiting its usefulness as an assessment tool in intervention studies. Accuracy varies depending on the placement of the electrodes (hand-foot, foot-foot, or hand-hand placement), the measurement conditions (fasting, bladder emptying), the predictive equations used, and the characteristics of the population being assessed. While BIA has been shown to correlate reasonably well with DXA in lean adult populations, agreement is poor in pediatric populations with both over- or underestimation of body fat percentage and increasing discrepancy in more obese cohorts [5–12]. A systematic review of BIA in pediatric populations found mean differences in percent body fat between BIA and the gold standard to be −12.3% and 13.7% [13]. In general, multi-site skinfold thickness measurements, which require no specialized electrical apparatus, are more accurate than BIA.

Anthropometric Assessments

Body mass index (BMI), waist circumference, waist-to-hip ratio, height-to-weight ratio, and abdominal height are easily accessible and inexpensive measures that have a moderate agreement with more robust measurements of total body (i.e., DXA) or regional adiposity. Although BMI is the most commonly used anthropometric outcome associated with disease risk, BMI is influenced by muscularity and provides no information regarding fat distribution. In children 8–19 years of age participating in the *National Health and Nutrition Examination Survey* (NHANES), BMI was correlated with body fat percentage, although the association was weaker than waist-to-hip ratio. Waist-to-hip ratio is a

good predictor of body fat percentage as measured by DXA [14]. Data from the Bogalusa Heart Study found that waist-to-hip ratio in children was a better predictor of adult obesity and other cardiovascular risk factors than BMI alone [15]. All anthropometric measurements suffer from inter-user variability and differences due to the type and calibration of instruments used for the measurements. However, because anthropometric assessments are inexpensive and relatively fast, they are commonly employed in large cross-sectional or multi-site studies where other methods are cost prohibitive.

Skinfold Thickness

Skinfold thickness can be used to predict total body fat, as 70–90% of total adipose tissue is located subcutaneously. The skinfold values can be compared to grouped values or norms or can be incorporated into specific equations that predict body density, total body fat mass, and percent body fat. Depending on the population, these equations can predict body fat with errors of 3.5–5% when compared to a reference model. Once again, skinfold thickness has its limitations—single-site measurements are not as accurate or reproducible as multi-site measurements, there can be significant inter-observer error, and there is variability between types of calipers [16]. Although skinfolds can be conducted in any pediatric population, results are more accurate in adolescents and less reliable in infants (particularly neonates) and very obese children [17–19]. Skinfold thickness remains a common method of estimating body composition, particularly in large, cross-sectional studies or studies where the risk of radiation is deemed inappropriate.

Regional Body Composition Assessments

As you will read later in this chapter, not all adipose tissue is alike. The location of adipose deposition matters in the overall contribution to cardiometabolic risk [20]. Therefore, we need methods of assessing visceral adiposity specifically. Whereas DXA and certain anthropometric measurements (waist circumference and skinfolds) are able to estimate regional adiposity, computed tomography (CT) and magnetic resonance imaging (MRI) are the most accurate methods for quantifying visceral fat, especially for prospective studies looking for small changes. CT and MRI imaging are expensive and investigators are usually charged per single cross-sectional image (slice). So, many investigators estimate visceral fat using single-slice CT or MRI imaging at the umbilicus or between specific vertebrae (usually L4–L5). Some 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. Expense and availability limit the application of CT and MRI in pediatric studies and differences in methodology limit the ability to compare data between studies.

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, with a pediatric emphasis, see Brodie and Stewart [21]. In short, DXA is the most accurate measurement of total body composition, especially when looking for small changes, with CT or MRI for regional adiposity assessment. However, it is common to see validated surrogate methods to estimate body fat, such as air displacement plethysmography, bioelectrical impedance, or anthropometric measurements in the literature, particularly for population studies or large, multicenter protocols. Importantly, absolute values of body composition measurements should not be compared between different methods, as the agreements between these different methods are generally poor.

Changes in Body Composition Throughout the Lifespan

The association between adiposity and insulin resistance can be appreciated at all ages. Generally, body fat increases with age, and, as fat mass increases, so does insulin resistance (Fig. 9.1). Individuals with higher body fat for a given age consistently have greater insulin resistance. This relationship is so strong that body composition can be thought of as a surrogate measure of cardiometabolic risk.

Infancy

Infancy is a time of significant fat accumulation and is also likely to be a critical period for lifelong metabolic programming. Rapid weight gain in infancy has been associated with increased adiposity and insulin resistance in childhood. Infants born small-for-gestational-age (SGA) are one population at a particularly high risk for rapid gain of adiposity during this critical period. Catch-up growth, whether in low birth weight infants or individuals who have regained after

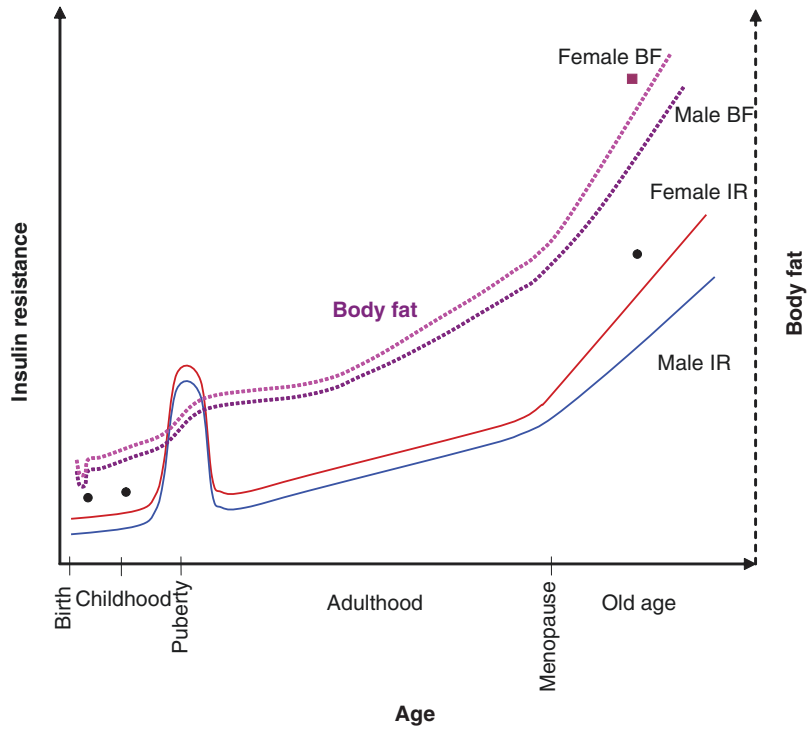

Fig. 9.1 Representation of insulin resistance and total body fat (BF) throughout life. Over all stages of the life cycle, from birth to old age, insulin resistance (solid lines) and adiposity (dotted lines) exhibit parallel trends. The only exception is puberty, a time of profound insulin resistance that does not directly correlate with the adiposity trajectory. 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

weight loss, is primarily associated with a gain in fat mass without an equivalent gain in fat-free mass (i.e., muscle mass) in a manner that tracks throughout the lifespan. Compared to controls matched for age and weight, SGA children have greater total and visceral fat mass [22, 23]. Further, healthy, young adult males who were born SGA have higher body fat—in particular higher visceral fat mass—than age-matched controls [24]. Similar findings were seen in elderly populations [25]. Importantly, this higher fat mass is associated with insulin resistance. Early catch-up growth (<3 years of age) in SGA cohorts is predictive of adult fat mass and can be correlated with central adiposity and hyperinsulinemia in young adulthood [26–29]. Even lean children who experience a catch-up growth period exhibit hyperinsulinemia during this period of rapid weight gain [30, 31]. These data indicate that a period of rapid fat accumulation is associated with impaired insulin sensitivity. Despite this strong relationship, it is difficult to determine if it is insulin resistance that causes fat accumulation during catch-up growth or vice versa.

Although studies in SGA cohorts provide strong evidence for the correlation between low birth weight, catch-up growth, increased adiposity, and insulin resistance, these data are not without caveats. This group of infants has some form of in utero and/or ex utero growth retardation; therefore, the data from this population must also be interpreted with caution and may not be generalizable to normal weight infants. In healthy children, the literature on rapid adiposity gain in infancy is equivocal. Whereas some studies have found infant weight gain in the first 1–2 years of life is associated with childhood or adulthood adiposity [32, 33], other studies refute these findings [34, 35]. One study compared glucose metabolism in adolescents who were either born SGA (n = 30) or normal weight (n = 57) [36]. Weight gain in the first 3 months of life was predictive of insulin resistance in the SGA cohort, but not the normal weight cohort. Therefore, the relative contribution of adiposity gain in infancy to lifelong metabolic dysfunction in normal birth weight infants does not prove to be as great as it is in SGA infants.

Childhood

Childhood is a period of remarkable growth and development. There is a rapid growth rate for both height and weight in childhood, but insulin sensitivity remains high. Insulin sensitivity continues to inversely correlate with body fat percentage, however, even in early childhood. For example, in very young, healthy children between birth and 4 years of age, insulin resistance is associated with higher z-scores for height and weight [37]. The same association can be found in older children, 8–13 years of age, where insulin sensitivity is negatively correlated with waist circumference [38]. In longitudinal studies of pediatric cohorts, current whole-body adiposity (measured via BMI and skinfolds or DXA) was associated with insulin resistance [39] and with a clustering of metabolic syndrome risk factors, including elevated blood pressure, fasting glucose, and triglycerides, and low HDL cholesterol [40–42]. In girls, this higher metabolic syndrome risk (i.e., insulin resistance) was also correlated with fat mass gain from 5 to 9 years of age [41]. Thin children who have rapid gain in weight in early school years have higher rates of type 2 diabetes and coronary heart diseases in adulthood than their peers [43]. Taken together, these studies show that there is a strong link between body fat and insulin sensitivity in childhood, and this is observed even in normal weight, healthy children.

In overweight and obese children, the correlation of adiposity and insulin sensitivity appears to be even stronger. In 9- to 11-year-old obese and lean boys and girls, insulin resistance was positively correlated with total body fat and waist circumference [44]; 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 [44]. These data point to visceral fat as a major determinant of insulin sensitivity in this population, as has also been found in other childhood cohorts [45]. Also, in 9-year-old lean and obese children who snore, insulin sen-

sitivity and dyslipidemia were not associated with sleep-disordered breathing or the degree of apnea, as expected, but were positively correlated with whole-body adiposity [46].

Puberty and Adolescence

Puberty is a time of growth and increasing fat mass, particularly in females. Puberty is also a period of profound insulin resistance that ameliorates as the adolescent transitions to adulthood [47–49]. Insulin resistance increases in puberty regardless of whole-body adiposity; however, there is still a strong correlation between insulin resistance and adiposity at this time [49–53]. This relationship appears to be greater in overweight and obese adolescents. For example, in overweight Hispanic adolescents, the change in insulin sensitivity across puberty was abolished after adjusting for body composition, suggesting that the effect of puberty on insulin sensitivity is mediated by adiposity [54]. Furthermore, total body adiposity in adolescents is still strongly associated with other metabolic risk factors, including dyslipidemia and high blood pressure [55–57]. Visceral adiposity, in particular, appears to have the strongest correlation with insulin resistance in adolescents [51, 58, 59]. In addition to adiposity, growth hormone and sex steroids have both been implicated as probable mechanisms by which insulin resistance increases in puberty [60].

Cross-sectional data using the gold standard hyperinsulinemic-euglycemic clamp to assess insulin sensitivity show a decline in insulin sensitivity early in puberty, with a nadir at Tanner 3, which is about 20% lower than Tanner 1, and near recovery by Tanner 5 [53]. In healthy individuals, the dramatic decrease in insulin sensitivity during puberty is offset by an increase in insulin secretion, facilitating glucose homeostasis [50]. However, in obese youth, puberty can precipitate loss of pancreatic β (beta)-cell compensation and the onset of dysglycemia, including type 2 diabetes [60]. In many obese children, insulin sensitivity does not recover at the end of puberty as it does in lean children. Among youth who have type 2 diabetes, who are highly insulin resistant and obese, adiposity is still correlated with lower insulin sensitivity, [61] giving greater credence to the fact that adiposity promotes insulin resistance regardless of initial body weight or health status.

Taken together, data from lean and obese adolescents show that adiposity is associated with profound 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.

Adulthood

The transient period of insulin resistance that occurs during puberty has 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, postmenopause. 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. 9.1).

Many studies demonstrate the association between current, adult adiposity and decreased insulin sensitivity at various ages—demonstrating the rigorous nature of this relationship [62–64]. In a study by Bryhni et al. [65], adult males were split into three groups: (1) elderly men, 71–77 years of age; (2) young men, in their early 30s matched to the elderly men for BMI and waist circumference; and (3) young men, in their early 30s with BMI and waist circumference that was average for age. This study unequivocally showed that insulin sensitivity is related to body fat 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) [65]. In sum, body fatness is associated with insulin resistance in adults; and weight gain through the years, whether the subject was lean or obese during childhood, is detrimental to insulin sensitivity.

Sex Differences in Body Composition

Sex differences in body composition and insulin sensitivity exist throughout the lifespan. Boys have lower body fat that can be appreciated in infancy [66]. As young as age 4, there is evidence that this lower body fat in males is associated with greater insulin sensitivity [37, 67–69]. Starting around 6–8 years, girls start gaining more body fat than boys, and after puberty, these differences are exaggerated [69]. For a similar BMI, women have twice the body fat percentage as men. There are also sex differences in body fat distribution, where women have proportionally more extremity/subcutaneous fat compared to abdominal/visceral fat—sometimes referred to as "gynoid" versus "android" fat distribution [70]. Even in children, a high android-to-gynoid fat ratio is strongly associated with insulin resistance [71, 72].

Racial and Ethnic Differences in Body Composition

In addition to sex differences, race and ethnicity also play a large role in body composition. African American children were found to have 26% less fat than Caucasian children, whereas Hispanic and Native American children have higher body fat than Caucasian children [73–75]. These differences track through adulthood and are associated with the risk for metabolic syndrome and type 2 diabetes. South Asian adolescents 14–17 years of age have been reported to be more insulin resistant and have higher body fat (as estimated by DXA) and central adiposity (measured via waist-hip ratio) than white European adolescents [76, 77].

Adiposity Leads to Insulin Resistance: Mechanisms

Adipose tissue is not simply a depot for storing excess energy, but a complex endocrine organ playing a critical role in energy metabolism, immune function, and bone metabolism. Over the past decade, there has been great advancement in our understanding of the mechanisms mediating the relationship between adiposity and insulin resistance; however, our knowledge is still evolving. There are multiple theories, none of which are mutually exclusive, for the mechanisms underlying the development of insulin resistance in the context of increased adiposity. For a more in-depth review of the postulated mechanisms, see Castro et al. [78].

Fat Sub-type

There is now abundant evidence to support the notion that visceral fat is the key factor relating adiposity to insulin resistance [79, 80]. While visceral fat does correlate with total body fat, around half of the variance in visceral adipose tissue mass in children is not explained by total body adiposity, and other variables, such as age, sex, ethnicity, physical activity, hormone concentrations, and genetics, clearly play a large role [20, 73, 81]. Studies have shown that visceral fat is associated with insulin resistance regardless of BMI, with little contribution from subcutaneous fat [64, 82, 83]. Furthermore, visceral fat is highly correlated with cardiovascular risk markers in children, including blood pressure, serum triglycerides and HDL, carotid artery intimal media thickness, inflammatory markers, and left ventricular mass [84, 85]. The data in adults is even stronger, consistently showing visceral adiposity is a stronger correlate of insulin resistance than total body fat [63, 64, 86–88].

So what makes visceral fat the enemy? Unique to visceral adipose tissue, free fatty acids (FFA) and cytokines produced by adipocytes are delivered directly to the liver via portal circulation and cause hepatic insulin resistance. For example,

circulating FFAs inhibit insulin clearance, resulting in downregulation of insulin receptors and a decrease in insulin receptor signaling, resulting in tissue-specific insulin resistance [78, 89]. Ectopic fat accumulation in peripheral tissues (liver, muscle, pancreas, etc.) is more likely to occur in those with high visceral adipose tissue mass, resulting in local exposure to adipocyte products, such as FFA, that induce insulin resistance within the exposed tissues [90–92].

Adipocyte Size

It has long been appreciated that hypertrophy of adipocytes is associated with insulin resistance in both lean and obese cohorts [93–96]. Hypertrophied adipocytes appear to be dysfunctional, with greater secretion of FFA, altered secretion of adipokines, and increased macrophage infiltration [97–100]. In a study of 171 healthy German children, insulin resistance was four times higher in subjects with large adipocytes compared to small adipocytes, and in a multivariate analysis, the insulin resistance by homeostatic model assessment (HOMA-IR) was most strongly affected by adipocyte size [101]. As visceral adipose tissue has lower capability to proliferate and differentiate than subcutaneous adipose tissue, expansion is primarily by hypertrophy to larger adipocytes [102–104], which could be another factor explaining the detrimental effect of visceral fat on insulin sensitivity.

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, and intrahepatic lipid content (assessed via magnetic resonance spectroscopy) [105]. Improvement in insulin sensitivity most strongly correlated with fat-cell size, although this parameter was also linked with visceral and hepatic adiposity. So, large fat cells seem to increase 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, 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 abdominal or femoral fat [106], implying that adipocyte cell size, regardless of location, plays a role in the modulation of insulin sensitivity.

Inflammation

One of the most prominent postulated mechanisms for how adiposity causes insulin resistance is through chronic inflammation. Many studies find an association between chronic low-grade systemic inflammation and insulin resistance. Macrophage infiltration into adipose tissue occurs in obesity, even in young children [101]. Macrophages secrete pro-inflammatory cytokines that then enter the circulation, producing systemic inflammation [78, 107]. Indeed, tissue necrosis factor-alpha (TNF-α [alpha]), C-reactive protein (CRP), interleukin 6 (IL-6), plasminogen activator inhibitor-1 (PAI-I), and many other inflammatory cytokines are found in higher concentrations in obese than in lean individuals, and levels are highly correlated with insulin resistance. In fact, TNF-α (alpha) infusion in healthy adults compromised insulin sensitivity within 2 hours [108].

Activation of inflammatory pathways, directly or indirectly, inhibits insulin signaling, ultimately resulting in insulin resistance. Increased inflammatory mediators from obesity can be seen in childhood [101]. In a study of obese adolescents, high visceral fat content was associated with higher macrophage infiltration, and this, in turn, was associated with an increase in expression of inflammation-related genes and a parallel decrease in expression of genes related to insulin sensitivity [109].

Hormones

Both leptin and adiponectin are hormones produced by and secreted from adipocytes, and obesity alters their function. Circulating leptin levels directly reflect the amount of total body adipose tissue. In healthy individuals, activation of leptin receptors in the brain decreases appetite and increases activity of the sympathetic nervous system, serving to both decrease energy intake and increase energy expenditure. Leptin receptors in adipose, hepatic, and muscle tissues stimulate pathways to increase glucose uptake and oxidation. Paradoxically, in obese individuals, high leptin levels do not reduce appetite or increase metabolism. Instead, they seem to be less sensitive to both endogenous and exogenous leptin—a phenomenon known as leptin resistance. Leptin deficiency and leptin resistance both contribute to insulin resistance, likely due to crosstalk between leptin and insulin intracellular signaling pathways [110]. Hyperleptinemia strongly correlates with insulin resistance, even in pediatric cohorts [111–113], and high leptin levels may predict poor cardiometabolic outcomes later in life [114, 115]. In contrast to leptin, adiponectin concentrations are not reflective of adipose tissue mass and instead are lower in children and adults with insulin resistance [116, 117]. A decline in adiponectin levels precedes total body insulin resistance, suggesting a causative relationship [118].

Contribution of Bone

The "bone-fat-pancreas axis" is the concept of cross talk between bone and adipose tissue in energy metabolism. It is now known that bones, particularly osteoblasts, are insulin sensitive [119]. In the setting of obesity, osteoblasts become insulin resistant through many of the same mechanisms described above (local elevated FFA, hyperinsulinemia, etc.). Insulin-resistant osteoblasts form less bone, but also produce less osteocalcin protein, which becomes activated by osteoclasts. Activated osteocalcin is now being recognized as a hormone that, in animal models, appears to be necessary for normal body composition and insulin secretion and sensitivity. Activated osteocalcin directly acts on the pancreas to promote β (beta)-cell proliferation and increase insulin secretion in a feed-forward manner [120, 121]. In muscle and adipose tissue, osteocalcin increases genes that are important for insulin sensitivity and energy expenditure. In adipose tissue, it decreases fat accumulation and increases expression of adiponectin, which exerts its own insulin-sensitizing effects on tissues. Numerous studies report low osteocalcin levels in insulin-resistant populations, including obese children, suggesting a disruption in the feedback between insulin and osteocalcin [122–126]. Adiposity is also negatively associated with bone density and bone strength in youth, likely due to fewer and/or poorer functioning osteoblasts needed for building healthy bone [127–129]. A current gap in knowledge relates to the time course of these relationships. Namely, it has yet to be determined whether slowed early bone development can predispose a person to increased adiposity and insulin resistance, or whether increased adiposity occurs prior to the decreased osteocalcin and resulting impairments in insulin secretion and sensitivity.

Our understanding of the mechanisms responsible for the effects of adiposity on insulin resistance is complex and continues to evolve. Additionally, the mechanisms that cause insulin resistance may not be equivalent to those that persist once insulin resistance is established/maintained over the years. Better understanding of the pathophysiology underlying these relationships, especially early in life, would be for development of early childhood prevention and treatment efforts.

Child Adiposity Is a Predictor of Adult Disease

Large retrospective and prospective trials have shown a strong link between childhood adiposity and the presence of the metabolic syndrome in later life. In a retrospective analysis of data from the United Kingdom, BMI at 2–14 years of age was negatively correlated with insulin sensitivity

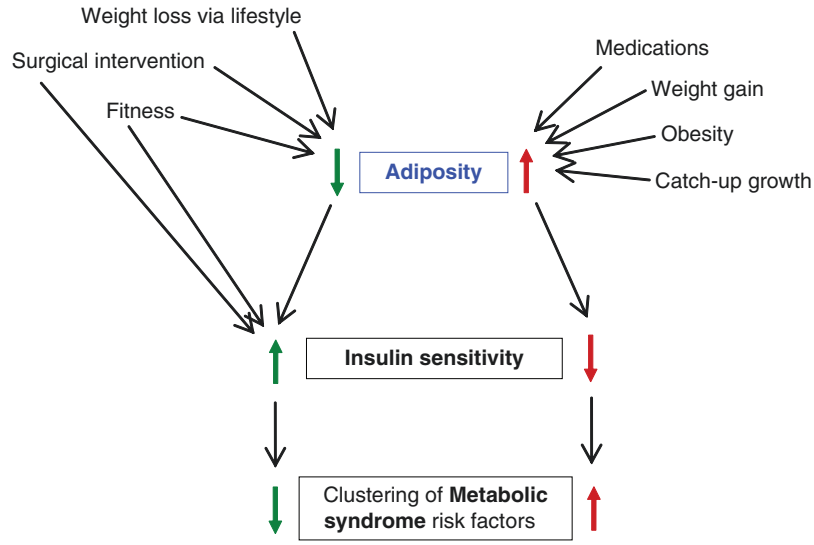

Fig. 9.2 Representation of the factors influencing whole-body adiposity and, henceforth, insulin sensitivity. Factors that increase adiposity act to decrease insulin sensitivity and are associated with the development of metabolic syndrome. The associations shown in this diagram apply through all stages of life—from childhood through adolescence and into adulthood

in lean adults at age 71 [130]. In addition, childhood BMI is the strongest predictive factor for the clustering of metabolic syndrome risk factors in adulthood [131]. In prospective studies, visceral adiposity, measured using waist circumference, and elevated fasting triglycerides at age 9–10 years predict metabolic syndrome in 18- to 19-year-old black and white women [132]. Finally, the large epidemiological Bogalusa Heart Study showed that children with higher body fat (BMI and skinfolds) exhibited increasing fasting glucose concentrations throughout adolescence, as well as greater adiposity, lower insulin sensitivity, and higher blood pressure as adults [133, 134].

The data directly linking early body composition to metabolic syndrome in adults is even more abundant in adolescents. 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 [135]. Adolescent BMI and fasting insulin concentration have been shown to be the strongest predictors of the clustering of metabolic syndrome risk factors at adulthood [131]. Also, decreased cardiovascular health at 27 years of age was dependent on weight gain since age 13 [136]. As all of these factors are intrinsically linked with development of the metabolic syndrome, adolescent adiposity is a good predictor of adult insulin sensitivity and progression to the disease state.

Collectively, these data indicate that early adiposity is strongly associated with the development of insulin resistance, hypertension, and cardiovascular disease in adulthood. Thus, the influence of childhood adiposity on these key risk factors and upon 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 (Fig. 9.2). 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 the metabolic syndrome in adults. Given these data, prevention strategies for the metabolic syndrome could include childhood interventions aimed at decreasing body weight and total adiposity.

How Can We Alter Body Composition?

Given the strong association between adiposity and insulin sensitivity and the plausible mechanisms by which adiposity may directly cause insulin resistance, it is important to understand which factors influencing body composition are modifiable. Interventions that reduce body fat should increase insulin sensitivity and improve metabolic health. Factors postulated to influence

body composition include weight change, exercise, diet/nutrition, hormones, and medications (Fig. 9.2). Medications affecting body weight and composition are discussed in a separate chapter.

Weight Change

It has been established in the adult literature that weight loss, physical activity, or a combination of these factors can positively alter body composition by decreasing total body fat mass, increasing lean mass, and ultimately result in lower body fat percentage. Several recent studies have also demonstrated this in children [137, 138]. Weight loss or gain can also change the distribution of body fat. There are few studies that do not report a change in body composition with significant fluctuations in weight, making weight change one of the most compelling ways to alter body composition.

Even better, weight loss leads to improved insulin sensitivity in both children and adults. In a sample of prepubertal obese boys and girls, significant weight loss over 1 year was associated with an increase in insulin sensitivity as assessed by HOMA and quantitative *insulin* sensitivity check index (QUIKI) assessment [139, 140]. Conversely, children in the same cohort who gained weight over the one-year period suffered significant impairment of insulin sensitivity. In obese middle school children randomized to intensive lifestyle intervention for weight loss or regular physical education classes, the lifestyle group lost weight and decreased total and percent body fat and increased insulin sensitivity [141].

Modest weight loss (5–11% of initial body weight) leads to a significant improvement in insulin sensitivity in adults [87, 142–144]. In all cases, 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 [63], abdominal height [87], or waist circumference [142]. 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 [143]. 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, there is no difference in the total amount of weight lost, fat lost, or change in insulin sensitivity, regardless of the method used to achieve weight loss [144].

Although we have discussed weight loss through lifestyle interventions, bariatric surgery is becoming increasingly common, even in adolescents. Bariatric surgery causes dramatic weight loss, with a majority (more than 75%) of lost weight consisting of fat [145]. In a study of weight-loss surgery in 242 adolescents, remission of pre-existing comorbidities at 3 years was high: 95% remission of diabetes, 76% remission of prediabetes, and 66% remission of dyslipidemia [146]. Although it is likely the enormous decrease in adiposity explains much of the decrease in insulin resistance, we must recognize that mechanisms beyond weight loss are also involved. A systematic review in adults concluded insulin sensitivity improves by 33% within 2 weeks of bariatric surgery, often preceding significant fat loss [147]. The effect of bariatric surgery on insulin resistance outcomes in adolescents is sure to be a dominant area of research in the upcoming decade.

Lifestyle Change

There is unequivocal evidence that diet and physical activity changes lead to weight and adiposity loss [148]. In children and adolescents, there are strong correlations between both diet and physical activity and metabolic health, as well as improvement in body composition and insulin sensitivity. In 8- to 10-year-old Danish children, physical activity (assessed using accelerometers) was independently and negatively associated with metabolic risk factors—notably adiposity and fasting insulin—which were used as surrogate measures of insulin sensitivity [149]. In children, fitness is directly correlated with insulin

sensitivity, total body fat (DXA), and abdominal fat (CT) [150]. However, this relationship between fitness and insulin sensitivity was lost when body fatness was taken into account, indicating that this relationship is, at least in part, mediated by adiposity. 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 decrease in percent body fat was associated with improved insulin sensitivity [151]. In a controlled trial, 44 obese adolescent girls were randomized to 1 of 3 isocaloric treatments, including aerobic exercise, resistance exercise, or no exercise for 3 months [152]. The aerobic exercise group had a significant decrease in visceral adiposity (measured by MRI) and insulin resistance (measured by hyperinsulinemic-euglycemic clamp), providing robust evidence that exercise can alter body composition and insulin sensitivity in youth.

It has been known for many years that dietary intake can effect body composition and is a major contributor to obesity. Hypocaloric diets cause weight and fat loss. It appears that any dietary strategy that decreases adiposity is effective at improving insulin sensitivity. For example, a study assessing the effects of hypocaloric weight loss diets that were either high glycemic index or low glycemic index over 6 months reported that both groups lost similar amounts of body weight and showed similar improvements in insulin sensitivity [153]. A longitudinal observational study of a birth cohort of more than 1000 children in the United Kingdom found poor diet quality at 1 and 3 years of age was strongly associated with higher fat mass (by DXA), but not BMI, at 6 years of age [154]. In children, a strategy to decrease the amount of food consumed away from home results in decreased body weight and adiposity, with overall diet quality, fiber, added sugars, and added fats responsible for observed body fat reductions [155].

Although some of the benefit of lifestyle change on insulin sensitivity can be attributed to reduced body fatness, there is also adiposity-independent effects on insulin sensitivity. It has been shown that a complete lifestyle intervention in obese youth with metabolic syndrome 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 [156]. Likewise, a 16-week resistance training program in overweight adolescent Latino males showed that training improved insulin sensitivity compared to the control group, independent of changes in fat mass or fat-free mass [157]. Utilizing a 12-week aerobic training program in obese girls increased fitness and improved insulin sensitivity, without any change in total body weight or percent body fat [158]. In adults, women with high cardiovascular fitness, whether lean or obese, have only marginally different insulin sensitivity indices despite the obese women having twice as much body fat as lean women [159]. In short, lifestyle interventions have both adiposity-dependent and adiposity-independent effects on insulin sensitivity. Regardless of whether the effects are mediated through reduced adiposity or not, lifestyle changes have robust effects on insulin sensitivity.

Hormones

Clinically, hormone deficiencies can alter body composition. Hypothyroidism, hypogonadism, and growth hormone deficiency all cause increased fat stores and decreased lean mass. Replacement of these hormones to normal concentrations improves body composition. Even without deficiencies, exogenous androgens and growth hormone can change body composition and are frequently abused to gain muscle mass and reduce adiposity. Sex steroids have a complex relationship with body composition, with probable sex differences. For example, although testosterone in men decreases adipose tissue and builds muscle, high testosterone in women is often associated with increased adiposity and insulin resistance (polycystic ovarian syndrome). Likewise, estrogen seems to have benefits on body composition and cardioprotection in women, while this does not seem to be the case for men. In addition, adipose tissue plays a role in regulation of both growth hormone secretion and

sex steroid concentrations, so this relationship is intertwined [160].

Cortisol also alters body composition. Excess cortisol (or exogenous glucocorticoids such as prednisone) results in increased adiposity, particularly central adiposity, along with muscle wasting. Chronic, low-grade hypercortisolemia from physiological or psychological stress may induce adiposity [161] and, because adiposity itself is a stressor, may be a mechanism by which adiposity worsens and contributes to insulin resistance. There is a strong association among cortisol, obesity, and insulin resistance in multiple cohorts. For example, obese adolescent girls have higher cortisol, and this is correlated with visceral adiposity and insulin resistance [162]. Whether hypercortisolemia is truly causative of obesity is plausible but difficult to prove.

Finally, there is emerging evidence that incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP), play crucial roles in adipose metabolism. Manipulating the action of incretins is the target of several medications for type 2 diabetes and could be potential targets for changing body composition in other populations also.

Summary: Adiposity Is the Enemy

There is a strong link between adiposity and insulin sensitivity that shows that adiposity is, indeed, the enemy (Fig. 9.1). This relationship is apparent even during the earliest years of life and persists through the whole of childhood, adolescence, adulthood, and 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 is a key influence on insulin sensitivity and development of the metabolic syndrome—from childhood precursor to adult disease. While adiposity, and, with it, insulin resistance, increases with age, the loss of body fat at any age improves insulin sensitivity. Given these data, prevention strategies for the metabolic syndrome should include childhood interventions aimed at decreasing adiposity. Of those interventions, physical activity has shown the most promise in the pediatric population and could be continued throughout the lifespan.

References

1. Helba M, Binkovitz LA. Pediatric body composition analysis with dual-energy X-ray absorptiometry. Pediatr Radiol. 2009;39(7):647–56.
2. Dempster P, Aitkens S. A new air displacement method for the determination of human body composition. Med Sci Sports Exerc. 1995;27(12):1692–7.
3. Fields DA, Gunatilake R, Kalaitzoglou E. Air displacement plethysmography: cradle to grave. Nutr Clin Pract. 2015;30(2):219–26.
4. Minderico CS, Silva AM, Teixeira PJ, Sardinha LB, Hull HR, Fields DA. Validity of air-displacement plethysmography in the assessment of body composition changes in a 16-month weight loss program. Nutr Metab (Lond). 2006;3:32.
5. Wang JG, Zhang Y, Chen HE, Li Y, Cheng XG, Xu L, et al. Comparison of two bioelectrical impedance analysis devices with dual energy X-ray absorptiometry and magnetic resonance imaging in the estimation of body composition. J Strength Cond Res. 2013;27(1):236–43.
6. Verney J, Schwartz C, Amiche S, Pereira B, Thivel D. Comparisons of a multi-frequency bioelectrical impedance analysis to the dual-energy X-ray absorptiometry scan in healthy young adults depending on their physical activity level. J Hum Kinet. 2015;47:73–80.
7. Eisenmann JC, Heelan KA, Welk GJ. Assessing body composition among 3- to 8-year-old children: anthropometry, BIA, and DXA. Obes Res. 2004;12(10):1633–40.
8. Fors H, Gelander L, Bjarnason R, Albertsson-Wikland K, Bosaeus I. Body composition, as assessed by bioelectrical impedance spectroscopy and dual-energy X-ray absorptiometry, in a healthy paediatric population. Acta Paediatr. 2002;91(7):755–60.
9. Campanozzi A, Dabbas M, Ruiz JC, Ricour C, Goulet O. Evaluation of lean body mass in obese children. Eur J Pediatr. 2008;167(5):533–40.
10. Wan CS, Ward LC, Halim J, Gow ML, Ho M, Briody JN, et al. Bioelectrical impedance analysis to estimate body composition, and change in adiposity, in overweight and obese adolescents: comparison with dual-energy x-ray absorptiometry. BMC Pediatr. 2014;14:249.
11. Kasvis P, Cohen TR, Loiselle SE, Kim N, Hazell TJ, Vanstone CA, et al. Foot-to-foot bioelectrical impedance accurately tracks direction of adiposity change in overweight and obese 7- to 13-year-old children. Nutr Res. 2015;35(3):206–13.

12. Verney J, Metz L, Chaplais E, Cardenoux C, Pereira B, Thivel D. Bioelectrical impedance is an accurate method to assess body composition in obese but not severely obese adolescents. Nutr Res. 2016;36(7):663–70.
13. Talma H, Chinapaw MJ, Bakker B, HiraSing RA, Terwee CB, Altenburg TM. Bioelectrical impedance analysis to estimate body composition in children and adolescents: a systematic review and evidence appraisal of validity, responsiveness, reliability and measurement error. Obes Rev. 2013;14(11):895–905.
14. Tuan NT, Wang Y. Adiposity assessments: agreement between dual-energy X-ray absorptiometry and anthropometric measures in U.S. children. Obesity (Silver Spring). 2014;22(6):1495–504.
15. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. Risk factors and adult body mass index among overweight children: the Bogalusa Heart Study. Pediatrics. 2009;123(3):750–7.
16. de Bruin NC, van Velthoven KA, Stijnen T, Juttmann RE, Degenhart HJ, Visser HK. Body fat and fat-free mass in infants: new and classic anthropometric indexes and prediction equations compared with total-body electrical conductivity. Am J Clin Nutr. 1995;61(6):1195–205.
17. Lingwood BE, Storm van Leeuwen AM, Carberry AE, Fitzgerald EC, Callaway LK, Colditz PB, et al. Prediction of fat-free mass and percentage of body fat in neonates using bioelectrical impedance analysis and anthropometric measures: validation against the PEA POD. Br J Nutr. 2012;107(10):1545–52.
18. Freedman DS, Horlick M, Berenson GS. A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. Am J Clin Nutr. 2013;98(6):1417–24.
19. De Miguel-Etayo P, Moreno LA, Santabarbara J, Martin-Matillas M, Piqueras MJ, Rocha-Silva D, et al. Anthropometric indices to assess body-fat changes during a multidisciplinary obesity treatment in adolescents: EVASYON Study. Clin Nutr. [Clinical Study Research Support, Non-U.S. Gov't]. 2015;34(3):523–8.
20. Suliga E. Visceral adipose tissue in children and adolescents: a review. Nutr Res Rev. 2009;22(2):137–47.
21. Brodie DA, Stewart AD. Body composition measurement: a hierarchy of methods. J Pediatr Endocrinol Metab. 1999;12(6):801–16.
22. Jornayvaz FR, Selz R, Tappy L, Theintz GE. Metabolism of oral glucose in children born small for gestational age: evidence for an impaired whole body glucose oxidation. Metabolism. 2004;53(7):847–51.
23. Ibanez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91(6):2153–8.
24. Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, et al. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. J Clin Endocrinol Metab. 2003;88(8):3645–50.
25. Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M, et al. Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. Am J Clin Nutr. 2005;82(5):980–7.
26. Euser AM, Finken MJ, Keijzer-Veen MG, Hille ET, Wit JM, Dekker FW, et al. Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. Am J Clin Nutr. 2005;81(2):480–7.
27. Finken MJ, Keijzer-Veen MG, Dekker FW, Frolich M, Hille ET, Romijn JA, et al. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. Diabetologia. 2006;49(3):478–85.
28. Bhargava SK, Sachdev HS, Fall CH, Osmond C, Lakshmy R, Barker DJ, et al. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. [see comment]. N Engl J Med. 2004;350(9):865–75.
29. Wadsworth M, Butterworth S, Marmot M, Ecob R, Hardy R. Early growth and type 2 diabetes: evidence from the 1946 British birth cohort. Diabetologia. 2005;48(12):2505–10.
30. Crowther NJ, Cameron N, Trusler J, Gray IP. Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. Diabetologia. 1998;41(10):1163–7.
31. Ong KK, Dunger DB. Birth weight, infant growth and insulin resistance. Eur J Endocrinol. 2004;151(Suppl 3):U131–9.
32. Salgin B, Norris SA, Prentice P, Pettifor JM, Richter LM, Ong KK, et al. Even transient rapid infancy weight gain is associated with higher BMI in young adults and earlier menarche. Int J Obes (Lond). 2015;39(6):939–44.
33. Demerath EW, Reed D, Choh AC, Soloway L, Lee M, Czerwinski SA, et al. Rapid postnatal weight gain and visceral adiposity in adulthood: the Fels Longitudinal Study. Obesity (Silver Spring). 2009;17(11):2060–6.
34. Jeffery AN, Metcalf BS, Hosking J, Murphy MJ, Voss LD, Wilkin TJ. Little evidence for early programming of weight and insulin resistance for contemporary children: EarlyBird Diabetes Study report 19. Pediatrics. 2006;118(3):1118–23.
35. Dwyer T, Blizzard L, Venn A, Stankovich JM, Ponsonby AL, Morley R. Syndrome X in 8-y-old Australian children: stronger associations with current body fatness than with infant size or growth. Int J Obes Relat Metab Disord. 2002;26(10):1301–9.
36. Fabricius-Bjerre S, Jensen RB, Faerch K, Larsen T, Molgaard C, Michaelsen KF, et al. Impact of birth

weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. PLoS One. 2011;6(6):e20595.
37. Garemo M, Palsdottir V, Strandvik B. Metabolic markers in relation to nutrition and growth in healthy 4-y-old children in Sweden. Am J Clin Nutr. 2006;84(5):1021–6.
38. Cruz ML, Goran MI. The metabolic syndrome in children and adolescents. Curr Diab Rep. 2004;4(1):53–62.
39. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, et al. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. [see comment][erratum appears in N Engl J Med 2002 May 30;346(22):1756 Note: Correction of dosage error in abstract.]. N Engl J Med. 2002;346(11):802–10.
40. Lambert M, Paradis G, O'Loughlin J, Delvin EE, Hanley JA, Levy E. Insulin resistance syndrome in a representative sample of children and adolescents from Quebec, Canada. [see comment]. Int J Obes Relat Metab Disord. 2004;28(7):833–41.
41. Ventura AK, Loken E, Birch LL. Risk profiles for metabolic syndrome in a nonclinical sample of adolescent girls. Pediatrics. 2006;118(6):2434–42.
42. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. [see comment]. N Engl J Med. 2004;350(23):2362–74.
43. Rolland-Cachera MF, Deheeger M, Maillot M, Bellisle F. Early adiposity rebound: causes and consequences for obesity in children and adults. Int J Obes (Lond). 2006;30(Suppl 4):S11–7.
44. Krekoukia M, Nassis G, Psarra G, Skenderi K, Chrousos G, Sidossis L. Elevated total and central adiposity and low physical activity are associated with insulin resistance in children. Metabolism. 2007;56:206–13.
45. Tanaka Y, Kikuchi T, Nagasaki K, Hiura M, Ogawa Y, Uchiyama M. Lower birth weight and visceral fat accumulation are related to hyperinsulinemia and insulin resistance in obese Japanese children. Hypertens Res. 2005;28(6):529–36.
46. Tauman R, O'Brien LM, Ivanenko A, Gozal D. Obesity rather than severity of sleep-disordered breathing as the major determinant of insulin resistance and altered lipidemia in snoring children. Pediatrics. 2005;116(1):e66–73.
47. Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH. Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. J Clin Endocrinol Metab. 1995;80(1):172–8.
48. Cook JS, Hoffman RP, Stene MA, Hansen JR. Effects of maturational stage on insulin sensitivity during puberty. J Clin Endocrinol Metab. 1993;77(3):725–30.
49. Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes. 2001;50(11):2444–50.
50. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. Pediatr Res. 2006;60(6):759–63.
51. Roemmich JN, Clark PA, Lusk M, Friel A, Weltman A, Epstein LH, et al. Pubertal alterations in growth and body composition. VI. Pubertal insulin resistance: relation to adiposity, body fat distribution and hormone release. Int J Obes Relat Metab Disord. 2002;26(5):701–9.
52. Wu DM, Chu NF, Shen MH, Wang SC. Obesity, plasma high sensitivity C-reactive protein levels and insulin resistance status among school children in Taiwan. Clin Biochem. 2006;39(8):810–5.
53. Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes. 1999;48(10):2039–44.
54. Ball GD, Weigensberg MJ, Cruz ML, Shaibi GQ, Kobaissi HA, Goran MI. Insulin sensitivity, insulin secretion and beta-cell function during puberty in overweight Hispanic children with a family history of type 2 diabetes. Int J Obes. 2005;29(12):1471–7.
55. Sinaiko AR, Jacobs DR Jr, Steinberger J, Moran A, Luepker R, Rocchini AP, et al. Insulin resistance syndrome in childhood: associations of the euglycemic insulin clamp and fasting insulin with fatness and other risk factors. J Pediatr. 2001;139(5):700–7.
56. Liu J, Wade T, Tan H. Cardiovascular risk factors and anthropometric measurements of adolescent body composition: a cross-sectional analysis of the third National Health and Nutrition Survey. Int J Obes (Lond). 2007;31:59–64.
57. Retnakaran R, Zinman B, Connelly PW, Harris SB, Hanley AJ. Nontraditional cardiovascular risk factors in pediatric metabolic syndrome. [see comment]. J Pediatr. 2006;148(2):176–82.
58. Lee S, Bacha F, Gungor N, Arslanian SA. Waist circumference is an independent predictor of insulin resistance in black and white youths. J Pediatr. 2006;148(2):188–94.
59. Bacha F, Saad R, Gungor N, Arslanian SA. Are obesity-related metabolic risk factors modulated by the degree of insulin resistance in adolescents? Diabetes Care. 2006;29(7):1599–604.
60. Kelsey MM, Zeitler PS. Insulin resistance of puberty. Curr Diab Rep. 2016;16(7):64.
61. RISE Consortium. Metabolic contrasts between youth and adults with impaired glucose tolerance or recently diagnosed type 2 diabetes: I. Observations using the hyperglycemic clamp. Diabetes Care. 2018;41(8):1696–706.
62. Pearce MS, Unwin NC, Parker L, Alberti KG. Life course determinants of insulin secretion and

sensitivity at age 50 years: the Newcastle thousand families study. Diabetes Metab Res Rev. 2006;22(2):118–25.
63. Vega GL, Adams-Huet B, Peshock R, Willett D, Shah B, Grundy SM. Influence of body fat content and distribution on variation in metabolic risk. J Clin Endocrinol Metab. 2006;91(11):4459–66.
64. dos Santos RE, Aldrighi JM, Lanz JR, Ferezin PC, Marone MM. Relationship of body fat distribution by waist circumference, dual-energy X-ray absorptiometry and ultrasonography to insulin resistance by homeostasis model assessment and lipid profile in obese and non-obese postmenopausal women. Gynecol Endocrinol. 2005;21(5):295–301.
65. Bryhni B, Jenssen TG, Olafsen K, Bendikssen A. Oxidative and nonoxidative glucose disposal in elderly vs younger men with similar and smaller body mass indices and waist circumferences. Metabolism. 2005;54(6):748–55.
66. Fields DA, Gilchrist JM, Catalano PM, Gianni ML, Roggero PM, Mosca F. Longitudinal body composition data in exclusively breast-fed infants: a multicenter study. Obesity (Silver Spring). 2011;19(9):1887–91.
67. Slinger J, van Breda E, Keizer H, Rump P, Hornstra G, Kuipers H. Insulin resistance, physical fitness, body composition and leptin concentration in 7–8 year-old children. J Sci Med Sport. 2008;11(2):132–8. (Epub ahead of print).
68. Garnett SP, Hogler W, Blades B, Baur LA, Peat J, Lee J, et al. Relation between hormones and body composition, including bone, in prepubertal children. Am J Clin Nutr. 2004;80(4):966–72.
69. He Q, Horlick M, Thornton J, Wang J, Pierson RN Jr, Heshka S, et al. Sex-specific fat distribution is not linear across pubertal groups in a multiethnic study. Obes Res. 2004;12(4):725–33.
70. Arfai K, Pitukcheewanont PD, Goran MI, Tavare CJ, Heller L, Gilsanz V. Bone, muscle, and fat: sex-related differences in prepubertal children. Radiology. 2002;224(2):338–44.
71. Samsell L, Regier M, Walton C, Cottrell L. Importance of android/gynoid fat ratio in predicting metabolic and cardiovascular disease risk in normal weight as well as overweight and obese children. J Obes. 2014;2014:846578.
72. Aucouturier J, Meyer M, Thivel D, Taillardat M, Duche P. Effect of android to gynoid fat ratio on insulin resistance in obese youth. Arch Pediatr Adolesc Med. 2009;163(9):826–31.
73. Goran MI, Nagy TR, Treuth MS, Trowbridge C, Dezenberg C, McGloin A, et al. Visceral fat in white and African American prepubertal children. Am J Clin Nutr. 1997;65(6):1703–8.
74. Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS, et al. Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab. 2002;87(12):5575–80.
75. Brambilla P, Bedogni G, Moreno LA, Goran MI, Gutin B, Fox KR, et al. Crossvalidation of anthropometry against magnetic resonance imaging for the assessment of visceral and subcutaneous adipose tissue in children. Int J Obes (Lond). 2006;30(1):23–30.
76. Ehtisham S, Crabtree N, Clark P, Shaw N, Barrett T. Ethnic differences in insulin resistance and body composition in United Kingdom adolescents. J Clin Endocrinol Metab. 2005;90(7):3963–9.
77. Whincup PH, Gilg JA, Owen CG, Odoki K, Alberti KG, Cook DG. British South Asians aged 13–16 years have higher fasting glucose and insulin levels than Europeans. Diabet Med. 2005;22(9):1275–7.
78. Castro AV, Kolka CM, Kim SP, Bergman RN. Obesity, insulin resistance and comorbidities? Mechanisms of association. Arq Bras Endocrinol Metabol. 2014;58(6):600–9.
79. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116(1):39–48.
80. Bosch TA, Steinberger J, Sinaiko AR, Moran A, Jacobs DR Jr, Kelly AS, et al. Identification of sex-specific thresholds for accumulation of visceral adipose tissue in adults. Obesity (Silver Spring). 2015;23(2):375–82.
81. Saelens BE, Seeley RJ, van Schaick K, Donnelly LF, O'Brien KJ. Visceral abdominal fat is correlated with whole-body fat and physical activity among 8-y-old children at risk of obesity. Am J Clin Nutr. 2007;85(1):46–53.
82. Guldiken S, Tuncbilek N, Okten O, Arikan E, Tugrul A. Visceral fat thickness determined using ultrasonography is associated with anthropometric and clinical parameters of metabolic syndrome. Int J Clin Pract. 2006;60:1576–81.
83. Owens S, Gutin B, Barbeau P, Litaker M, Allison J, Humphries M, et al. Visceral adipose tissue and markers of the insulin resistance syndrome in obese black and white teenagers. Obes Res. 2000;8(4):287–93.
84. Daniels SR, Jacobson MS, McCrindle BW, Eckel RH, Sanner BM. American Heart Association childhood obesity research summit report. Circulation. 2009;119(15):e489–517.
85. Syme C, Abrahamowicz M, Leonard GT, Perron M, Pitiot A, Qiu X, et al. Intra-abdominal adiposity and individual components of the metabolic syndrome in adolescence: sex differences and underlying mechanisms. Arch Pediatr Adolesc Med. 2008;162(5):453–61.
86. Sierra-Johnson J, Johnson BD, Bailey KR, Turner ST. Relationships between insulin sensitivity and measures of body fat in asymptomatic men and women. Obes Res. 2004;12(12):2070–7.
87. Mazzali G, Di Francesco V, Zoico E, Fantin F, Zamboni G, Benati C, et al. Interrelations between

fat distribution, muscle lipid content, adipocytokines, and insulin resistance: effect of moderate weight loss in older women. Am J Clin Nutr. 2006;84(5):1193–9.
88. Salmenniemi U, Ruotsalainen E, Vanttinen M, Vauhkonen I, Pihlajamaki J, Kainulainen S, et al. High amount of visceral fat mass is associated with multiple metabolic changes in offspring of type 2 diabetic patients. Int J Obes. 2005;29(12):1464–70.
89. Stears A, O'Rahilly S, Semple RK, Savage DB. Metabolic insights from extreme human insulin resistance phenotypes. Best Pract Res Clin Endocrinol Metab. 2012;26(2):145–57.
90. Cali AM, Caprio S. Ectopic fat deposition and the metabolic syndrome in obese children and adolescents. Horm Res. 2009;71(Suppl 1):2–7.
91. Bennett B, Larson-Meyer DE, Ravussin E, Volaufova J, Soros A, Cefalu WT, et al. Impaired insulin sensitivity and elevated ectopic fat in healthy obese vs. nonobese prepubertal children. Obesity (Silver Spring). 2012;20(2):371–5.
92. Pacifico L, Di Martino M, Anania C, Andreoli GM, Bezzi M, Catalano C, et al. Pancreatic fat and beta-cell function in overweight/obese children with non-alcoholic fatty liver disease. World J Gastroenterol. 2015;21(15):4688–95.
93. Salans LB, Knittle JL, Hirsch J. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. J Clin Invest. 1968;47(1):153–65.
94. Stern JS, Batchelor BR, Hollander N, Cohn CK, Hirsch J. Adipose-cell size and immunoreactive insulin levels in obese and normal-weight adults. Lancet. 1972;2(7784):948–51.
95. Henninger AM, Eliasson B, Jenndahl LE, Hammarstedt A. Adipocyte hypertrophy, inflammation and fibrosis characterize subcutaneous adipose tissue of healthy, non-obese subjects predisposed to type 2 diabetes. PLoS One. 2014;9(8):e105262.
96. Ryden M, Andersson DP, Bergstrom IB, Arner P. Adipose tissue and metabolic alterations: regional differences in fat cell size and number matter, but differently: a cross-sectional study. J Clin Endocrinol Metab. 2014;99(10):E1870–6.
97. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab. 2007;92(3):1023–33.
98. Drolet R, Belanger C, Fortier M, Huot C, Mailloux J, Legare D, et al. Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women. Obesity (Silver Spring). 2009;17(3):424–30.
99. Michaud A, Boulet MM, Veilleux A, Noel S, Paris G, Tchernof A. Abdominal subcutaneous and omental adipocyte morphology and its relation to gene expression, lipolysis and adipocytokine levels in women. Metabolism. 2014;63(3):372–81.
100. Gornicka A, Fettig J, Eguchi A, Berk MP, Thapaliya S, Dixon LJ, et al. Adipocyte hypertrophy is associated with lysosomal permeability both in vivo and in vitro: role in adipose tissue inflammation. Am J Physiol Endocrinol Metab. 2012;303(5):E597–606.
101. Landgraf K, Rockstroh D, Wagner IV, Weise S, Tauscher R, Schwartze JT, et al. Evidence of early alterations in adipose tissue biology and function and its association with obesity-related inflammation and insulin resistance in children. Diabetes. 2015;64(4):1249–61.
102. Lessard J, Laforest S, Pelletier M, Leboeuf M, Blackburn L, Tchernof A. Low abdominal subcutaneous preadipocyte adipogenesis is associated with visceral obesity, visceral adipocyte hypertrophy, and a dysmetabolic state. Adipocytes. 2014;3(3):197–205.
103. Van Harmelen V, Rohrig K, Hauner H. Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. Metabolism. 2004;53(5):632–7.
104. Kursawe R, Eszlinger M, Narayan D, Liu T, Bazuine M, Cali AM, et al. Cellularity and adipogenic profile of the abdominal subcutaneous adipose tissue from obese adolescents: association with insulin resistance and hepatic steatosis. Diabetes. 2010;59(9):2288–96.
105. Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, et al. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes Care. 2006;29(6):1337–44.
106. Shadid S, Stehouwer CD, Jensen MD. Diet/exercise versus pioglitazone: effects of insulin sensitization with decreasing or increasing fat mass on adipokines and inflammatory markers. J Clin Endocrinol Metab. 2006;91(9):3418–25.
107. Ye J. Mechanisms of insulin resistance in obesity. Front Med. 2013;7(1):14–24.
108. Nielsen ST, Lehrskov-Schmidt L, Krogh-Madsen R, Solomon TP, Lehrskov-Schmidt L, Holst JJ, et al. Tumour necrosis factor-alpha infusion produced insulin resistance but no change in the incretin effect in healthy volunteers. Diabetes Metab Res Rev. 2013;29(8):655–63.
109. Kursawe R, Dixit VD, Scherer PE, Santoro N, Narayan D, Gordillo R, et al. A role of the inflammasome in the low storage capacity of the abdominal subcutaneous adipose tissue in obese adolescents. Diabetes. 2016;65(3):610–8.
110. Szanto I, Kahn CR. Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. Proc Natl Acad Sci USA. 2000;97(5):2355–60.
111. Gonzaga NC, Medeiros CC, de Carvalho DF, Alves JG. Leptin and cardiometabolic risk factors in obese

children and adolescents. J Paediatr Child Health. 2014;50(9):707–12.
112. Jois A, Navarro P, Ortega-Senovilla H, Gavela-Perez T, Soriano-Guillen L, Garces C. Relationship of high leptin levels with an adverse lipid and insulin profile in 6–8 year-old children in Spain. Nutr Metab Cardiovasc Dis. 2015;25(12):1111–6.
113. Catli G, Anik A, Tuhan HU, Kume T, Bober E, Abaci A. The relation of leptin and soluble leptin receptor levels with metabolic and clinical parameters in obese and healthy children. Peptides. 2014;56:72–6.
114. Miras M, Ochetti M, Martin S, Silvano L, Sobrero G, Castro L, et al. Serum levels of adiponectin and leptin in children born small for gestational age: relation to insulin sensitivity parameters. J Pediatr Endocrinol Metab. 2010;23(5):463–71.
115. Martinez-Aguayo A, Capurro T, Pena V, Iniguez G, Hernandez MI, Avila A, et al. Comparison of leptin levels, body composition and insulin sensitivity and secretion by OGTT in healthy, early pubertal girls born at either appropriate- or small-for-gestational age. Clin Endocrinol (Oxf). 2007;67(4):526–32.
116. Bacha F, Saad R, Gungor N, Arslanian SA. Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care. 2004;27(2):547–52.
117. Lee S, Bacha F, Gungor N, Arslanian SA. Racial differences in adiponectin in youth: relationship to visceral fat and insulin sensitivity. Diabetes Care. 2006;29(1):51–6.
118. Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Weyer C, Lindsay RS, et al. Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. Diabetes. 2002;51(6):1884–8.
119. Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell. 2010;142(2):296–308.
120. Levinger I, Lin X, Zhang X, Brennan-Speranza TC, Volpato B, Hayes A, et al. The effects of muscle contraction and recombinant osteocalcin on insulin sensitivity ex vivo. Osteoporos Int. 2016;27(2):653–63.
121. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone. 2012;50(2):568–75.
122. Saleem U, Mosley TH Jr, Kullo IJ. Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. Arterioscler Thromb Vasc Biol. 2010;30(7):1474–8.
123. Reinehr T, Roth CL. A new link between skeleton, obesity and insulin resistance: relationships between osteocalcin, leptin and insulin resistance in obese children before and after weight loss. Int J Obes (Lond). 2010;34(5):852–8.
124. Garanty-Bogacka B, Syrenicz M, Rac M, Krupa B, Czaja-Bulsa G, Walczak M, et al. Association between serum osteocalcin, adiposity and metabolic risk in obese children and adolescents. Endokrynol Pol. 2013;64(5):346–52.
125. Poomthavorn P, Nantarakchaikul P, Mahachoklertwattana P, Chailurkit LO, Khlairit P. Effects of correction of vitamin D insufficiency on serum osteocalcin and glucose metabolism in obese children. Clin Endocrinol (Oxf). 2014;80(4):516–23.
126. Kanazawa I, Yamaguchi T, Tada Y, Yamauchi M, Yano S, Sugimoto T. Serum osteocalcin level is positively associated with insulin sensitivity and secretion in patients with type 2 diabetes. Bone. 2011;48(4):720–5.
127. Gao Y, Zong K, Gao Z, Rubin MR, Chen J, Heymsfield SB, et al. Magnetic resonance imaging-measured bone marrow adipose tissue area is inversely related to cortical bone area in children and adolescents aged 5–18 years. J Clin Densitom. [Research Support, N.I.H., Extramural]. 2015;18(2):203–8.
128. Cole ZA, Harvey NC, Kim M, Ntani G, Robinson SM, Inskip HM, et al. Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children. Bone. [Research Support, Non-U.S. Gov't]. 2012;50(2):562–7.
129. Dimitri P, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. J Bone Miner Res. [Research Support, Non-U.S. Gov't]. 2010;25(3):527–36.
130. Martin RM, Holly JM, Davey Smith G, Gunnell D. Associations of adiposity from childhood into adulthood with insulin resistance and the insulin-like growth factor system: 65-year follow-up of the Boyd Orr Cohort. J Clin Endocrinol Metab. 2006;91(9):3287–95.
131. Srinivasan SR, Myers L, Berenson GS. Predictability of childhood adiposity and insulin for developing insulin resistance syndrome (syndrome X) in young adulthood: the Bogalusa Heart Study. Diabetes. 2002;51(1):204–9.
132. Morrison JA, Friedman LA, Harlan WR, Harlan LC, Barton BA, Schreiber GB, et al. Development of the metabolic syndrome in black and white adolescent girls: a longitudinal assessment. Pediatrics. 2005;116(5):1178–82.
133. Srinivasan SR, Myers L, Berenson GS. Changes in metabolic syndrome variables since childhood in prehypertensive and hypertensive subjects: the Bogalusa Heart Study. [see comment]. Hypertension. 2006;48(1):33–9.
134. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. The relation of child-

hood BMI to adult adiposity: the Bogalusa Heart Study. Pediatrics. 2005;115(1):22–7.
135. Eisenmann JC, Welk GJ, Wickel EE, Blair SN, Aerobics Center Longitudinal Study. Stability of variables associated with the metabolic syndrome from adolescence to adulthood: the Aerobics Center Longitudinal Study. Am J Hum Biol. 2004;16(6):690–6.
136. Sivanandam S, Sinaiko AR, Jacobs DR Jr, Steffen L, Moran A, Steinberger J. Relation of increase in adiposity to increase in left ventricular mass from childhood to young adulthood. Am J Cardiol. 2006;98(3):411–5.
137. Treuth MS, Hunter GR, Figueroa-Colon R, Goran MI. Effects of strength training on intra-abdominal adipose tissue in obese prepubertal girls. Med Sci Sports Exerc. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. 1998;30(12):1738–43.
138. Figueroa-Colon R, Mayo MS, Aldridge RA, Winder T, Weinsier RL. Body composition changes in Caucasian and African American children and adolescents with obesity using dual-energy X-ray absorptiometry measurements after a 10-week weight loss program. Obes Res. [Research Support, U.S. Gov't, P.H.S.]. 1998;6(5):326–31.
139. Reinehr T, Kiess W, Kapellen T, Andler W. Insulin sensitivity among obese children and adolescents, according to degree of weight loss. [see comment]. Pediatrics. 2004;114(6):1569–73.
140. Reinehr T, Roth CL, Menke T, Andler W. Resistin concentrations before and after weight loss in obese children. Int J Obes. 2006;30(2):297–301.
141. Carrel AL, Clark RR, Peterson SE, Nemeth BA, Sullivan J, Allen DB. Improvement of fitness, body composition, and insulin sensitivity in overweight children in a school-based exercise program: a randomized, controlled study. [see comment]. Arch Pediatr Adolesc Med. 2005;159(10):963–8.
142. Racette SB, Evans EM, Weiss EP, Hagberg JM, Holloszy JO. Abdominal adiposity is a stronger predictor of insulin resistance than fitness among 50–95 year olds. Diabetes Care. 2006;29(3):673–8.
143. Dengel DR, Kelly AS, Olson TP, Kaiser DR, Dengel JL, Bank AJ. Effects of weight loss on insulin sensitivity and arterial stiffness in overweight adults. Metabolism. 2006;55(7):907–11.
144. Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, et al. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr. 2006;84(5):1033–42.
145. Carey DG, Pliego GJ, Raymond RL, Skau KB. Body composition and metabolic changes following bariatric surgery: effects on fat mass, lean mass and basal metabolic rate. Obes Surg. 2006;16(4):469–77.
146. Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Helmrath MA, Brandt ML, et al. Weight loss and health status 3 years after bariatric surgery in adolescents. N Engl J Med. 2016;374(2):113–23.
147. Rao RS, Yanagisawa R, Kini S. Insulin resistance and bariatric surgery. Obes Rev. 2012;13(4):316–28.
148. Sun Y, You W, Almeida F, Estabrooks P, Davy B. The effectiveness and cost of lifestyle interventions including nutrition education for diabetes prevention: a systematic review and meta-analysis. J Acad Nutr Diet. [Meta-Analysis Research Support, N.I.H., Extramural Review]. 2017;117(3):404–21.e36.
149. Brage S, Wedderkopp N, Ekelund U, Franks PW, Wareham NJ, Andersen LB, et al. Features of the metabolic syndrome are associated with objectively measured physical activity and fitness in Danish children: the European Youth Heart Study (EYHS). Diabetes Care. 2004;27(9):2141–8.
150. Lee S, Bacha F, Gungor N, Arslanian SA. Cardiorespiratory fitness in youth: relationship to insulin sensitivity and beta-cell function. Obesity. 2006;14(9):1579–85.
151. Balagopal P, George D, Patton N, Yarandi H, Roberts WL, Bayne E, et al. Lifestyle-only intervention attenuates the inflammatory state associated with obesity: a randomized controlled study in adolescents. [see comment]. J Pediatr. 2005;146(3):342–8.
152. Lee S, Deldin AR, White D, Kim Y, Libman I, Rivera-Vega M, et al. Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. Am J Physiol Endocrinol Metab. 2013;305(10):E1222–9.
153. Visuthranukul C, Sirimongkol P, Prachansuwan A, Pruksananonda C, Chomtho S. Low-glycemic index diet may improve insulin sensitivity in obese children. Pediatr Res. [Randomized Controlled Trial Research Support, Non-U.S. Gov't]. 2015;78(5):567–73.
154. Okubo H, Crozier SR, Harvey NC, Godfrey KM, Inskip HM, Cooper C, et al. Diet quality across early childhood and adiposity at 6 years: the Southampton Women's Survey. Int J Obes (Lond). 2015;39(10):1456–62.
155. Altman M, Cahill Holland J, Lundeen D, Kolko RP, Stein RI, Saelens BE, et al. Reduction in food away from home is associated with improved child relative weight and body composition outcomes and this relation is mediated by changes in diet quality. J Acad Nutr Diet. [Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural]. 2015;115(9):1400–7.
156. Chen AK, Roberts CK, Barnard RJ. Effect of a short-term diet and exercise intervention on metabolic syndrome in overweight children. Metabolism. 2006;55(7):871–8.

157. Shaibi GQ, Cruz ML, Ball GD, Weigensberg MJ, Salem GJ, Crespo NC, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. Med Sci Sports Exerc. 2006;38(7):1208–15.
158. Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA, Yannakoulia M, et al. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. Metabolism. 2005;54(11):1472–9.
159. Gerson LS, Braun B. Effect of high cardiorespiratory fitness and high body fat on insulin resistance. Med Sci Sports Exerc. 2006;38(10):1709–15.
160. Casanueva FF, Dieguez C. 7 Interaction between body composition, leptin and growth hormone status. Baillieres Clin Endocrinol Metab. 1998;12(2):297–314.
161. Schorr M, Lawson EA, Dichtel LE, Klibanski A, Miller KK. Cortisol measures across the weight spectrum. J Clin Endocrinol Metab. 2015;100(9):3313–21.
162. Misra M, Bredella MA, Tsai P, Mendes N, Miller KK, Klibanski A. Lower growth hormone and higher cortisol are associated with greater visceral adiposity, intramyocellular lipids, and insulin resistance in overweight girls. Am J Physiol Endocrinol Metab. 2008;295(2):E385–92.

Ectopic Fat and Insulin Resistance in Youth

10

Giuseppina Rosaria Umano and Sonia Caprio

Introduction

The unabated rise in the prevalence of pediatric obesity represents a major health issue of the twenty-first century [1, 2]. In the United States, the most recent data from the National Health and Nutrition Examination Survey (NHANES) reported a mean prevalence of 17% in children and adolescents [2], being higher among youth aged 12–19 years (20.8%) [1]. Moreover, it has been reported that the increase in prevalence from 1999 to 2014 mainly depends on children aged 6–19 years, while the rate tends to be stable among children aged 2–5 years [2]. Economically, obesity is costly not only due to the direct medical costs of treating the large number of affected youth [3], but also for treating its indirect sequelae [4]. In fact, obese youth are more prone to develop impaired glucose tolerance [5], type 2 diabetes (T2D) [6], dyslipidemia [6], hypertension [6], and cardiovascular disease (CVD) in adulthood [7].

The pathogenic link between obesity and its related comorbidities is insulin resistance (IR) [8]. Insulin signaling is essential for glucose uptake in insulin-responsive organs, such as skeletal muscle and liver. The persistence over time of IR might impair β(beta)-cell function leading to hyperglycemia and finally to T2D [9]. Thus, the prevention of IR is a key point in the natural history of obesity-related sequelae.

Furthermore, a growing body of evidence has pointed out the existence of two different metabolic phenotypes among obese adults [10, 11] and children [12, 13], also referred to as metabolically healthy and metabolically unhealthy obese (MHO and MUO, respectively). The MHO phenotype is characterized by a relatively higher insulin sensitivity and lower prevalence of comorbidity than the MUO [10, 12]. Among the predictors of MHO, waist circumference is negatively associated with insulin sensitivity independent of obesity both in adults and in children [10, 12]. This evidence supports the hypothesis that body fat distribution, rather than total adiposity, is the major determinant of IR.

In this chapter, we review the evidence for the association between ectopic fat depots and obesity-related comorbidities in youth. Moreover, we describe the molecular pathways involved in the link between ectopic lipid accumulation and

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IR. Finally, we review the possible biological mechanisms that underlie ectopic fat deposition.

Diagnostic Tools for Body Fat Distribution

The method by which fat deposition is assessed is crucial for the reliability of measurement. The growing evidence that body fat distribution rather than total adiposity per se plays a major role in IR has led to the development of noninvasive techniques for characterizing these depots (see Table 10.1).

Anthropometry

Waist circumference measurement, waist-to-hip ratio, and waist-to-height ratio are easily accessible and cost-effective methods to assess visceral adiposity. Moreover, anthropometric measurements do not expose the patient to radiation. These measurements have been reported to correlate with body mass index (BMI) and to be predictive of prediabetes and other components of metabolic syndrome in children and adolescents [14, 15]. Nevertheless, anthropometric measurements have a low interindividual reproducibility and do not allow the clinicians to distinguish between fat and lean body mass, or to assess ectopic fat deposition.

Bioimpedance

Bioelectrical impedance analysis (BIA) [16] allows distinction between fat and lean body mass. It is a cost-effective and easily accessible method and has a good reproducibility. It has been widely used for body composition analysis, but it is not useful for body fat distribution or ectopic fat deposition assessment [17]. Moreover, it has been shown to be less reliable than dual-energy X-ray absorptiometry (DEXA) for body fat composition analysis in obese youth [18].

Dual-Energy X-Ray Absorptiometry

DEXA characterizes lean, fat, and bone mineral mass with high accuracy. It is more accurate than BIA and anthropometry for total body fat estimation [17, 18] and has a good reliability. Nevertheless, it is unable to distinguish among the different fat depots and exposes the patient to a small amount of radiation [19].

Table 10.1 Diagnostic tools for body fat distribution

Method	Fat depot	Pros	Cons
Anthropometry (waist circumference, waist-to-hip ratio, waist-to-height ratio)	Indirect measures of visceral adiposity and body fat distribution	Easily accessible, cost-effective, no radiation	Do not allow to characterize body fat composition and ectopic fat deposition, poor reproducibility
BIA	Whole body composition	Easily accessible, cost-effective, no radiation	Does not detect ectopic fat depots, poor accuracy and specificity
DEXA	Whole body composition and abdominal fat depot	Easily accessible, cost-effective, good accuracy in body composition	Does not distinguish between visceral and subcutaneous abdominal fat, small amount of radiations, lower accuracy in severe obese patients
US	Liver fat content and subcutaneous fat	Easily accessible, cost-effective, no radiation	Poor reproducibility, poor accuracy in obese patients
MRI	Whole body composition, ectopic fat deposition in all tissues	No radiations	Expensive, time-consuming, not easily accessible

BIA bioelectrical impedance analysis, *DEXA* dual-energy X-ray absorptiometry, *US* ultrasound, *MRI*, magnetic resonance imaging

Ultrasound

Ultrasound (US) is commonly used in clinical practice for screening of nonalcoholic fatty liver disease (NAFLD). This imaging tool is less expensive, noninvasive, and does not expose the patient to radiation. However, it is less reliable and accurate than magnetic resonance imaging (MRI) [20]. Moreover, it has been shown that US overestimates the presence of liver fat content in severely obese youth, while it has a good negative predictive value for the disease [21]. Thus, US should not be used for diagnosis and grading of NAFLD [20]. Moreover, the technique is not able to quantify visceral or skeletal muscle adiposity.

Magnetic Resonance Imaging

MRI techniques use different resonance responses of the tissues to magnetic fields to characterize the fat content. It is the only nonradiation technique that allows quantification of total adipose tissue and its subdepots. It has been widely used in research for assessing visceral and subcutaneous adipose tissue [19]. Moreover, it has been shown to have a good accuracy in detecting liver fat content compared to liver histology in youth [20]. Additionally, with the proton magnetic spectroscopy (^{1}H-MRS) technique, it is possible to evaluate ectopic fat deposition in muscle and liver. ^{1}H-MRS is the only noninvasive technique that differentiates between the intramyocellular and extramyocellular lipid contents [19]. Nevertheless, the technique is expensive and time-consuming, so its use in clinical practice is limited.

Visceral Fat Accumulation

Visceral adipose tissue (VAT) refers to intra-abdominal fat that surrounds internal organs and is deposited in greater and lesser omentum. Subcutaneous adipose tissue (SAT) is stored under the skin, and in the abdominal region the *fascia superficialis* separates the superficial layer from the deep layer of SAT. The deep layer has been more closely correlated with IR than superficial SAT [22]. VAT and SAT differ in their biology, and several studies have evaluated their role in determining obesity-related comorbidities and IR in both adults and children [23–30].

Findings from the Framingham Heart Study (FHS), a study based on a large cohort of the U.S. adults of European descent, have shown that VAT and SAT are differently correlated with metabolic risk factors, including dyslipidemia and hypertension [31]. The authors also examined the association between abdominal SAT, VAT, and various measures of IR and found that both VAT and SAT were correlated with fasting insulin, proinsulin, and homeostasis model assessment (HOMA) [32]. However, VAT showed a stronger correlation with these metabolic parameters than SAT, especially among younger subjects [23]. Similarly, in the Jackson Heart Study [24] cohort of African Americans, VAT was a stronger correlate than SAT for most cardiometabolic risk factors independent of BMI [33]. Moreover, VAT was inversely correlated with serum adiponectin in African-American women, while SAT showed a positive correlation [24].

Similar findings have been reported for the pediatric population. Bennett et al. reported a significantly higher prevalence of metabolic syndrome and IR in obese prepubertal children compared to lean prepubertal children. Notably, obese children showed a higher VAT and intrahepatic fat, and these two depots significantly correlated with the degree of IR, independent of BMI [34].

Several studies have proposed the ratio of VAT to SAT (VAT/SAT) as a better predictor of insulin resistance rather than total amount of VAT or overall adiposity [25–27]. The VAT/SAT ratio describes the proportion of visceral to subcutaneous abdominal fat and defines the propensity to store the fat viscerally, thus it better characterizes the subject's body fat distribution. Kaess et al. have reported a direct correlation between high VAT/SAT ratio and higher prevalence of cardiometabolic risk factors in a group of adults [25]. Subjects with a high VAT/SAT ratio showed higher prevalence of dyslipidemia,

elevated systolic and diastolic blood pressure, and IR independently from total VAT [25]. Moreover, Gastaldelli et al. found that VAT/SAT was inversely correlated with β-cell function assessed by a 3-hour oral glucose tolerance test (OGTT) [26]. In this cohort of nondiabetic adults, the VAT/SAT ratio was directly correlated with fasting insulin and insulin secretion rates, suggesting a higher hormone release both in basal and stimulated conditions [26]. Similarly, in a population of 36 adult males with T2D, high VAT/SAT ratios were associated with a reduced suppression of endogenous glucose production during euglycemic hyperinsulinemic clamp [27]. These studies support the hypothesis that high VAT/SAT is an important risk factor for adverse cardiometabolic profile. Recently, Gyllenhammer and colleagues reported that, in a cohort of Hispanic youth, changes in visceral fat and intrahepatic fat content were negatively correlated with β-cell function and features of IR over 2 years of follow-up. Conversely, changes in SAT were positively correlated with improvement of β-cell function in obese youth [30]. Moreover, Cali et al. reported that an increased relative proportion of VAT to SAT (VAT/[VAT+SAT]) in obese children and adolescents was associated with higher hepatic fat content and IR and lower high-density lipoprotein (HDL) [29]. Additionally, the authors observed that with increasing degrees of the relative proportion of VAT, there was a significant increased risk for metabolic syndrome [29]. Similarly, Taksali et al. enrolled 118 obese adolescents who were stratified into tertiles according to VAT/(VAT+SAT) distribution [28]. They observed that, as the relative proportion of VAT to SAT increased, insulin sensitivity was reduced independently of total adiposity. Additionally, serum adiponectin and leptin showed a different profile in the three tertile groups; in fact, the obese adolescents belonging to the third VAT/(VAT+SAT) tertile group showed lower adiponectin and total leptin levels compared to adolescents in the first tertile. The higher risk of showing metabolic syndrome was confirmed in this study [28]. These findings suggest that expandability of SAT instead of VAT exerts a protective action against IR in obese adolescents. Indeed, the same group reported that adolescents with a high VAT/(VAT+SAT) ratio showed impaired adipogenesis/lipogenesis and a lower quantity of large adipocytes [35]. Along with the insulin-resistant phenotype that characterizes the high ratio group, the in vivo analyses showed impaired expression of genes involved in the regulation of insulin signaling and de novo lipogenesis. Thus, it might be suggested that this inability to store fat in the subcutaneous depot enhances the flux of free fatty acids (FFA) toward ectopic tissues, such as VAT, liver, and muscle. The key differences that characterize the two distinct endophenotypes in the obese adolescents with a high or a low VAT/(VAT+SAT) are illustrated in Fig. 10.1.

The endocrine function of adipose tissue has been well recognized [36]. Adipocytes actively secrete several hormones that influence whole body insulin sensitivity, for example, leptin, adiponectin, resistin, interleukin 6 (IL-6), and plasminogen activator inhibitor 1 (PAI-1) [36]. Leptin is secreted in direct proportion to adipocyte mass. SAT is a relatively higher source of leptin. This hormone acts as a peripheral sensor for energy storage, thus in the case of adipose tissue hypertrophy, leptin induces satiety and enhances energy expenditure. Moreover, it has been shown to induce lipid β oxidation in liver and pancreas; thus, it is a protective hormone against IR. Nevertheless, it has been shown that essential obesity is characterized by leptin resistance [36]. Conversely, adiponectin is paradoxically reduced in obese subjects. Adiponectin is an adipocyte-derived hormone whose receptors are highly expressed in skeletal muscle and liver [36]. Adiponectin has been shown to reduce inflammation and IR. In particular, its signaling in the liver is associated with increased fatty acid oxidation, reduced FFA influx, and suppression of hepatic glucose production. In skeletal muscle, adiponectin activates fatty acid oxidation and glucose uptake. Moreover, it has been shown to counteract atherogenesis through several mechanisms: lower macrophage activation, lower proliferation, and migration of smooth muscle cells, and increased nitric oxide production [36]. The typical obesity-related adipose tissue hypertrophy alters the

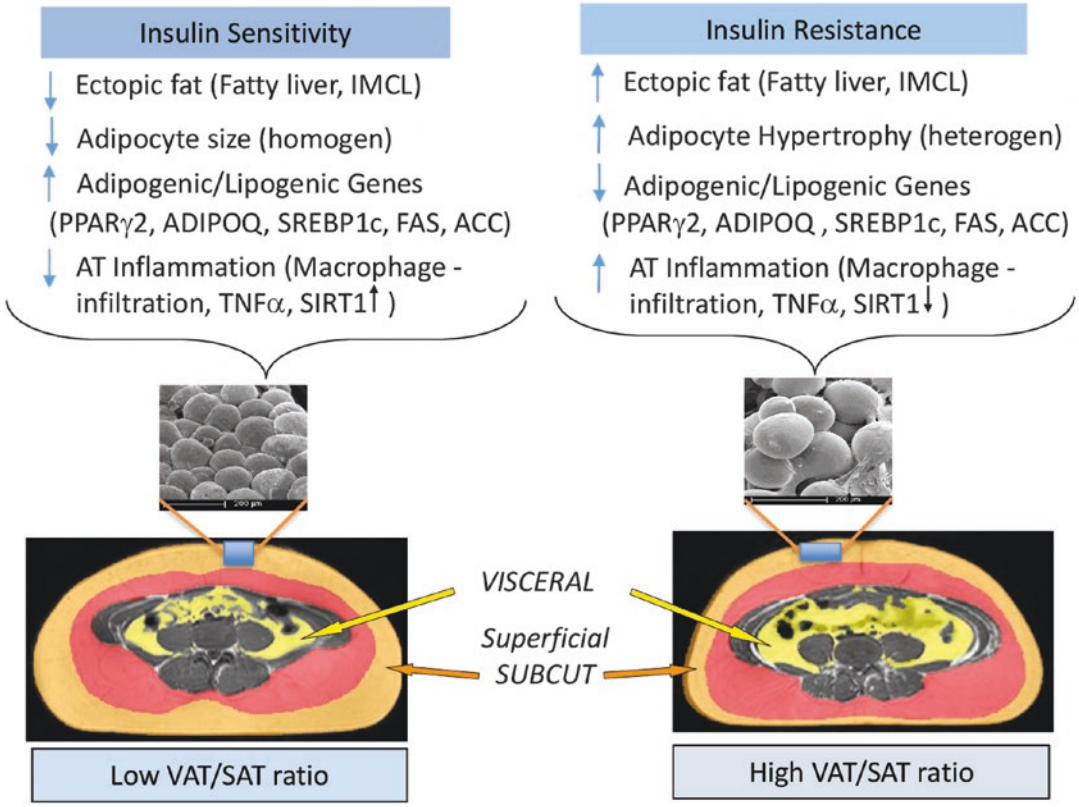

Fig. 10.1 Major differences between the two phenotypes of low and high VAT/SAT ratio in obese adolescents. Low VAT/SAT ratio is associated with lower ectopic fat deposition, lower homogeneity of adipocytes' size, higher expression of lipogenic genes, and lower adipose tissue inflammation compared to the high VAT/SAT ratio phenotype. *Abbreviations:* VAT visceral adipose tissue, SAT subcutaneous adipose tissue, IMCL intramyocellular lipid

secretion pattern of these hormones leading to IR. VAT and SAT show different secretion patterns, possibly explaining why they exert different effects on IR [37]. VAT is more negatively associated with adiponectin levels than SAT and more positively associated with proinflammatory cytokine secretion. Altogether, these differences might account for a stronger association of VAT with IR.

Another possible link between adipose tissue and IR is impaired FFA metabolism. It is known that both SAT and VAT release FFA into the circulation, and elevated levels of FFA have been associated with IR [38]. Physiologically, adipose protects other tissues from FFA accumulation after daily lipid intake. Postprandial lipids reach adipose tissues where lipoprotein lipase (LPL) converts them into FFA that usually are stored in adipocytes and reconverted into triglycerides. In hypertrophic adipose tissue, adipocytes are unable to store and reconvert FFA, leading to an increased flux of FFA into the systemic circulation [38]. Elevated FFA levels have several adverse metabolic effects, including inhibition of insulin-stimulated glucose uptake, glycogen synthesis, and glucose oxidation [38]. VAT and SAT show a different pattern of FFA metabolism. Visceral fat cells have a more pronounced lipolytic activity than subcutaneous fat cells and are less sensitive to the antilipolytic and fatty acid re-esterification effects of insulin [39]. This phenomenon might further enhance FFA secretion in subjects that tend to store fat viscerally. Furthermore, the circulation of visceral fat depots leads directly into the portal vein, resulting in greater FFA delivery to the liver in viscerally obese individuals and to increased hepatic insulin resistance.

Hepatic Fat Deposition

Several studies support the association between intrahepatic lipid content (HFF%) and metabolic derangement in children and adolescents [40–43]. The first evidence came from the comparison between obese children with nonalcoholic fatty liver disease (NAFLD) and obese children without NAFLD. The presence of a 5% fat infiltration in hepatocytes at biopsy defines fatty liver disease [44]. In 2005, Szczepaniak et al. assessed intrahepatic fat content on 345 subjects without any risk factors for NAFLD to characterize the distribution of liver fat infiltration in healthy subjects [45]. Since then, it has been established that HFF% ≥5.5% is the threshold to define NAFLD by MRI [45]. NAFLD is the most common chronic liver disease in Western countries in adults and children and is associated with higher risk of end-stage liver disease and mortality [46]. However, it is also associated with other nonhepatic comorbidities. In fact, obese children and adolescents with fatty liver show a more pronounced proatherogenic lipid profile, characterized by low high-density lipoproteins, high small dense low-density lipoprotein, and large very low-density lipoprotein [40, 41]. Moreover, increased serum alanine transaminase, a surrogate of NAFLD, has been associated with T2D in children and adolescents [47], since NAFLD has been reported to be the hepatic manifestation of IR [16]. Whether NAFLD is a cause or a consequence of IR is still unclear. However, the association between HFF% and hyperglycemia is already present in the early stage of the disease. Cali et al. reported that obese youth with high HFF% showed higher rates of prediabetes, that is, impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) [42]. In this cohort, the HFF% was inversely correlated with whole body insulin sensitivity and serum adiponectin, independent of the degree of obesity. Moreover, the authors observed a parallel rise in metabolic syndrome rates with the increase of HFF% [29]. Similarly, Toledo and coworkers reported that obese adolescents with IGT showed significantly higher HFF% than normoglycemic obese adolescents [43]. Furthermore, the HFF% was predictive of IGT independent of total adiposity and VAT in Latino adolescents, but not in African Americans [43]. The influence of fatty liver on IR seems to start during the early stages of life. In fact, IR and fasting insulin have been positively associated with intrahepatic fat content and intramyocellular lipid content in prepubertal children [48]. Fatty liver infiltration is associated with lower whole body insulin sensitivity as well as higher hepatic insulin resistance. D'Adamo et al. have reported that obese children with high and low HFF% showed a similar hepatic glucose production suppression in the basal condition, while children with high HFF% had impaired suppression after a low-dose insulin infusion compared to obese adolescents with low HFF% [49]. It should be emphasized that the two groups did not differ in VAT or intramyocellular (IMCL) fat content, thus the author concluded that hepatic steatosis exerts an independent effect on IR [49]. The NAFLD phenotype tends to be stable over time and to affect future metabolic outcomes in youth. In a group of 76 obese children and adolescents who were longitudinally followed for an average of 2 years, the baseline HFF% was predictive of the presence of NAFLD at follow-up. Moreover, baseline hepatic fat content was strongly predictive of impaired insulin sensitivity, 2-hour glucose levels, adiponectin levels, and β-cell function at follow-up [50].

Altogether, these data strongly suggest that hepatic steatosis plays a key role in the development of local IR—probably impairing intracellular insulin signaling. Insulin signaling starts with the activation of the insulin receptor tyrosine kinase that phosphorylates the insulin receptor substrate 1 (IRS-1). Then, IRS-1 starts a kinase cascade that transmits the insulin signal, culminating in activation of glycogen synthesis and suppression of glucose production [51]. Tyrosine phosphorylation of IRS-1 is critical for its activity. In mice, high intracellular lipid content has been associated with increased hepatic diacylglycerol (DAG) levels. Hepatic DAG activates the epsilon form of protein kinase C (PKCε) that leads to serine–threonine phosphorylation of IRS-1 and IRS-2, ultimately inhibiting them [52]. The role of PKCε in hepatic IR has been further

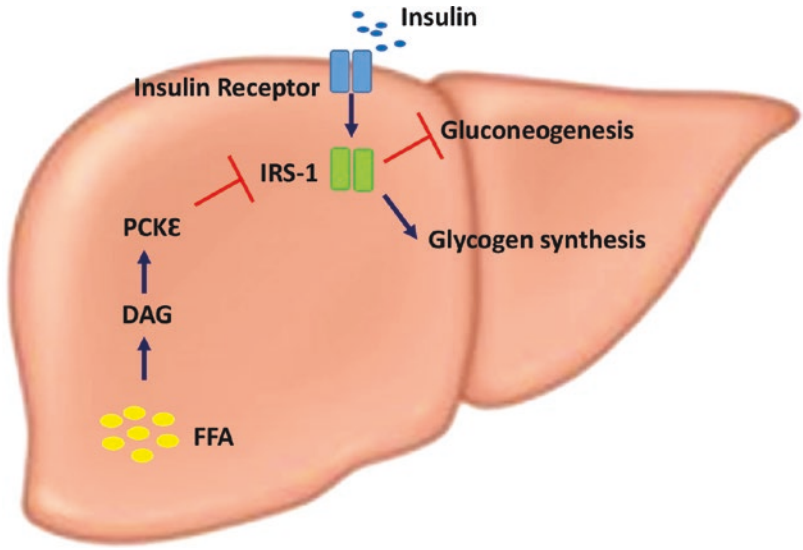

Fig. 10.2 Mechanisms of insulin resistance in liver. Insulin binds its receptors in the liver that activates IRS-1 that in turn activates a kinase cascade that culminates in promotion of glycogen synthesis and suppression of gluconeogenesis. The high amount of lipids in the hepatocytes increases the concentration of DAG that activates PCKε, a serine–threonine kinase that inhibits IRS-1. *Abbreviations:* IRS-1 insulin receptor substrate 1, DAG diacylglycerol, PCKε protein kinase C epsilon, FFA free fatty acids

validated in a knockout mice model. In this model, high-fat-fed knockout mice showed no impaired hepatic insulin activity despite similar intrahepatic lipid content as control and knockdown PKCε mice [53] (see Fig. 10.2).

Additionally, it has been shown that in insulin-resistant subjects, hyperinsulinemia depends both on higher secretion and reduced hepatic clearance [54]. Hepatic insulin extraction accounts for a reduction of approximately 50% of pancreatic insulin, thus leading to a substantial increase of plasma insulin levels. Animal models have shown that infusion of physiological FFA concentrations leads to a decline of insulin clearance in the liver [55]. Probably, FFAs interfere with insulin receptor activity, leading to a reduced receptor turnover and impairment of its signaling via PKC. These mechanisms result in low-insulin binding and internalization in the liver [56].

In conclusion, hepatic steatosis plays a key role in the development of local IR by way of intracellular impairment of insulin signal transduction pathways and a compensatory hyperinsulinemia that may lead to eventual β-cell stress and demise and ultimately to T2D.

Intramyocellular Fat Deposition

The first evidence supporting a relationship between intramyocellular lipid (IMCL) and IR was provided by Falholt et al. who found increased triglyceride content in skeletal muscle in normoglycemic, hyperinsulinemic dogs with low plasma triglycerides [57]. In 1991, Storlien et al. reported that high triglyceride content in skeletal muscle was associated with reduced insulin-stimulated glucose uptake measured in the same muscle [58]. This association was also reported in humans. In 1988, in a cohort of hyperinsulinemic adults with T2D, skeletal muscle lipid content was significantly higher than in controls [59]. In these studies, the lipid accumulation was assessed by muscle biopsy, restricting the studies to small cohorts of adults. With the increasing availability of noninvasive diagnostic techniques, the evidence regarding the association between IMCL and IR in youth increased rapidly. Sinha et al. first described the accumulation of fat in skeletal muscle in children [60]. The authors observed that obese adolescents had a higher intramyocellular lipid (IMCL) content

assessed by magnetic resonance spectroscopy (MRS) than lean adolescents [60]. Moreover, the authors, and others, have reported that IMCL content is positively associated with the accumulation of lipids in other ectopic depots, such as VAT [60] and hepatic fat [41].

Several pieces of evidence have underlined the association between ectopic fat accumulation in skeletal muscle and impaired insulin sensitivity in children and adolescents [61–63]. In 2003, Weiss and coworkers showed that children and adolescents with impaired glucose tolerance had a significantly higher IMCL content than obese children with normal glucose tolerance [61]. Moreover, IMCL was inversely correlated with plasma adiponectin and insulin sensitivity, independent of total and visceral adiposity, in a cohort of obese and normal weight youth [63]. This association was stronger in obese than in lean adolescents, suggesting that IMCL is crucial in determining the effect on insulin sensitivity [63]. Later, the same group reported differences in IMCL content and plasma adiponectin between insulin-sensitive and insulin-resistant obese adolescents [62]. Consistent with these findings, the obese insulin-resistant group showed a higher IMCL content and lower adiponectin compared to insulin-sensitive obese youth independent of other adiposity measures [62]. IMCL has been associated with other biochemical markers of CVD. In a large cohort of prepubertal and early pubertal children, the IMCL was shown to be associated with elevated triglycerides and high triglyceride-to-HDL ratio independent of VAT, SAT, and BMI [64].

The molecular mechanisms by which increased triglyceride content impairs insulin sensitivity in skeletal muscle are not completely clarified. In skeletal muscle, as well as in the liver, insulin binds the insulin receptor tyrosine kinase that phosphorylates IRS-1, which in turn activates phosphatidylinositol 3-kinase (PI3K). PI3K, through signaling intermediaries, induces the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane and, finally, glucose uptake into the myocyte. When ectopic lipid accumulation occurs, the intracellular concentrations of DAG, long-chain acyl-CoA, and malonyl CoA increase [65, 66]. These lipids, in particular DAG, activate the theta form of protein kinase C (PKCθ), which activates a threonine–serine kinase cascade that inhibits insulin receptor signaling (see Fig. 10.3). Fatty acids can interfere with glycolysis and hexokinase activity. In fact, Randle described that enhanced β oxidation in muscle cell leads to increased concentration of

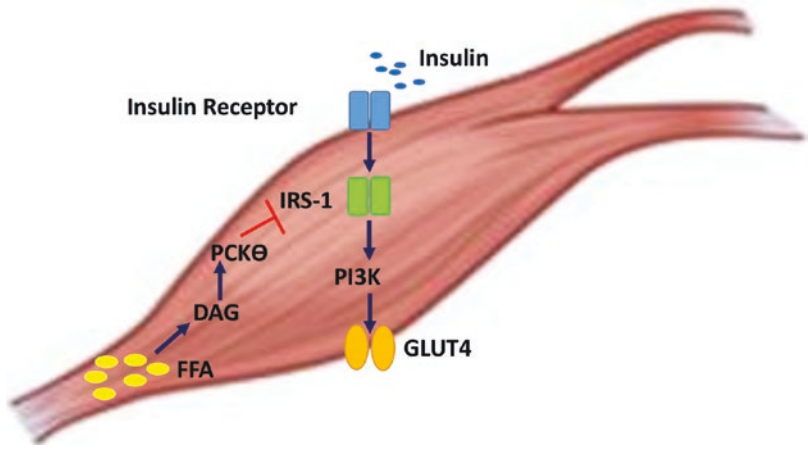

Fig. 10.3 Mechanisms of insulin resistance in skeletal muscle. Insulin binds its receptors that activate IRS-1 that in turn activate PI3K that promotes the migration of GLUT4 from the cytosol to the plasmatic membrane surface. The high amount of FFA increases the concentration of DAG that activates PCKθ, a serine–threonine kinase that inhibits IRS-1. *Abbreviations:* IRS-1 insulin receptor substrate 1, PI3K phosphatidylinositol 3-kinase, FFA free fatty acids, DAG diacylglycerol, PCKθ protein kinase C theta

acetyl CoA and of NADH to NAD^+ ratio, which impairs pyruvate dehydrogenase activity and glucose oxidation [67]. This, through the increase of cytosolic citrate, might increase the intracellular concentrations of glucose-6-phosphate, which might inhibit hexokinase II activity leading to increased intracellular glucose levels and finally to a lower glucose uptake [51].

Another pathway that may be involved is oxidative stress. FFAs can directly increase reactive oxygen species (ROS) via peroxidation reactions [68] and via mitochondrial production [69]. FFAs can also indirectly increase ROS via hexosamine biosynthetic products [70]. Several studies have reported an association between oxidative stress and hyperglycemia and the consequent reduction of glucose levels after antioxidant infusion in rats and human experimental models [68, 71, 72]. The biochemical mechanisms of oxidative stress-induced insulin resistance are not completely understood. ROS can affect signal transduction and gene expression via redox modification of proteins. Moreover, it has been shown that ROS can activate the PKC pathway leading to inactivation of IRS-1 [56].

These data support the role of IMCL in the pathogenesis of impaired insulin sensitivity, thus, it is important to look at it as potential risk factor leading to the development of metabolic syndrome.

Possible Mechanisms of Ectopic Fat Deposition

Lipodystrophy is a heritable disease characterized by a defect in adipose tissue mass. The inability to store fat in white adipose tissue leads to ectopic fat deposition in liver, skeletal muscle, and pancreas in affected subjects. In humans and mice models of lipodystrophy, the ectopic intracellular triglyceride accumulation is associated with IR [73–75]. Similarly, experimental models of Ob/Ob mice lacking aP2, a fatty acid binding protein, show reduced adipose tissue lipolysis and enlarged fat mass with a consequent increased insulin sensitivity and reduced circulating plasma lipoproteins [76].

Obesogenic adipose tissue hypertrophy might share these pathogenic mechanisms for ectopic fat deposition. Animals and in vivo models have shown that adipocytes have a genetically preestablished expandability potential [35, 77]. The reduced expression of lipogenic genes in SAT might be seen as an impaired storage function in adipocytes. In line with this theory are the findings proposed by Kursawe et al. that evaluated the pattern of gene expression of subcutaneous adipocytes in obese adolescents stratified for visceral adiposity. They reported that adolescents with high relative proportion of VAT to SAT, along with the downregulation of lipogenic gene expression, showed lower expression of the *LIPIN1* gene that is involved in adipocyte differentiation and lipid accumulation [35]. Thus, it might be hypothesized that in the case of obesity and increased need for fat storage, certain individuals are unable to mature new adipose cells owing to an impairment in differentiation, leading to triglyceride spillover into ectopic depots. Subsequently, the same group added another piece to this complex and still unsolved puzzle. The authors reported that SAT of obese youth with altered body partitioning showed higher expression of the inflammosome components (*NLR3*, *CASP1*, *IL1B*, and *TLR4*) and macrophage infiltration and activation markers (CD68 and CD115) [78]. Moreover, they found that among the markers of inflammation, CASP-1 and CD68 expression in SAT were the best predictors of body fat distribution in this cohort. Similarly, IL-1b has been showed to impair both adipogenesis and reduce adipocyte lipid content in human cells [79]. Thus, it could be hypothesized that SAT of an obese adolescent with high VAT/(VAT+SAT) might more readily secrete proinflammatory proteins that may further alter body fat distribution [78].

As mentioned above, VAT hypertrophy has been associated with an increased flux of FFAs into the portal vein toward the liver where FFAs are extracted by hepatocytes through a nonhormone-dependent mechanism [56]. After hepatocyte uptake, FFA can be hydrolyzed or re-esterified into triglycerides, which in turn might be stored in the cytosolic pool or secreted as

VLDL [56]. The imbalance between uptake and oxidation/secretion leads to lipid accumulation. Nevertheless, intrahepatic fat accumulation is not only due to an excess of lipid uptake, but also due to enhanced hepatic de novo lipogenesis (DNL). Recent studies suggest that insulin stimulates transcription of sterol-regulatory element-binding protein-1c (SREBP-1c) that in turn positively regulates the transcription of lipogenic enzymes [80]. The molecular pathways involved in lipid metabolism are not affected by IR, thus in condition of hyperinsulinemia, such as obese insulin-resistant youth, the DNL is increased in insulin-responsive organs, for example, the liver.

The high rate of DNL is not the only player in hepatic ectopic lipid deposition. Lipid deposition in skeletal muscle depends on a net balance between the rate of FFA uptake and the rate of lipid oxidation. Mitochondria exert a central role in regulating fatty acid oxidation. In elderly subjects, high muscular lipid content has been associated with lower mitochondrial oxidation and phosphorylation activity compared to young controls [81]. In obese adults affected by T2D, muscle tissue has been shown to have reduced oxidation of fatty acids derived from the plasma during fasting and exercise [82–84]. Moreover, increased IMCL has been associated with impaired mitochondrial phosphorylation in insulin-resistant offspring of T2D patients [85]. Taken together, these findings support the hypothesis that impaired mitochondrial oxidation and phosphorylation activity might be an important predisposing factor for intramyocellular fat accumulation in insulin-resistant youth.

References

1. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of obesity among adults and youth: United States, 2011–2014. NCHS data brief, no 219. Hyattsville, MD: National Center for Health Statistics; 2015.
2. Ogden CL, Carroll MD, Lawman HG, Fryar CD, Kruszon-Moran D, Kit BK, Flegal KM. Trends in obesity prevalence among children and adolescents in the United States, 1988–1994 through 2013–2014. JAMA. 2016;315(21):2292–9.
3. Finkelstein EA, Graham WC, Malhotra R. Lifetime direct medical costs of childhood obesity. Pediatrics. 2014;133(5):854–62.
4. Sonntag D, Ali S, De Bock F. Lifetime indirect cost of childhood overweight and obesity: a decision analytic model. Obesity (Silver Spring). 2016;24(1):200–6.
5. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, Savoye M, Rieger V, Taksali S, Barbetta G, Sherwin RS, Caprio S. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. N Engl J Med. 2002;346(11):802–10.
6. Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. Int J Obes (Lond). 2010;35(7):891–8.
7. Twig G, Kark JD. Body-mass index in adolescence and cardiovascular death in adulthood. N Engl J Med. 2016;375(13):1298–9.
8. D'Adamo E, Santoro N, Caprio S. Metabolic syndrome in pediatrics: old concepts revised, new concepts discussed. Pediatr Clin North Am. 2011;58(5):1241–55, xi.
9. Giannini C, Caprio S. Progression of β-cell dysfunction in obese youth. Curr Diab Rep. 2013;13(1):89–95.
10. Stefan N, Häring HU, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. Lancet Diabetes Endocrinol. 2013;1(2):152–62.
11. van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L, Gaye A, Gögele M, Heier M, Hiekkalinna T, Joensuu A, Newby C, Pang C, Partinen E, Reischl E, Schwienbacher C, Tammesoo ML, Swertz MA, Burton P, Ferretti V, Fortier I, Giepmans L, Harris JR, Hillege HL, Holmen J, Jula A, Kootstra-Ros JE, Kvaløy K, Holmen TL, Männistö S, Metspalu A, Midthjell K, Murtagh MJ, Peters A, Pramstaller PP, Saaristo T, Salomaa V, Stolk RP, Uusitupa M, van der Harst P, van der Klauw MM, Waldenberger M, Perola M, Wolffenbuttel BH. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. BMC Endocr Disord. 2014;14(1):14.
12. Prince RL, Kuk JL, Ambler KA, Dhaliwal J, Ball GD. Predictors of metabolically healthy obesity in children. Diabetes Care. 2014;13(5):1462–8.
13. Bervoets L, Massa G. Classification and clinical characterization of metabolically "healthy" obese children and adolescents. J Pediatr Endocrinol Metab. 2016;29(5):553–60.
14. Santoro N, Amato A, Grandone A, Brienza C, Savarese P, Tartaglione N, Marzuillo P, Perrone L, Miraglia Del Giudice E. Predicting metabolic syndrome in obese children and adolescents: look, measure and ask. Obes Facts. 2013;6(1):48–56.
15. Samouda H, de Beaufort C, Stranges S, Guinhouya BC, Gilson G, Hirsch M, Jacobs J, Leite S, Vaillant M, Dadoun F. Adding anthropometric measures of regional adiposity to BMI improves prediction of cardiometabolic, inflammatory and adipokines profiles in youths: a cross-sectional study. BMC Pediatr. 2015;15:168.
16. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S,

Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes. 2001;50(8):1844–50.
17. Verney J, Metz L, Chaplais E, Cardenoux C, Pereira B, Thivel D. Bioelectrical impedance is an accurate method to assess body composition in obese but not severely obese adolescents. Nutr Res. 2016;36(7):663–70.
18. Wan CS, Ward LC, Halim J, Gow ML, Ho M, Briody JN, Leung K, Cowell CT, Garnett SP. Bioelectrical impedance analysis to estimate body composition, and change in adiposity, in overweight and obese adolescents: comparison with dual-energy x-ray absorptiometry. BMC Pediatr. 2014;14:249.
19. Lemos T, Gallagher D. Current body composition measurement techniques. Curr Opin Endocrinol Diabetes Obes. 2017;24(5):310–4.
20. Awai HI, Newton KP, Sirlin CB, Behling C, Schwimmer JB. Evidence and recommendations for imaging liver fat in children, based on systematic review. Clin Gastroenterol Hepatol. 2014;12(5):765–73.
21. Bohte AE, Koot BG, van der Baan-Slootweg OH, van Werven JR, Bipat S, Nederveen AJ, Jansen PL, Benninga MA, Stoker J. US cannot be used to predict the presence or severity of hepatic steatosis in severely obese adolescents. Radiology. 2012;262(1):327–34.
22. Smith SR, Lovejoy JC, Greenway F, Ryan D, de Jonge L, de la Bretonne J, Volafova J, Bray GA. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metabolism. 2001;50(4):425–35.
23. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, et al. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. Obesity (Silver Spring). 2010;18(11):2191–8.
24. Bidulescu A, Liu J, Hickson DA, Hairston KG, Fox ER, Arnett DK, Sumner AE, Taylor HA, Gibbons GH. Gender differences in the association of visceral and subcutaneous adiposity with adiponectin in African Americans: the Jackson Heart Study. BMC Cardiovasc Disord. 2013;13(1):9. https://doi.org/10.1186/1471-2261-13-9.
25. Kaess BM, Pedley A, Massaro JM, Murabito J, Hoffmann U, Fox CS. The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk. Diabetologia. 2012;55(10):2622–30.
26. Gastaldelli A, Sironi AM, Ciociaro D, Positano V, Buzzigoli E, Giannessi D, et al. Visceral fat and beta cell function in non-diabetic humans. Diabetologia. 2005;48(10):2090–6.
27. Miyazaki Y, DeFronzo RA. Visceral fat dominant distribution in male type 2 diabetic patients is closely related to hepatic insulin resistance, irrespective of body type. Cardiovasc Diabetol. 2009;8:44.
28. Taksali SE, Caprio S, Dziura J, Dufour S, Cali AM, Goodman TR, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. Diabetes. 2008;57(2):367–71.
29. Cali AM, Caprio S. Ectopic fat deposition and the metabolic syndrome in obese children and adolescents. Horm Res. 2009;71(Suppl 1):2–7.
30. Gyllenhammer LE, Alderete TL, Toledo-Corral CM, Weigensberg M, Goran MI. Saturation of subcutaneous adipose tissue expansion and accumulation of ectopic fat associated with metabolic dysfunction during late and post-pubertal growth. Int J Obes (Lond). 2016;40(4):601–6.
31. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116(1):39–48.
32. Kusters DM, Caceres M, Coll M, Cuffie C, Gagné C, Jacobson MS, et al. Efficacy and safety of ezetimibe monotherapy in children with heterozygous familial or nonfamilial hypercholesterolemia. J Pediatr. 2015;166(6):1377–84.e3.
33. Liu J, Fox CS, Hickson DA, May WD, Hairston KG, Carr JJ, et al. Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study. J Clin Endocrinol Metab. 2010;95(12):5419–26.
34. Bennett B, Larson-Meyer DE, Ravussin E, Volaufova J, Soros A, Cefalu WT, Chalew S, Gordon S, Smith SR, Newcomer BR, Goran M, Sothern M. Impaired insulin sensitivity and elevated ectopic fat in healthy obese vs. nonobese prepubertal children. Obesity (Silver Spring). 2012;20(2):371–5.
35. Kursawe R, Eszlinger M, Narayan D, Liu T, Bazuine M, Cali AM, et al. Cellularity and adipogenic profile of the abdominal subcutaneous adipose tissue from obese adolescents: association with insulin resistance and hepatic steatosis. Diabetes. 2010;59(9):2288–96.
36. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–56.
37. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881–7.
38. Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia. 2002;45(9):1201–10.
39. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porter D Jr. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes. 1993;42(11):1663–72.
40. Toledo FG, Sniderman AD, Kelley DE. Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. Diabetes Care. 2006;29(8):1845–50.
41. Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Otvos JD, Caprio S. Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. Diabetes Care. 2007;30(12):3093–8.
42. Cali AM, De Oliveira AM, Kim H, Chen S, Reyes-Mugica M, Escalera S, et al. Glucose dysregulation

and hepatic steatosis in obese adolescents: is there a link? Hepatology. 2009;49(6):1896–903.
43. Toledo-Corral CM, Alderete TL, Hu HH, Nayak K, Esplana S, Liu T, et al. Ectopic fat deposition in prediabetic overweight and obese minority adolescents. J Clin Endocrinol Metab. 2013;98(3):1115–21.
44. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology. 2012;142(7):1592–609.
45. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288(2):E462–8.
46. Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology. 2015;62(6):1723–30.
47. Nadeau KJ, Klingensmith G, Zeitler P. Type 2 diabetes in children is frequently associated with elevated alanine aminotransferase. J Pediatr Gastroenterol Nutr. 2005;41(1):94–8.
48. Larson-Meyer DE, Newcomer BR, Ravussin E, Volaufova J, Bennett B, Chalew S, Cefalu WT, Sothern M. Intrahepatic and intramyocellular lipids are determinants of insulin resistance in prepubertal children. Diabetologia. 2011;54(4):869–75.
49. D'Adamo E, Cali AM, Weiss R, Santoro N, Pierpont B, Northrup V, et al. Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. Diabetes Care. 2010;33(8):1817–22.
50. Kim G, Giannini C, Pierpont B, Feldstein AE, Santoro N, Kursawe R, et al. Longitudinal effects of MRI-measured hepatic steatosis on biomarkers of glucose homeostasis and hepatic apoptosis in obese youth. Diabetes Care. 2013;36(1):130–6.
51. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med. 2014;371(12):1131–41.
52. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem. 2004;279(31):32345–53.
53. Raddatz K, Turner N, Frangioudakis G, Liao BM, Pedersen DJ, Cantley J, et al. Time-dependent effects of Prkce deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. Diabetologia. 2011;54(6):1447–56.
54. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Cauter E. Quantitative study of insulin secretion and clearance in normal and obese subjects. J Clin Invest. 1988;81(2):435–41.
55. Svedberg J, Strömblad G, Wirth A, Smith U, Björntorp P. Fatty acids in the portal vein of the rat regulate hepatic insulin clearance. J Clin Invest. 1991;88(6):2054–8.
56. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev. 2002;23(2):201–29.
57. Falholt K, Cutfield R, Alejandro R, Heding L, Mintz D. The effects of hyperinsulinemia on arterial wall and peripheral muscle metabolism in dogs. Metabolism. 1985;34(12):1146–9.
58. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. Diabetes. 1991;40(2):280–9.
59. Falholt K, Jensen I, Lindkaer Jensen S, Mortensen H, Vølund A, Heding LG, Noerskov Petersen P, Falholt W. Carbohydrate and lipid metabolism of skeletal muscle in type 2 diabetic patients. Diabet Med. 1988;5(1):27–31.
60. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, Savoye M, Rothman DL, Shulman GI, Caprio S. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. Diabetes. 2002;51(4):1022–7.
61. Weiss R, Dufour S, Taksali SE, Tamborlane WV, Petersen KF, Bonadonna RC, et al. Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. Lancet. 2003;362(9388):951–7.
62. Weiss R, Taksali SE, Dufour S, Yeckel CW, Papademetris X, Cline G, et al. The "obese insulin-sensitive" adolescent: importance of adiponectin and lipid partitioning. J Clin Endocrinol Metab. 2005;90(6):3731–7.
63. Weiss R, Dufour S, Groszmann A, Petersen K, Dziura J, Taksali SE, et al. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. J Clin Endocrinol Metab. 2003;88(5):2014–8.
64. Brumbaugh DE, Crume TL, Nadeau K, Scherzinger A, Dabelea D. Intramyocellular lipid is associated with visceral adiposity, markers of insulin resistance, and cardiovascular risk in prepubertal children: the EPOCH study. J Clin Endocrinol Metab. 2012;97(7):E1099–105.
65. Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur J Clin Invest. 2002;32(3):1–23.
66. Petersen KF, Shulman GI. Cellular mechanism of insulin resistance in skeletal muscle. J R Soc Med. 2002;42:8–13.

67. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963;1(7285):785–9.
68. Paolisso G, Giugliano D. Oxidative stress and insulin action: is there a relationship? Diabetologia. 1996;39(3):357–63.
69. Bakker SJ, IJzerman RG, Teerlink T, Westerhoff HV, Gans RO, Heine RJ. Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and beta-cell failure? Atherosclerosis. 2000;148(1):17–21.
70. Taniguchi N, Kaneto H, Asahi M, Takahashi M, Wenyi C, Higashiyama S, Fujii J, Suzuki K, Kayanoki Y. Involvement of glycation and oxidative stress in diabetic macroangiopathy. Diabetes. 1996;45(3):S81–3.
71. Paolisso G, Gambardella A, Tagliamonte MR, Saccomanno F, Salvatore T, Gualdiero P, D'Onofrio MV, Howard BV. Does free fatty acid infusion impair insulin action also through an increase in oxidative stress? J Clin Endocrinol Metab. 1996;81(12):4244–8.
72. Haber CA, Lam TK, Yu Z, Gupta N, Goh T, Bogdanovic E, Giacca A, Fantus IG. N-acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance in vivo: possible role of oxidative stress. Am J Physiol Endocrinol Metab. 2003;285(4):E744–53.
73. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest. 2000;105(3):271–8.
74. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev. 1998;12(20):3182–94.
75. Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. Ann N Y Acad Sci. 2002;967:363–78.
76. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. Endocrinology. 2000;141(9):3388–96.
77. Hoffstedt J, Förster D, Löfgren P. Impaired subcutaneous adipocyte lipogenesis is associated with systemic insulin resistance and increased apolipoprotein B/AI ratio in men and women. J Intern Med. 2007;262(1):131–9.
78. Kursawe R, Dixit VD, Scherer PE, Santoro N, Narayan D, Gordillo R, Giannini C, Lopez X, Pierpont B, Nouws J, Shulman GI, Caprio S. A role of the inflammasome in the low storage capacity of the abdominal subcutaneous adipose tissue in obese adolescents. Diabetes. 2016;65(3):610–8.
79. Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulangé A, Capeau J, Caron M. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. Diabetologia. 2006;49(9):2162–73.
80. Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, Le Lièpvre X, Berthelier-Lubrano C, Spiegelman B, Kim JB, Ferré P, Foufelle F. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Mol Cell Biol. 1999;19(5):3760–8.
81. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science. 2003;300(5622):1140–2.
82. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Phys. 1999;277(6):E1130–41.
83. Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB J. 1999;13(14):2051–60.
84. Turpeinen AK, Takala TO, Nuutila P, Axelin T, Luotolahti M, Haaparanta M, Bergman J, Hämäläinen H, Iida H, Mäki M, Uusitupa MI, Knuuti J. Impaired free fatty acid uptake in skeletal muscle but not in myocardium in patients with impaired glucose tolerance: studies with PET and 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid. Diabetes. 1999;48(6):1245–50.
85. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med. 2004;350(7):664–71.

Biomarkers of Insulin Resistance

11

Kimberly A. Cox-York and Rocio I. Pereira

Introduction

The prevalence of insulin resistance (IR) in children and adolescents has increased globally in the past two decades, in association with similar increases in the prevalence of obesity. IR is a precursor to a number of diseases, including type 2 diabetes mellitus (T2DM), polycystic ovary syndrome, hypertension, hypertriglyceridemia, and cardiovascular disease. The hallmark of IR is the inability of the liver, fat, and muscle to respond to insulin. To maintain glucose control, pancreatic β(beta)-cells produce increasing amounts of insulin until they can no longer keep up and excess glucose accumulates in the blood, resulting eventually in type 2 diabetes mellitus. Muscle, fat, and the liver communicate to maintain metabolic homeostasis and adapt to metabolic dysregulation via small signaling molecules. The rate of production and the capacity to respond to these signals likely depend on metabolic disposition, including the spectrum of circulating plasma glucose, but the details of these responses are still poorly understood. Most of these molecules are expressed in multiple tissues and act in both paracrine and endocrine fashion to regulate metabolic homeostasis. For the purposes of this chapter, they are presented in the classification of their original identification, but cross-tissue communication should be assumed for most. Alterations in circulating concentrations of some of these molecules may suggest IR, and measurement of these biomarkers shows promise for early identification of this condition.

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Biomarker Identification

A key strategy for identifying a successful treatment for any disease is detecting the disease in its early stages. IR is a particularly difficult condition to identify due to variability in time of onset, the contribution of multiple genes, and multi-organ pathophysiology. Moreover, by the time hyperglycemia or type 2 diabetes are diagnosed, there is already substantial β(beta)-cell dysfunction [1] and increased risk for other cardiometabolic disease [2]. Therefore, markers of IR identifiable prior to onset of cardiovascular risk are desirable. The gold standard for determining IR is the hyperinsulinemic-euglycemic clamp. This is an invasive, expensive procedure requiring a clinical research setting, making it costly and difficult to do on a routine basis. The oral glucose tolerance test is an easier but less precise method that also involves multiple blood draws and several hours to complete. These tests can be especially challenging in children. However, with

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advanced methodology and understanding of the molecular basis of IR, other predictive markers are emerging.

Metabolomics has had a particularly profound impact on biomarker discovery and assessment [3]. Metabolomics is the analysis of a large number of low-molecular-weight products of metabolism [4] that attempts to quantify and profile these products in biological samples, i.e., blood, urine, feces, and tissues [5]. The samples required for metabolomics are relatively small and noninvasive, making this approach more suitable for children and adolescents, as well as adults. Several classes of metabolites have been associated with IR, including those involved in central carbon metabolism, amino acid and lipid pathways, and some secondary metabolites.

Proton magnetic resonance spectroscopy (^{1}H MRS) is a noninvasive imaging method capable of detecting lipid moieties in tissues other than adipose tissue in adults and children. As ectopic lipid deposition is associated with IR in several populations, ^{1}H MRS may also be a promising strategy for early detection of metabolic derangements associated with IR.

Amino Acids

In addition to acting as building blocks of proteins, amino acids function as signaling molecules in the regulation of growth and metabolism [6–8]. The essential branched-chain amino acids (BCAA; leucine, isoleucine, and valine) have the potential to regulate food intake and glycemia indirectly by modulating the release of hormones, such as leptin, glucagon-like peptide-1 (GLP-1), and ghrelin [9–13], and directly by affecting insulin sensitivity via glucose transport and regulation of insulin receptor phosphorylation through the mTOR/p70S6 kinase pathway [14, 15]. Administration of BCAA in clinical studies suggests improvements in metabolic parameters, such as body weight, satiety, and glycemia through action at multiple tissues, including brain, liver, muscle, and adipose [16–18], as well as increased insulin secretion [19, 20].

Hyperaminoacidemia has been described in obese compared to lean adults [21]. Moreover, elevated levels of BCAA, their breakdown products, glutamate, C3 and C5 acylcarnitines [22], and aromatic amino acids (AAA) have been associated with an increased risk of IR and T2DM in humans [23–26], possibly independent of obesity [8]. BCAA and AAA concentrations have also been found to be positively associated with BMI in children (8–12 years old) [27–32]; and children of obese mothers tend to have increased levels of BCAA relative to children of nonobese mothers, implicating an epigenetic component [33]. Mihalik et al., however, have reported significantly lower concentrations of most of the profiled amino acids, including leucine/isoleucine and tyrosine, in overweight and T2DM adolescents (13–15 years old) [34], and improved β(beta)-cell function with elevated plasma amino acids [35]. It has been suggested that these discrepancies are due to differences in age and the result of the metabolic plasticity of children [28, 34].

Given the seemingly discordant data in interventional versus observational studies, it is not known if amino acids contribute directly to IR or are a by-product of metabolic dysregulation. BCAA rates of appearance depend on protein intake and degradation, and rates of disappearance depend on synthesis, excretion, and oxidation. Understanding the mechanisms by which BCAA affect insulin sensitivity is complicated by the fact that BCAA and insulin act additively or synergistically to activate the mTOR pathway [36–39]. Two proposed mechanisms of BCAA influence on IR are as follows: (1) persistent activation of the mTOR complex 1 (mTORC1) by excess BCAA leads to serine phosphorylation of insulin receptor substrate 1 (IRS-1) and IRS-2 and, along with lipotoxicity and oxidative stress, promotes β(beta)-cell exhaustion; and (2) BCAA dysmetabolism leads to accumulation of toxic by-products, in turn leading to β(beta)-cell mitochondrial dysfunction and apoptosis (reviewed in [40]). Evidence for persistent mTOR activation and β(beta)-cell exhaustion [22, 41–43] is countered by studies showing that BCAA supplementation improves metabolic parameters despite

increased mTORC1 signaling [44, 45] and that activation of mTORC1 in β(beta)-cells is associated with increases in islet and β(beta)-cell mass and protection from T2DM [46–51]. The BCAA dysregulation model is supported by consistent observation of decreased expression of genes and proteins involved in BCAA metabolism in insulin-sensitive tissues (adipose tissue, muscle, liver) over a broad range of obesity models and correlation with insulin sensitivity [40]. The accumulation of BCAA metabolites can lead to mitochondrial oxidative stress and apoptosis and IR [8, 52–54].

Overall, additional investigations are required to determine if BCAA contribute directly to IR or if elevated levels are secondary to established disease. Amino acids and their metabolites alone, or in combination with other metabolic by-products, may hold promise as biomarkers for early identification of IR prior to overt glycemic symptoms. Recent evidence supports an interaction between BCAA and increased free fatty acid (FFA) to negatively affect insulin sensitivity [41].

Lipids

Given the close association of IR and T2DM with obesity, it is not surprising that lipids and lipid intermediates were among the first mediators of IR identified [55]. Spillover of FFA from adipose tissue that is unable to adequately store excess energy leads to deposition of lipids in other insulin-sensitive tissues, such as liver and muscle. In human and animal models of lipodystrophy, lack of adipose tissue also results in ectopic deposition of lipids [56, 57]. Recently, the "obesity paradox" has described normal-weight/lean subjects with diabetes and a higher mortality rate than their obese counterparts [58]. Moreover, acute IR can be induced with infusion of FFA [59, 60], and pharmacological lowering of FFA improves insulin sensitivity [61]. Whether due to lifestyle or genetic predisposition, FFAs are a central focus of IR etiology [62–65]. The mechanisms associated with FFA-induced IR are tissue-specific and include deposition of diacylglycerol (DAG), reactive oxygen species production, and mitochondrial dysfunction (reviewed in [66]).

The by-products of incomplete FFA oxidation have also been identified as potential mediators of IR. Acylcarnitines, for instance, are by-products of incomplete FA oxidation and have been found to be associated with IR in adults [67, 68] and children/adolescents [28, 31, 33, 69].

Lipids are the major source of fuel and fuel storage for the human body. They also act as intracellular signaling molecules regulating metabolic pathways. When there is a disparity between lipid oxidation and lipid delivery to muscle or the liver, intramyocellular lipid (IMCL) or intrahepatic lipid (IHL) accumulates. As mentioned above, ^{1}H MRS is a noninvasive method for measuring IMCL and IHL. In healthy lean [70], nonobese insulin-resistant [71] adults and children [72], IMCL predicted muscle IR better than fat mass. Upon further analysis, specific lipid species have been defined as the primary contributors to impaired signaling in these insulin-responsive tissues. Diacylglycerol (DAG) accumulation is believed to activate PKCθ(theta) [73, 74] in muscle and PKCδ(delta) in the liver [75, 76], thus altering IRS-1 phosphorylation and resulting in IR. It is now believed that specific side chains on a DAG determine the extent of toxicity. Fatty acyl-CoAs that are not incorporated into mono-, di-, or triacylglycerols can esterify with sphingosine to form ceramides. Ceramides formed from saturated fatty acids have been postulated to act as second messengers in the response to inflammatory cytokines and are also implicated in the development of IR [77, 78]. Recent studies have shown that improvement of hepatic mitochondrial function can improve ectopic lipid deposition and the associated IR [79–81].

While effective at detecting ectopic lipid, ^{1}H MRS is not available as a screening tool, and the cellular effects must be studied in cell or animal models or in tissue biopsies from humans. Lipidomics is beginning to identify serum markers and profiles to corroborate these findings noninvasively in humans and may hold promise as an early biomarker of IR [82].

Sex Steroids

There are well-established, fundamental differences between males and females in the regulation of metabolic homeostasis (reviewed in [83]), with implications for the development of metabolic disease and response to interventions. While men are considered more prone to impaired fasting glucose, impaired glucose tolerance is more prevalent in women [84–87]. The disparity arises at the level of the gamete and the evolutionary reproductive roles of males and females [88] and is triggered by the sex hormones (androgens and estrogens) with the onset of puberty. Indeed, a large body of literature describes decreased insulin sensitivity during puberty that resolves shortly thereafter in healthy adolescents but can continue in obese children [89]. The mechanisms are still poorly understood, but sex hormones have direct effects on the development, integrity, and function of β(beta)-cells [90].

In a longitudinal study following girls and boys from 11 to 19 years of age, insulin sensitivity was measured via hyperinsulinemic-euglycemic clamp [91]. At baseline, the boys were more insulin sensitive than the girls. Despite gaining less fat mass and more lean mass, by age 19, the boys became *less* insulin sensitive than the girls. Several studies have reported increased androgen precursors in obese versus lean children and associations with markers of insulin sensitivity [28, 33, 92]. Sex differences have also been observed in the relative levels of the other biomarkers discussed in this chapter, i.e., BCAA, lipids, adipokines, etc. [69, 93] Together, these results support a role for sex steroids in the regulation of insulin sensitivity.

Importantly, androgens and estrogens are produced and active in brain and adipose tissue, as well as reproductive tissues (reviewed in [83, 94]). Adipose tissue distribution is markedly different in males versus females and may contain as much as one hundred times more sex steroid than plasma [95]. Moreover, β(beta)-cells may produce sex steroids for local control of insulin sensitivity [90]. Consequently, while sex hormones are vital constituents of insulin homeostasis, it is problematic to use their circulating levels as predictors of IR in youth.

Adipokines and Cytokines

Over the last 30 years, adipose tissue has been ascertained to be a highly regulated endocrine organ. Through its production of a variety of cell signaling proteins, hormones, cytokines, and growth factors—collectively termed "adipokines" or "adipocytokines"—adipose tissue is a central modulator of metabolic homeostasis [96, 97]. The majority of adipose tissue in humans is white adipose tissue of two distinct types—subcutaneous (SAT) and visceral (VAT)—with depot-specific adipokine/cytokine profiles. Further, SAT is associated with insulin sensitivity and VAT with IR [98]. There is a strong association between obesity-related IR and increased adipose tissue inflammation characterized by a greater pro-inflammatory-to-anti-inflammatory profile [99]. Most of what is known to date has emerged from studies in adults, but the increasing prevalence of childhood obesity is driving the investigation of adipokine action in this population. The relative levels of pro-to-anti-inflammatory adipokines are associated with IR in obese children of all ages [100, 101]. Recently, some adipokines have been measured in saliva, and comparisons to plasma levels are currently underway [102]. While there are more than 50 adipokines identified [103], only the best-studied and those most closely associated to IR will be covered here.

Pro-inflammatory

Leptin

Leptin is the most studied and best-known adipokine. It is a 16 kD polypeptide hormone mainly secreted from white adipose tissue, which acts in the central nervous system to regulate appetite, body weight, neuroendocrine functions, and glucose-insulin homeostasis [104] via modulation

of hepatic and muscle insulin action [56], glucagon secretion [105], glucose production [106], and glucocorticoid secretion [107, 108]. With increasing obesity, overproduction of leptin or hyperleptinemia can lead to leptin resistance and central leptin insufficiency resulting in the loss of this control mechanism [109]. Hyperleptinemia is associated with IR and T2DM [99, 110, 111]. The association between leptin and IR in children and adolescents is mixed. A recent study by Soliman et al. found that plasma leptin was lower in obese adolescents with T2DM relative to age-, gender-, and BMI-matched controls without T2DM [109]. This is in line with results from one study in adults [112]; however, it contrasts with increased [110] or no difference [113, 114] in plasma leptin in other studies of obese children and adolescents with and without T2DM. In addition to its metabolic effects, leptin is involved in the regulation of normal growth and puberty [109], complicating its use as a biomarker of IR in obese children and adolescents.

Resistin

Named because of its ability to interfere with or resist insulin action [115], human resistin is a 12.5 kDa polypeptide primarily released from circulating blood monocytes [116]. Resistin has been found to be increased in peripheral blood mononuclear cells (PBMC) of women with T2DM relative to healthy women [117] and in plasma levels of Jordanian patients with T2DM compared to nondiabetic controls [118]. Others, however, have found no association between resistin and insulin sensitivity in adults [119, 120]. The picture is much the same in the pediatric population, with some studies finding positive associations between resistin and IR [121, 122], whereas others have not [123–125], including a large, multicountry comparison study in 2290 children ages 8–11 and 12–15 [126]. Importantly, in this and other studies, resistin appears to vary by age, gender, and country of origin [121, 123, 126], which complicates its use as a dependable biomarker for IR.

Retinol-Binding Protein 4

The canonical role of retinol-binding protein 4 (RBP4) is to carry retinol (vitamin A alcohol) from stores in the liver to peripheral tissues. In the last 10 years, RBP4 has been linked to IR and T2DM [127–129]; a single nucleotide polymorphism in humans is associated with a twofold risk for T2DM [130]. In clinical studies, RBP4 is used as a biomarker for metabolic disease in adults [131–133]. RBP4 impairs insulin signaling in adipocytes by inducing proinflammatory cytokine production from macrophages via activation of the JNK (c-Jun N-terminal kinase) pathway and is mediated though Toll-like receptor 4 [134]. In obese and nonobese adolescents, RBP4 was independently associated with insulin sensitivity (HOMA-IR) [135], and physical activity intervention decreased RBP4 levels by 30% in obese 14- to 18-year-olds [136]. The predictive power of RBP4 is strengthened by comparing its ratio to retinol [137] making it one of the strongest candidates for an IR biomarker.

Visfatin

Predominantly expressed in adipose tissue and leukocytes, visfatin (also called pre-β[beta]-cell colony-enhancing factor and NAMPT; nicotinamide phosphoribosyltransferase) affects the sensitivity of liver cells to insulin action [138] and can regulate insulin secretion from β(beta)-cells via NAD production [139]. Visfatin acts as an insulin sensitizer by binding to the insulin receptor and activating the PI3-kinase/AKT pathway [140]. Infusion of varying levels of glucose in healthy male subjects caused an increase in circulating visfatin, and somatostatin-induced decrease in endogenous insulin secretion resulted in a decrease in visfatin [141], suggesting that visfatin may be a better marker for glucose regulation per se rather than just an indicator of adiposity. In children and adolescents, visfatin is positively correlated to BMI, HOMA-IR, and fasting insulin [142] and can be decreased with weight loss [143]. The ability to

monitor metabolic improvements across interventions in the pediatric population makes visfatin a promising biomarker.

Chronic Inflammation

It is now well-accepted that obesity is associated with chronic low-grade, sterile inflammation that is believed to play a major role in obesity-related IR in adults [144–146]. A number of proinflammatory chemokines and cytokines have been implicated in obesity-related inflammation. This is a complex network involving multiple signaling cascades, making it difficult to pinpoint individual molecules or pathways. Daniele et al. have attempted to develop an "inflammatory status score" to evaluate the level of inflammation and predict IR and β(beta)-cell dysfunction for better treatment of T2DM [147]. Among the most studied are tumor necrosis factor-alpha (TNF-α[alpha]), interleukin (IL)-6 (IL-6), IL-1β(beta), IL-18, and monocyte chemoattractant protein-1 (MCP1), all of which are increased in obesity and are positively associated with IR in multiple models (reviewed in [66, 146, 148]). The multi-organ, multi-process nature of chronic inflammation in obesity and diabetes poses a challenge in using inflammatory markers to diagnose and/or predict metabolic disease [149]. Furthermore, changes in adipose tissue accumulation and distribution with the onset of puberty make establishing an adipokine profile to identify and evaluate IR in children and adolescents challenging.

Anti-inflammatory

Adiponectin

Adiponectin is one of the best-studied adipokines. It is widely recognized as an insulin sensitizer and has been associated with improved insulin sensitivity and reduced risk of T2DM [150]. Through interaction with its receptors, ADIPOR1/2, adiponectin improves insulin sensitivity by activating AMP-dependent protein kinase (AMPK) and downstream signaling pathways (p38MAPK, PPAR-α[alpha], PPAR-γ[gamma]) to augment fatty acid oxidation and glucose uptake in muscle and to suppress gluconeogenic genes (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) in the liver [151]. Adiponectin also acts on other organs (kidney, heart), tissues (central nervous system), and cell types (pancreatic β[beta]-cells, immune cells) with effects on inflammation and insulin sensitivity [150, 152]. Some of these effects may be mediated via T-cadherin receptor signaling; however, the molecular mechanisms of this activity are not clear [153].

Despite being highly expressed by adipocytes, adiponectin is decreased with obesity, likely as a result of increased expression of pro-inflammatory factors (TNF-α[alpha], IL-6) [154] and possibly iron overload via forkhead box protein O1 (FOXO1) [155, 156]. In circulation, adiponectin exists as low-molecular-weight (LMW) trimers, medium-molecular-weight (MMW) hexamers, and high-molecular-weight (HMW) oligomers of four to six trimers formed by disulfide bonds, as well as a proteolytic fragment termed globular adiponectin (gAd). The stable HMW form is generally considered as the most biologically active form in the regulation of glucose and insulin sensitivity and metabolic homeostasis; however, total adiponectin, as well as the ratio of total to the different molecular weight forms of the protein, may dictate diabetes risk [157–160].

A study by Ohman-Hansen et al. investigated ethnic and sex differences in adiponectin levels across the lifespan (8–57 years) [161]. Fasting adiponectin was lower in all Hispanic (H) participants relative to non-Hispanic whites (NHW) and lower in males than females independent of ethnicity. Confirming the results of studies in discrete populations, this comprehensive study found an inverse correlation between adiponectin and IR across ages and stages in H and NHW participants of both sexes. There was a downward trend in the association in late puberty, which rebounded in NHW women, but not in H women. These results are supported by a study in 2290 children from four different countries, in which adiponectin was higher in younger (8–11 years

old) than older (12–15 years old) [126]. In general, there was a positive correlation between adiponectin and insulin sensitivity; however, there appeared to be some age, sex, and country of origin differences.

Adiponectin may also be valuable as a predictor of the development of T2DM and as a marker to track metabolic response to treatment. In a prospective study of 1300 nondiabetic Chinese (Hong Kong Cardiovascular Risk Factor Prevalence Study; CRISPS), serum adiponectin and TNF-α(alpha) receptor 2 (TNF-α[alpha]-R2) were as effective in predicting 5-year diabetes development as a diabetes risk factor score (age, sex, family history, smoking, physical activity hypertension, waist circumference, fasting glucose, and dyslipidemia) plus an oral glucose tolerance test (OGTT) [162]. In the TODAY (Treatment Options for type 2 Diabetes in Adolescents and Youth) study of children aged 10–17, non-Hispanic black participants had significantly lower HMW adiponectin than non-Hispanic whites at baseline, and HMW adiponectin increased in the group with improved glycemic control achieved with pharmaceutical and/or lifestyle intervention [163]. There was, however, a 50% lower increase in non-Hispanic black children, further supporting racial and/or ethnic disparities. These results are also supported by increases in HMW adiponectin in prepubertal children following weight loss, which also corresponded to an inverse association between HMW adiponectin and HOMA-IR [164]. Together, these data suggest that current measures of adiponectin may function as an effective biomarker of IR. Further investigation of the mechanistic actions of the specific adiponectin oligomers may provide further resolution and expand its use in predicting and monitoring disease.

Secreted Frizzled-Related Protein 5 (SFRP5)

The frizzled proteins are cell surface receptors for wingless-type MMTV integration site (WNT) proteins involved in adipogenesis and inflammation [165]. The N-terminal, cysteine-rich domain of secreted frizzled-related protein 5 (SFRP5) is homologous to the frizzled proteins and has been identified as an anti-inflammatory adipokine [166]. It is highly expressed in adipose tissue and, like its family members SFRP1–4, opposes the WNT proteins to regulate adipogenesis and inflammation. In obese rodents and humans, WNT5A in particular is increased and initiates inflammation through activation of c-Jun N-terminal kinase-1 (JNK1) [167, 168]. SFRP5 acts as a decoy receptor to bind and sequester WNT5A in the adipose tissue extracellular space and diminish Wnt signaling, attenuating inflammation and improving insulin signaling [166, 169]. At the level of the adipocyte, mitochondrial metabolism is suppressed and adipocyte growth is stimulated [170].

Expression of SFRP5 mRNA has been reported to both increase [170–173] and decrease [166] with obesity in mice. In human obesity, SFRP5 is consistently decreased [166, 174–177]; however, associations with IR and diabetes are less consistent, with some demonstrating a positive association [177], some a negative association [176], while others report variability in SFRP5 levels with the degree of IR [178]. This is not surprising, perhaps, given the nature of SFRP5 as a homeostatic control. In 7-year-old boys and girls, Prats-Puig et al. demonstrated that WNT5A and SFRP5 were positively correlated in serum and conditioned media from adipose tissue explants [179]. Moreover, in children with the lowest circulating SFRP5, the WNT5A association with IR was significantly strengthened. SFRP1–4 are also under investigation for their roles in metabolic dysfunction [180–182], so the entire SFRP family may be useful markers of metabolic status.

Interleukin-10

The immune system is composed of intricate signaling cascades designed to protect the host from foreign pathogens. The pro-inflammatory response launched to protect against pathogens is countered by release of anti-inflammatory

molecules to limit host tissue damage caused by the inflammation. As mentioned above, a state of chronic, systemic inflammation contributes to the myriad metabolic complications associated with obesity. The degree of adipose tissue macrophage (ATM) infiltration, and relative distribution of M1 (pro-inflammatory) to M2 (anti-inflammatory) ATM, is associated with the progression of IR [183]. Part of the counter-regulation of this inflammation is the release of the anti-inflammatory cytokine, interleukin-10 (IL-10), from multiple immune cell types including M2 macrophages and lymphocytes residing in several tissues, including muscle, liver, and adipose tissue [97]. In fact, IL-10 is implicated directly in macrophage polarization between M1 and M2. IL-10 is a Th2-type cytokine that inhibits proinflammatory cytokine synthesis and activity via various, tissue-specific mechanisms [184–186].

White adipose tissue insulin sensitivity is maintained by production of eosinophil-derived IL-4, which drives production of IL-10 by M2 macrophages [187]. Obesity-associated hyperinsulinemia is postulated to suppress IL-10 production by key immunosuppressive T-regulatory cells (Tregs) via insulin receptors on the cell surface, contributing to immune dysregulation [188]. Animal studies have demonstrated that exogenous treatment or overexpression of IL-10 improves insulin sensitivity [189–191]. In humans, the associations between circulating IL-10 and obesity and IR are mixed [192–195], likely due to many of the factors discussed throughout the chapter including age, sex, developmental stage, ethnicity, and race [192].

Evidence in children is similar to that in adults. The longitudinal Nepean study of boys and girls from ages 8 through 15 demonstrated no differences in IL-10 between overweight/obese girls and boys relative to one another or their normal-weight counterparts [196]. By age 15, regardless of pubertal stage, a significant increase in IL-10 was observed in overweight/obese girls relative to normal-weight girls, but there was no difference between normal-weight and obese boys. In Taiwanese children and adolescents with metabolic syndrome, circulating IL-10 was significantly lower in overweight/obese, relative to normal-weight children in all three age groups (8 years old, 11 years old, 13 years old) [197]. In normal-weight children, plasma IL-10 was positively correlated with fasting insulin and HOMA-IR. In the overweight/obese children, only fasting insulin was moderately associated with IL-10. The presence of other comorbidities (blood pressure, waist-hip circumference, lipid profiles) may affect IL-10 levels and complicate the use of IL-10 as a reliable biomarker.

Myokines and Cytokines

In nonobese individuals, muscle is the largest organ in the body and is fundamental to proper glucose control [198]. An important part of that control is the production and secretion of hundreds of chemical modulators termed "myokines" that communicate with other insulin-sensitive organs, i.e., the liver, adipose tissue, and the pancreas [199]. Given that myokine release is stimulated via muscle contraction, it is believed that myokines play a significant role in the metabolic benefits of exercise [200]. Myokines other than the ones discussed below may also be involved in IR and have potential as biomarkers including β(beta)-aminoisobutyric acid (BAIBA), myostatin, brain-derived neurotrophic factor (BDNF), and follistatin-related protein-1 (FST1) [165].

Interleukin-6

Interleukin-6 (IL-6) is the best-characterized myokine. As mentioned, IL-6 is expressed in multiple tissues and has tissue-specific effects. In exercising muscle, IL-6 is released from myofibers in amounts coinciding with exercise intensity and duration [201] and pre-exercise glycogen stores [202]. With regard to glucose sensitivity, it appears that in muscle the length of exposure dictates whether IL-6 results in increased glucose uptake (acute) or activation of JNK/activator protein-1 and insulin signaling impairment at IRS-1 (chronic) [203]. With acute exposure in myotubes, IL-6 results in increased glucose uptake,

GLUT4 translocation, and glycogen synthesis—effects that are not observed in myotubes from subjects with T2DM [204]. IL-6 also regulates insulin secretion by increasing the proliferation of α(alpha)- and β(beta)-cells and preventing apoptosis of α(alpha)-cells [205] and stimulating glucagon-like peptide-1 (GLP-1) synthesis and secretion in intestinal L cells and pancreatic α(alpha)-cells [206]. A paucity of data exists in children with regard to muscle production of IL-6. In a mixed sample of 9- to 11-year-olds, resting levels of IL-6 were elevated in normal-weight, low-fit and obese, and low-fit children relative to their normal-weight, high-fit counterparts [207]. These data support a positive effect of exercise on lowering circulating IL-6, but are not direct evidence of muscle involvement per se.

Interleukin-13 and Interleukin-15

Interleukin-13 (IL-13) was recently identified as being synthesized in muscle [208]. It is secreted by Th2 cells and is categorized as an anti-inflammatory cytokine that counteracts the actions of inflammatory cytokines with regard to insulin sensitivity [198]. In cultured myotubes from subjects with T2DM, IL-13 secretion is reduced, and treatment with an IL-13 neutralizing antibody resulted in a reduction in basal glycogen synthesis [208]. In muscle tissue, it is also associated with increased glucose uptake and oxidation [208]. In mice, IL-13 has been shown to act in an endocrine fashion to decrease glucose production in the liver via the Stat3 pathway [209]. Interleukin-15 (IL-15) is also secreted by muscle and may influence insulin sensitivity through improvements in lipid handling via regulation of mitochondrial activity and mass in adipocytes [210]. IL-15 reportedly increases fatty acid oxidation, decreases lipid accumulation, and increases adiponectin secretion [198].

Irisin

Irisin is a relative newcomer to the myokine family. Discovered in 2012, this hormone is a cleavage product of fibronectin type III domain contain 5 (FDNC5) [211]. Since its discovery, irisin has been controversial, with questions arising as to not only its function but also its existence (reviewed in [212]). The controversy arose mainly due to questions about the ability of commercially available antibodies to detect the peptide. Spiegelman et al. used mass spectrometry and stable isotope-enriched controls to identify irisin in human plasma [213].

FDNC5 is driven by the transcriptional coactivator PGC1-α(alpha) (ppar-γ[gamma] coactivator-1-α[alpha]) and is induced with exercise [214]. Irisin has since been determined to be secreted from adipose tissue as well as muscle and participates in organ cross-talk to regulate metabolic homeostasis [215]. Via upregulation of UCP1, irisin has been indicated in upregulation of subcutaneous adipose tissue browning and thermogenesis in response to exercise [211, 215]. This mechanism is supported in mice treated with fenofibrate, a PGC1-α(alpha) activator, which led to an increase in serum irisin levels and UCP1 expression [216]. In humans, similar to exercise, cold-induced thermogenesis induced irisin secretion proportional to shivering intensity [217].

With regard to insulin sensitivity, overexpression of FNDC5 in mice resulted in decreased fat mass and improved glucose tolerance in response to high-fat diet-induced IR [211]. Multiple human studies have reported decreased irisin in T2DM patients relative to euglycemic controls [215, 218–221]. However, others have found negative associations between irisin and fasting glucose [222] and HbA1c [219, 222]. The role of irisin may be difficult to ascertain since irisin, like leptin, likely varies with BMI and adiposity [223–225]. The picture is equally discordant in children and adolescents. In a group of 13- to 15-year-olds, obese teens had significantly higher levels of circulating irisin than their healthy controls [226], while Viitasalo et al. reported no significant differences in plasma irisin levels between overweight/obese and normal-weight children [227]. Yet in a separate study, irisin correlated negatively with fasting glucose in boys and girls; however, it showed a

negative correlation with HOMA-IR in girls alone, indicating sexual discordance [228]. More work is needed in adults and children to clarify this relationship.

Fibroblast Growth Factor 21

Though more highly expressed in the liver, fibroblast growth factor 21 (FGF21) is secreted in C2C12 myotubes [229] and FGF21 mRNA was increased in skeletal muscle corresponding to an increase in plasma FGF21 in healthy men in a hyperinsulinemic-euglycemic clamp study [230]. Experiments in mice treated with FGF21 suggest that it modulates insulin sensitivity by enhancing glucose uptake in skeletal muscle and adipose tissue, suppressing adipose tissue lipolysis and inducing adipose tissue browning [231]. Recent studies suggest that muscle produces FGF21 under conditions of mitochondrial stress as a mechanism for mediating metabolic adaptations [232]. This protein is discussed further in the following section under hepatokines.

Hepatokines

The liver is a key regulator of whole-body metabolic homeostasis. Through proteins produced in and secreted by the liver (hepatokines), the liver acts as a control center for the regulation of lipid and glucose metabolism in other metabolic tissues, such as adipose tissue and muscle. Hepatic lipid accumulation can result in glucotoxicity and lipotoxicity via inflammatory and signaling pathways in conditions of overnutrition. Various hepatokines have been proposed as predictive biomarkers of IR. The major hepatokines are discussed below, but others that may be of interest include leukocyte cell-derived chemotaxin 2 (LECT2), chimerin, and insulin-like growth factors [165, 233].

Fibroblast Growth Factor 21

As mentioned above, FGF21 is primarily produced in the liver and is, therefore, considered a hepatokine. It is a member of the FGF family of proteins responsible for regulating biological functions, such as cell differentiation, cell growth, and angiogenesis, and is now recognized as playing a key role in oxidative stress [234]. In the liver and adipose tissue, FGF21 is a potent activator of glucose uptake and lipid metabolism via cell surface FGF receptors [235], and studies in rats suggest that FGF21 may also act centrally [236]. FGF21 administration in mouse models of diabetes and obesity causes decreased body weight, improvements in lipid profiles, and improved insulin sensitivity [237]. Moreover, the glucose effects occur within 1 hour and are independent of weight loss. Similar effects were observed in nonhuman primates [238]. In humans, the role of FGF21 is less clear. Treatment of humans with a pharmacological FGF21 analog produced some weight loss and modestly lowered insulin; however, there were no effects on glucose [239]. Some, but not all, human studies have found associations between FGF21 levels and BMI, nonalcoholic fatty liver disease (NAFLD), and metabolic syndrome [240]. Similarly, in children, the relationship between FGF21 and obesity/IR is mixed. A study of a large cohort of children and adolescents aged 6–18 reported decreased FGF21 in those with obesity relative to their lean counterparts, and children in the lowest quintile of FGF21 were more likely to be insulin resistant [241]. However, studies of children more similar in age (10–14 years old [240] and 14–15 years old [242]) observed a positive association between FGF21 and obesity, but no significant association with IR. Further, in Thai children aged 9–14 years, serum FGF21 was highest in obese children with the most severe IR [243]. The mixed results highlight the complex nature of assessing metabolic parameters in growing children. The observation that FGF21 circulation is circadian-dependent [244] may also complicate its use as a biomarker.

Fetuin-A

Shim et al. very recently proposed fetuin-A (FetA) as a potential marker for IR and cardiovascular risk in prepubertal children [245]. FetA, also known as α(alpha)2-Heremans-Schmid glycoprotein (AHSG), is a glycoprotein

produced in the liver and adipose tissue. It acts in muscle and the liver as a natural inhibitor of insulin receptor tyrosine kinase activity [246, 247]. FetA has been shown to drive IR alone [248] or as an adapter molecule for saturated fatty acid-mediated Toll-like receptor 4 (TLR4) activation [248, 249], suggesting it might be a key player in lipid-induced inflammation [250]. The Toll-like receptors are a class of proteins with a key role in the innate immune system. FetA is a primary carrier of circulating free fatty acids (FFA) [251]. In the absence of FFA, FetA can bind directly to TLR4 [251, 252] and act as a FFA presenter to TLR4. Pal et al. demonstrated in mice that both FetA and FFA are required for the activation of TLR4 and the resulting proinflammatory cytokine expression [249]. Reducing the expression of FetA in mice on a high-fat diet prevented FFA-induced IR. In humans, the relationship between circulating FetA and BMI is not completely clear [245, 253–256]; however, FetA is consistently associated with IR and T2DM in adults [233, 249, 257, 258] and children [245, 256, 259, 260]. Circulating FetA can be modified with weight loss [260, 261] and is an independent predictor of abdominal adiposity [262]; both are characteristics of a potential candidate biomarker.

Betatrophin

To date, there are eight identified angiopoietin-like proteins. Betatrophin (ANGPTL8/lipasin) is unique among the members of the ANGPTL family as it lacks the typical fibrinogen-like domain as well as the glycosylation sites and amino acids required for forming disulfide bonds that are common to the others. Derived from hepatocytes, betatrophin regulates other ANGPTL proteins to control circulating triglycerides, potentially by trafficking postprandial free fatty acids from the liver to peripheral tissues [263]. In adipose tissue, betatrophin is upregulated by insulin and downregulated by lipolysis activators, such as forskolin and isoproterenol [264]. Clinical studies in humans with T2DM have shown both that betatrophin levels are increased [265–267] and decreased [268]. Abu-Farh recently reported that although betatrophin was increased in T2DM patients, betatrophin level did not correlate with fasting blood glucose or HOMA-IR in these subjects [269]. Early studies in animal models that suggested that betatrophin could stimulate β(beta)-cell mass and proliferation have since been disputed [270, 271]. The relation of circulating betatrophin to IR and T2DM in children and adolescents is unclear. Positive [272], negative [273], and neutral [274] associations have been reported potentially due to differences in age, sex, and pubertal status.

Selenoprotein P (SeP)

The trace mineral selenium is a key component of several enzymes that regulate redox homeostasis, thyroid hormone metabolism, and oxidative stress [275]. Selenoprotein P (SeP) transports selenium from the liver to extrahepatic tissues and contains 22–65% of the plasma selenium content [276, 277]. SeP was originally identified as an antioxidant enzyme that reduced reactive oxygen species by supplying cells with selenium [278], and early studies of selenium suggested it might act as an insulin mimetic [279]. Overproduction of SeP has been correlated with the severity of IR in adults with prediabetes and T2DM by impairing insulin signaling in the liver and muscle [280–283]. Further, downregulation of SeP in livers of mice with T2DM resulted in improvements in glucose tolerance and insulin sensitivity via the AMPK-FOXO1α(alpha) pathway. This mechanism is supported in animals treated with adiponectin and salsalate [284].

In young children (9 years old), however, increased SeP was associated with a *decreased* risk profile for metabolic syndrome and correlated negatively with insulin and HOMA-IR [285]. This negative correlation is supported by studies in mice, rats, and primary rat adipocytes showing inverse associations with adipogenesis, obesity, and IR—likely due to its role as an antioxidant [286]. The discrepancy may partially arise from wide ranges of selenium in various geological and climate environments, as well as sources of food and fodder resulting in extremes of deficiency and excess [275]. There is evidence that both conditions may contribute to IR and risk of

T2DM. Further complicating the matter, the expression of SeP and gluconeogenic enzymes, such as glucose-6-phosphatase, is intimately linked through PGC1-α(alpha)/Foxo1α(alpha) and S-adenosyl methionine-dependent protein methylation [275]. Given the role of SeP in oxidative stress, the degree of systemic inflammation may also explain differences in populations at different stages of metabolic dysfunction. Plasma selenium and the expression of SeP is typically reduced in the inflammatory state with implications for peripheral, insulin-sensitive tissues [275]. As with many nutrients, there may be a U-shaped association between selenium and its metabolic pathways and the associated pathologies.

Steroid Hormone-Binding Globulin (SHBG)

Canonically considered a sex hormone transport protein, steroid hormone-binding globulin (SHBG) has also been observed in relation to IR, namely, low SHBG levels are associated with increased risk of IR and T2DM [287–289]. SHBG is a 90 kDa glycoprotein primarily synthesized in the liver. It has two subunits and two steroid binding sites with varying affinity for testosterone and estradiol [290]. Liganded SHBG does not bind to the SHBG receptor, supporting the idea that SHBG has a role in activities unrelated to sex hormones [291]. Circulating SHBG is inversely associated with HbA1c in both men and women without diabetes [292, 293], and cross-sectional and longitudinal studies report increased risk of developing T2DM with low SHBG concentrations [294].

In children, SHBG rises, plateaus, and then declines before puberty, potentially because of changes in adiposity. Cross-sectional studies of children and adolescents have found an inverse relationship between SHBG levels and insulin sensitivity based on oral glucose tolerance tests [295] and HOMA-IR [296]. However, adiposity may also be implicated in decreased SHBG and IR in obese children [297]. The EarlyBird study, an observational study of the childhood origins of metabolic disease, measured SHBG in children longitudinally from ages 5 through 15 years [298]. They confirmed the decline in SHBG before puberty in girls and boys, but were unable to make a direct association between SHBG and insulin. Various models controlling for BMI revealed that adiposity was the driving factor behind SHBG levels across the study. As with the sex hormones themselves, the use of SHBG as a biomarker for IR is complicated by developmental stage and adiposity.

Gut Microbiota

Based initially on the observation that germ-free mice have reduced adiposity that can be reversed by colonization with gut microbiota from normal mice [299], the trillions of organisms that inhabit the gut are now recognized as significant contributors to metabolic health. The gut microbiota is integral to energy harvest and production and metabolism of numerous molecules entering the gut. Products of gut microbial metabolism have activities in the gut tissue itself as well as systemically via entry into portal and then systemic circulation. Therefore, gut ecology likely regulates metabolism both directly and indirectly. This is a complex and evolving relationship and less is known in children than animal studies and adults. The current state of knowledge is covered nicely in a series of papers in the *Journal of Physiology*. Some of the best-studied mediators include components of the bacteria themselves (lipopolysaccharides, LPS) and their metabolic products, angiopoietin-like protein-4 (ANGPTL4), bile acids (BA), and short-chain fatty acids (SCFA).

Much of this work has been in animal models and is inconsistent. However, human studies have linked bacterial profiles with increased capacity for branched-chain amino acid (BCAA) production and IR in nondiabetic subjects [300]. Mastrangelo et al. also observed greater concentrations of the conjugated bile acids, glycodeoxycholate and taurodeoxycholate, in obese boys and girls aged 5–11 years [32]. Primary bile acids are produced in the liver and are metabolized by gut microbiota to form secondary bile acids. In

addition to their role in the emulsification and absorption of dietary fatty acids, bile acids act through the farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 5 (TGR5) to modulate bile acid, lipid, glucose, and energy metabolism, as well as intestinal hormone secretion and inflammation. Disruptions in the gut microbiota may therefore play a significant role in metabolic disease [301]. New technologies in bacterial identification and metabolic profiling will enable thorough investigation of these pathways.

Summary

IR is a multi-organ disorder. Cross talk between organs (Fig. 11.1) [32] and compensatory mechanisms in different stages of obesity and IR [165, 210], as well as different stages of growth and puberty, makes establishing biomarkers that are predictive of dysfunction difficult in children and adolescents. While the body of work in this area is steadily growing, inconsistencies between studies arise from variations in the subject populations (age, developmental stage, race, geography), study design (cross-sectional, longitudinal, intervention), and methodology (mass spectroscopy, glucose tolerance test, hyperinsulinemic-euglycemic clamp). Despite these variables, central carbon metabolism and inflammation are the most consistent processes modified in obese children and adolescents with impaired insulin signaling.

Advancements in technology (i.e., metabolomics, metagenomics) are allowing more detailed analyses in minimally invasive samples, such as blood, saliva, and stool. These developments should improve study participation, compliance, and reliability in young participants and accelerate the rate of biomarker discovery. Extracellular RNAs (exRNAs) are also being

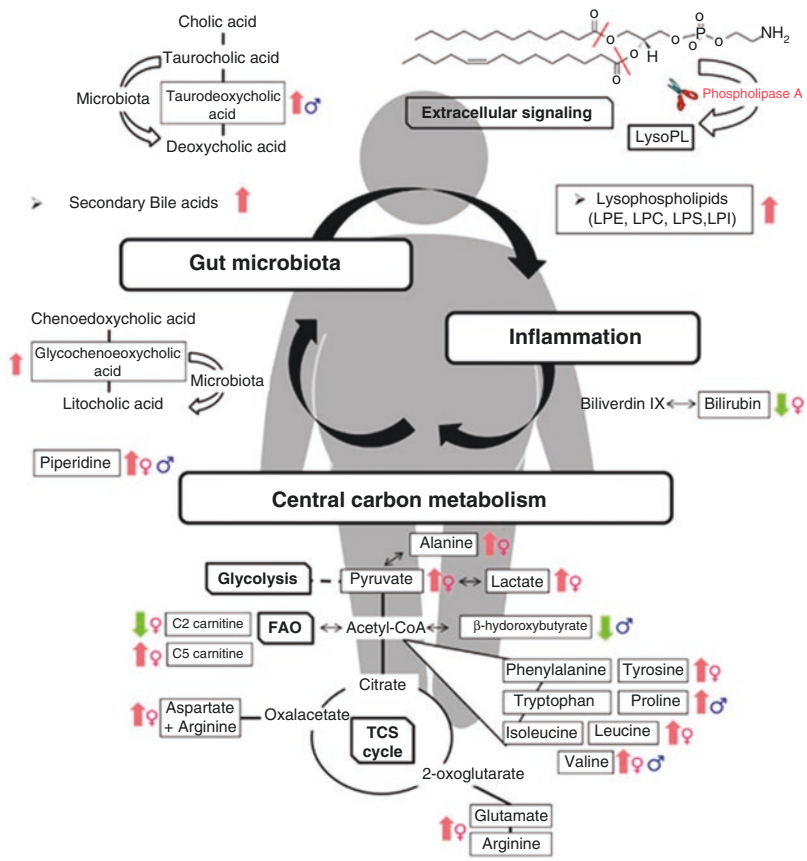

Fig. 11.1 Overview of the changes observed in the metabolic profile of obese children with and without IR. (Reprinted with permission from Mastrangelo et al. [32])

investigated for their role in glucose homeostasis, but there is much work to do in this burgeoning field [302]. Early identification of predictive biomarkers of IR and other metabolic derangements may help mitigate the development of chronic disease.

References

1. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet. 2009;373(9682):2215–21.
2. Faerch K, Witte DR, Tabak AG, Perreault L, Herder C, Brunner EJ, et al. Trajectories of cardiometabolic risk factors before diagnosis of three subtypes of type 2 diabetes: a post-hoc analysis of the longitudinal Whitehall II cohort study. Lancet Diabetes Endocrinol. 2013;1(1):43–51.
3. Kim OY, Lee JH, Sweeney G. Metabolomic profiling as a useful tool for diagnosis and treatment of chronic disease: focus on obesity, diabetes and cardiovascular diseases. Expert Rev Cardiovasc Ther. 2013;11(1):61–8.
4. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological nmr spectroscopic data. Xenobiotica. 1999;29(11):1181–9.
5. Moco S, Collino S, Rezzi S, Martin FP. Metabolomics perspectives in pediatric research. Pediatr Res. 2013;73(4 Pt 2):570–6.
6. Jewell JL, Russell RC, Guan KL. Amino acid signalling upstream of mtor. Nat Rev Mol Cell Biol. 2013;14(3):133–9.
7. Wiklund PK, Pekkala S, Autio R, Munukka E, Xu L, Saltevo J, et al. Serum metabolic profiles in overweight and obese women with and without metabolic syndrome. Diabetol Metab Syndr. 2014;6(1):40.
8. Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y, et al. Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. Sci Rep. 2016;6:24540.
9. Potier M, Darcel N, Tome D. Protein, amino acids and the control of food intake. Curr Opin Clin Nutr Metab Care. 2009;12(1):54–8.
10. Salehi A, Gunnerud U, Muhammed SJ, Ostman E, Holst JJ, Bjorck I, et al. The insulinogenic effect of whey protein is partially mediated by a direct effect of amino acids and gip on beta-cells. Nutr Metab (Lond). 2012;9(1):48.
11. Xu G, Li Y, An W, Li S, Guan Y, Wang N, et al. Gastric mammalian target of rapamycin signaling regulates ghrelin production and food intake. Endocrinology. 2009;150(8):3637–44.
12. Lynch CJ, Gern B, Lloyd C, Hutson SM, Eicher R, Vary TC. Leucine in food mediates some of the postprandial rise in plasma leptin concentrations. Am J Physiol Endocrinol Metab. 2006;291(3):E621–30.
13. Chen Q, Reimer RA. Dairy protein and leucine alter glp-1 release and mrna of genes involved in intestinal lipid metabolism in vitro. Nutrition. 2009;25(3):340–9.
14. Krebs M, Krssak M, Bernroider E, Anderwald C, Brehm A, Meyerspeer M, et al. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes. 2002;51(3):599–605.
15. Krebs M, Brunmair B, Brehm A, Artwohl M, Szendroedi J, Nowotny P, et al. The mammalian target of rapamycin pathway regulates nutrient-sensitive glucose uptake in man. Diabetes. 2007;56(6):1600–7.
16. Blouet C, Schwartz GJ. Brainstem nutrient sensing in the nucleus of the solitary tract inhibits feeding. Cell Metab. 2012;16(5):579–87.
17. Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, et al. Hypothalamic mtor signaling regulates food intake. Science. 2006;312(5775):927–30.
18. Laeger T, Reed SD, Henagan TM, Fernandez DH, Taghavi M, Addington A, et al. Leucine acts in the brain to suppress food intake but does not function as a physiological signal of low dietary protein. Am J Physiol Regul Integr Comp Physiol. 2014;307(3):R310–20.
19. Floyd JC Jr, Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. J Clin Invest. 1966;45(9):1487–502.
20. van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, Keizer HA. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. Diabetes Care. 2003;26(3):625–30.
21. Felig P, Marliss E, Cahill GF. Plasma amino acid levels and insulin secretion in obesity. N Engl J Med. 1969;281(15):811–6.
22. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009;9(4):311–26.
23. Palmer ND, Stevens RD, Antinozzi PA, Anderson A, Bergman RN, Wagenknecht LE, et al. Metabolomic profile associated with insulin resistance and conversion to diabetes in the insulin resistance atherosclerosis study. J Clin Endocrinol Metab. 2015;100(3):E463–8.
24. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011;17(4):448–53.
25. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, et al. Insulin resistance is associated with a metabolic profile of altered protein metabo-

lism in chinese and asian-indian men. Diabetologia. 2010;53(4):757–67.
26. Wurtz P, Soininen P, Kangas AJ, Ronnemaa T, Lehtimaki T, Kahonen M, et al. Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. Diabetes Care. 2013;36(3):648–55.
27. McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, Gerszten RE, et al. Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. Pediatr Obes. 2013;8(1):52–61.
28. Butte NF, Liu Y, Zakeri IF, Mohney RP, Mehta N, Voruganti VS, et al. Global metabolomic profiling targeting childhood obesity in the hispanic population. Am J Clin Nutr. 2015;102(2):256–67.
29. Hellmuth C, Kirchberg FF, Lass N, Harder U, Peissner W, Koletzko B, et al. Tyrosine is associated with insulin resistance in longitudinal metabolomic profiling of obese children. J Diabetes Res. 2016;2016:2108909.
30. Lee A, Jang HB, Ra M, Choi Y, Lee HJ, Park JY, et al. Prediction of future risk of insulin resistance and metabolic syndrome based on korean boy's metabolite profiling. Obes Res Clin Pract. 2015;9(4):336–45.
31. Wahl S, Yu Z, Kleber M, Singmann P, Holzapfel C, He Y, et al. Childhood obesity is associated with changes in the serum metabolite profile. Obes Facts. 2012;5(5):660–70.
32. Mastrangelo A, Martos-Moreno GA, Garcia A, Barrios V, Ruperez FJ, Chowen JA, et al. Insulin resistance in prepubertal obese children correlates with sex-dependent early onset metabolomic alterations. Int J Obes (Lond). 2016;40(10):1494–502.
33. Perng W, Gillman MW, Fleisch AF, Michalek RD, Watkins SM, Isganaitis E, et al. Metabolomic profiles and childhood obesity. Obesity (Silver Spring). 2014;22(12):2570–8.
34. Mihalik SJ, Michaliszyn SF, de las Heras J, Bacha F, Lee S, Chace DH, et al. Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation. Diabetes Care. 2012;35(3):605–11.
35. Michaliszyn SF, Sjaarda LA, Mihalik SJ, Lee S, Bacha F, Chace DH, et al. Metabolomic profiling of amino acids and beta-cell function relative to insulin sensitivity in youth. J Clin Endocrinol Metab. 2012;97(11):E2119–24.
36. Vary TC, Lynch CJ. Nutrient signaling components controlling protein synthesis in striated muscle. J Nutr. 2007;137(8):1835–43.
37. Wang X, Proud CG. The mtor pathway in the control of protein synthesis. Physiology (Bethesda). 2006;21:362–9.
38. Dodd KM, Tee AR. Leucine and mtorc1: a complex relationship. Am J Physiol Endocrinol Metab. 2012;302(11):E1329–42.
39. Laplante M, Sabatini DM. Regulation of mtorc1 and its impact on gene expression at a glance. J Cell Sci. 2013;126(Pt 8):1713–9.
40. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol. 2014;10(12):723–36.
41. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab. 2012;15(5):606–14.
42. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of s6k1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature. 2004;431(7005):200–5.
43. Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein s6 kinase 1, s6k1. Cell Metab. 2006;3(6):393–402.
44. Macotela Y, Emanuelli B, Bång AM, Espinoza DO, Boucher J, Beebe K, et al. Dietary leucine – an environmental modifier of insulin resistance acting on multiple levels of metabolism. PLoS One. 2011;6(6):e21187.
45. She P, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, et al. Disruption of bcatm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. Cell Metab. 2007;6(3):181–94.
46. Fraenkel M, Ketzinel-Gilad M, Ariav Y, Pappo O, Karaca M, Castel J, et al. Mtor inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. Diabetes. 2008;57(4):945–57.
47. Leibowitz G, Cerasi E, Ketzinel-Gilad M. The role of mtor in the adaptation and failure of beta-cells in type 2 diabetes. Diabetes Obes Metab. 2008;10(Suppl 4):157–69.
48. Lopes P, Fuhrmann A, Sereno J, Pereira MJ, Nunes P, Pedro J, et al. Effects of cyclosporine and sirolimus on insulin-stimulated glucose transport and glucose tolerance in a rat model. Transplant Proc. 2013;45(3):1142–8.
49. Gyurus E, Kaposztas Z, Kahan BD. Sirolimus therapy predisposes to new-onset diabetes mellitus after renal transplantation: a long-term analysis of various treatment regimens. Transplant Proc. 2011;43(5):1583–92.
50. Hughes KJ, Kennedy BK. Cell biology. Rapamycin paradox resolved. Science. 2012;335(6076):1578–9.
51. Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, et al. Rapamycin-induced insulin resistance is mediated by mtorc2 loss and uncoupled from longevity. Science. 2012;335(6076):1638–43.
52. Sonnet DS, O'Leary MN, Gutierrez MA, Nguyen SM, Mateen S, Hsu Y, et al. Metformin inhibits branched chain amino acid (bcaa) derived ketoacidosis and promotes metabolic homeostasis in msud. Sci Rep. 2016;6:28775.
53. Montgomery MK, Turner N. Mitochondrial dysfunction and insulin resistance: an update. Endocr Connect. 2015;4(1):R1–R15.

54. Adams SH, Hoppel CL, Lok KH, Zhao L, Wong SW, Minkler PE, et al. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic african-american women. J Nutr. 2009;139(6):1073–81.
55. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963;1(7285):785–9.
56. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest. 2002;109(10):1345–50.
57. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI. Mechanism of insulin resistance in a-zip/f-1 fatless mice. J Biol Chem. 2000;275(12):8456–60.
58. Carnethon MR, De Chavez PJ, Biggs ML, Lewis CE, Pankow JS, Bertoni AG, et al. Association of weight status with mortality in adults with incident diabetes. JAMA. 2012;308(6):581–90.
59. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, et al. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest. 1996;97(12):2859–65.
60. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, et al. Effects of free fatty acids on glucose transport and irs-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest. 1999;103(2):253–9.
61. Rigazio S, Lehto HR, Tuunanen H, Någren K, Kankaanpaa M, Simi C, et al. The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans. Am J Physiol Endocrinol Metab. 2008;295(2):E413–9.
62. Kashyap SR, Belfort R, Berria R, Suraamornkul S, Pratipranawatr T, Finlayson J, et al. Discordant effects of a chronic physiological increase in plasma ffa on insulin signaling in healthy subjects with or without a family history of type 2 diabetes. Am J Physiol Endocrinol Metab. 2004;287(3):E537–46.
63. Gulli G, Ferrannini E, Stern M, Haffner S, DeFronzo RA. The metabolic profile of niddm is fully established in glucose-tolerant offspring of two mexican-american niddm parents. Diabetes. 1992;41(12):1575–86.
64. Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes. 2007;56(5):1376–81.
65. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med. 2004;350(7):664–71.
66. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest. 2016;126(1):12–22.
67. Muoio DM, Newgard CB. Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(3):193–205.
68. D'Souza K, Nzirorera C, Kienesberger PC. Lipid metabolism and signaling in cardiac lipotoxicity. Biochim Biophys Acta. 2016;1860(10):1513–24.
69. Newbern D, Gumus Balikcioglu P, Balikcioglu M, Bain J, Muehlbauer M, Stevens R, et al. Sex differences in biomarkers associated with insulin resistance in obese adolescents: metabolomic profiling and principal components analysis. J Clin Endocrinol Metab. 2014;99(12):4730–9.
70. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1h nmr spectroscopy study. Diabetologia. 1999;42(1):113–6.
71. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1h-13c nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. Diabetes. 1999;48(8):1600–6.
72. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, et al. Assessment of skeletal muscle triglyceride content by (1)h nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. Diabetes. 2002;51(4):1022–7.
73. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, et al. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (irs-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem. 2002;277(52):50230–6.
74. Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, et al. Role of diacylglycerol activation of pkcθ in lipid-induced muscle insulin resistance in humans. Proc Natl Acad Sci U S A. 2014;111(26):9597–602.
75. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase c, and ikappab-alpha. Diabetes. 2002;51(7):2005–11.
76. Magkos F, Su X, Bradley D, Fabbrini E, Conte C, Eagon JC, et al. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. Gastroenterology. 2012;142(7):1444–1446.e1442.
77. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, et al. Cers2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. Cell Metab. 2014;20(4):687–95.
78. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-induced cers6-dependent c16:0 ceramide production promotes weight gain and glucose intolerance. Cell Metab. 2014;20(4):678–86.

79. Perry RJ, Kim T, Zhang XM, Lee HY, Pesta D, Popov VB, et al. Reversal of hypertriglyceridemia, fatty liver disease, and insulin resistance by a liver-targeted mitochondrial uncoupler. Cell Metab. 2013;18(5):740–8.
80. Perry RJ, Zhang D, Zhang XM, Boyer JL, Shulman GI. Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. Science. 2015;347(6227):1253–6.
81. Tao H, Zhang Y, Zeng X, Shulman GI, Jin S. Niclosamide ethanolamine-induced mild mitochondrial uncoupling improves diabetic symptoms in mice. Nat Med. 2014;20(11):1263–9.
82. Perreault L, Starling AP, Glueck D, Brozinick JT, Sanders P, Siddall P, et al. Biomarkers of ectopic fat deposition: the next frontier in serum lipidomics. J Clin Endocrinol Metab. 2016;101(1):176–82.
83. Mauvais-Jarvis F. Sex differences in metabolic homeostasis, diabetes, and obesity. Biol Sex Differ. 2015;6:14.
84. Glumer C, Jorgensen T, Borch-Johnsen K. Inter s. Prevalences of diabetes and impaired glucose regulation in a danish population: the inter99 study. Diabetes Care. 2003;26(8):2335–40.
85. Sicree RA, Zimmet PZ, Dunstan DW, Cameron AJ, Welborn TA, Shaw JE. Differences in height explain gender differences in the response to the oral glucose tolerance test- the ausdiab study. Diabet Med. 2008;25(3):296–302.
86. van Genugten RE, Utzschneider KM, Tong J, Gerchman F, Zraika S, Udayasankar J, et al. Effects of sex and hormone replacement therapy use on the prevalence of isolated impaired fasting glucose and isolated impaired glucose tolerance in subjects with a family history of type 2 diabetes. Diabetes. 2006;55(12):3529–35.
87. Williams JW, Zimmet PZ, Shaw JE, de Courten MP, Cameron AJ, Chitson P, et al. Gender differences in the prevalence of impaired fasting glycaemia and impaired glucose tolerance in mauritius. Does sex matter? Diabet Med. 2003;20(11):915–20.
88. Whitfield J. Everything you always wanted to know about sexes. PLoS Biol. 2004;2(6):718–21.
89. Kelsey MM, Zeitler PS. Insulin resistance of puberty. Curr Diab Rep. 2016;16(7):64.
90. Mauvais-Jarvis F. Role of sex steroids in beta cell function, growth, and survival. Trends Endocrinol Metab. 2016;27(12):844–55.
91. Moran A, Jacobs DR Jr, Steinberger J, Steffen LM, Pankow JS, Hong CP, et al. Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. Circulation. 2008;117(18):2361–8.
92. Utriainen P, Laakso S, Liimatta J, Jaaskelainen J, Voutilainen R. Premature adrenarche – a common condition with variable presentation. Horm Res Paediatr. 2015;83(4):221–31.
93. Wurtz P, Makinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, et al. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes. 2012;61(6):1372–80.
94. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev. [Research Support, N.I.H., Extramural Research Support, Non--U.S. Gov't Review]. 2013;34(3):309–38.
95. Deslypere JP, Verdonck L, Vermeulen A. Fat tissue: a steroid reservoir and site of steroid metabolism. J Clin Endocrinol Metab. 1985;61(3):564–70.
96. Falcao-Pires I, Castro-Chaves P, Miranda-Silva D, Lourenco AP, Leite-Moreira AF. Physiological, pathological and potential therapeutic roles of adipokines. Drug Discov Today. 2012;17(15-16):880–9.
97. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11(2):85–97.
98. Zhang M, Hu T, Zhang S, Zhou L. Associations of different adipose tissue depots with insulin resistance: a systematic review and meta-analysis of observational studies. Sci Rep. 2015;5:18495.
99. Al-Hamodi Z, Al-Habori M, Al-Meeri A, Saif-Ali R. Association of adipokines, leptin/adiponectin ratio and c-reactive protein with obesity and type 2 diabetes mellitus. Diabetol Metab Syndr. 2014;6(1):99.
100. Korner A, Kratzsch J, Gausche R, Schaab M, Erbs S, Kiess W. New predictors of the metabolic syndrome in children – role of adipocytokines. Pediatr Res. 2007;61(6):640–5.
101. Valle M, Martos R, Gascon F, Canete R, Zafra MA, Morales R. Low-grade systemic inflammation, hypoadiponectinemia and a high concentration of leptin are present in very young obese children, and correlate with metabolic syndrome. Diabetes Metab. 2005;31(1):55–62.
102. Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World J Diabetes. 2014;5(6):730–8.
103. Rodriguez A, Ezquerro S, Mendez-Gimenez L, Becerril S, Fruhbeck G. Revisiting the adipocyte: a model for integration of cytokine signaling in the regulation of energy metabolism. Am J Physiol Endocrinol Metab. 2015;309(8):E691–714.
104. Coppari R, Bjorbaek C. Leptin revisited: its mechanism of action and potential for treating diabetes. Nat Rev Drug Discov. 2012;11(9):692–708.
105. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J Clin Invest. 2012;122(1):4–12.
106. German JP, Thaler JP, Wisse BE, Oh IS, Sarruf DA, Matsen ME, et al. Leptin activates a novel cns mechanism for insulin-independent normalization of severe diabetic hyperglycemia. Endocrinology. 2011;152(2):394–404.
107. German JP, Wisse BE, Thaler JP, Oh IS, Sarruf DA, Ogimoto K, et al. Leptin deficiency causes insulin resistance induced by uncontrolled diabetes. Diabetes. 2010;59(7):1626–34.

108. Perry RJ, Zhang XM, Zhang D, Kumashiro N, Camporez JP, Cline GW, et al. Leptin reverses diabetes by suppression of the hypothalamic-pituitary-adrenal axis. Nat Med. 2014;20(7):759–63.
109. Soliman AT, Yasin M, Kassem A. Leptin in pediatrics: a hormone from adipocyte that wheels several functions in children. Indian J Endocrinol Metab. 2012;16(Suppl 3):S577–87.
110. Morales A, Wasserfall C, Brusko T, Carter C, Schatz D, Silverstein J, et al. Adiponectin and leptin concentrations may aid in discriminating disease forms in children and adolescents with type 1 and type 2 diabetes. Diabetes Care. 2004;27(8):2010–4.
111. Rajkovic N, Zamaklar M, Lalic K, Jotic A, Lukic L, Milicic T, et al. Relationship between obesity, adipocytokines and inflammatory markers in type 2 diabetes: relevance for cardiovascular risk prevention. Int J Environ Res Public Health. 2014;11(4):4049–65.
112. Abdelgadir M, Elbagir M, Eltom M, Berne C, Ahren B. Reduced leptin concentrations in subjects with type 2 diabetes mellitus in sudan. Metabolism. 2002;51(3):304–6.
113. Stringer DM, Sellers EA, Burr LL, Taylor CG. Altered plasma adipokines and markers of oxidative stress suggest increased risk of cardiovascular disease in first nation youth with obesity or type 2 diabetes mellitus. Pediatr Diabetes. 2009;10(4):269–77.
114. West NA, Hamman RF, Mayer-Davis EJ, D'Agostino RB, Marcovina SM, Liese AD, et al. Cardiovascular risk factors among youth with and without type 2 diabetes differences and possible mechanisms. Diabetes Care. 2009;32(1):175–80.
115. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature. 2001;409(6818):307–12.
116. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin / fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes. 2001;50(10):2199–202.
117. Tsiotra PC, Tsigos C, Anastasiou E, Yfanti E, Boutati E, Souvatzoglou E, et al. Peripheral mononuclear cell resistin mrna expression is increased in type 2 diabetic women. Mediators Inflamm. 2008;2008:892864.
118. Gharibeh MY, Al Tawallbeh GM, Abboud MM, Radaideh A, Alhader AA, Khabour OF. Correlation of plasma resistin with obesity and insulin resistance in type 2 diabetic patients. Diabetes Metab. 2010;36(6):443–9.
119. Hasegawa G, Ohta M, Ichida Y, Obayashi H, Shigeta M, Yamasaki M, et al. Increased serum resistin levels in patients with type 2 diabetes are not linked with markers of insulin resistance and adiposity. Acta Diabetol. 2005;42(2):104–9.
120. Laudes M, Oberhauser F, Schulte DM, Freude S, Bilkovski R, Mauer J, et al. Visfatin/pbef/nampt and resistin expressions in circulating blood monocytes are differentially related to obesity and type 2 diabetes in humans. Horm Metab Res. 2010;42(4):268–73.
121. Gerber M, Boettner A, Seidel B, Lammert A, Bar J, Schuster E, et al. Serum resistin levels of obese and lean children and adolescents: biochemical analysis and clinical relevance. J Clin Endocrinol Metab. 2005;90(8):4503–9.
122. Tso TK, Chang CK, Huang HY. Association of circulating adipokines with insulin resistance in obese children. FASEB J. 2008;22(1):880–3.
123. Reinehr T, Roth CL, Menke T, Andler W. Resistin concentrations before and after weight loss in obese children. Int J Obes (Lond). 2006;30(2):297–301.
124. Roth CL, Kratz M, Ralston MM, Reinehr T. Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children. Metabolism. 2011;60(4):445–52.
125. de Luis DA, Gonzalez Sagrado M, Conde R, Aller R, Izaola O, Perez Castrillon JL, et al. Relation of resistin levels with cardiovascular risk factors and insulin resistance in non-diabetes obese patients. Diabetes Res Clin Pract. 2009;84(2):174–8.
126. Takemoto K, Deckelbaum RJ, Saito I, Likitmaskul S, Morandi A, Pinelli L, et al. Adiponectin/resistin levels and insulin resistance in children: a four country comparison study. Int J Pediatr Endocrinol. 2015;2015(1):2.
127. Faghihi T, Radfar M, Abdoli E, Amini H, Hemami MR, Larijani B. Association of serum retinol-binding protein 4 with insulin resistance and metabolic parameters during olanzapine therapy. Clin Endocrinol (Oxf). 2012;76(2):207–11.
128. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med. 2006;354(24):2552–63.
129. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature. 2005;436(7049):356–62.
130. Munkhtulga L, Nagashima S, Nakayama K, Utsumi N, Yanagisawa Y, Gotoh T, et al. Regulatory SNP in the RBP4 gene modified the expression in adipocytes and associated with BMI. Obesity (Silver Spring). 2010;18(5):1006–14.
131. Meisinger C, Ruckert IM, Rathmann W, Doring A, Thorand B, Huth C, et al. Retinol-binding protein 4 is associated with prediabetes in adults from the general population the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study. Diabetes Care. 2011;34(7):1648–50.
132. Qi QB, Yu ZJ, Ye XW, Zhao F, Huang P, Hu FB, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in chinese people. J Clin Endocrinol Metab. 2007;92(12):4827–34.
133. Sun Q, Kiernan UA, Shi L, Phillips DA, Kahn BB, Hu FB, et al. Plasma retinol-binding protein 4 (rbp4) levels and risk of coronary heart disease: a prospec-

tive analysis among women in the nurses' health study. Circulation. 2013;127(19):1938–47.
134. Norseen J, Hosooka T, Hammarstedt A, Yore MM, Kant S, Aryal P, et al. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-jun n-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Mol Cell Biol. 2012;32(10):2010–9.
135. Lee DC, Lee JW, Im JA. Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents. Metabolism. 2007;56(3):327–31.
136. Balagopal P, Graham TE, Kahn BB, Altomare A, Funanage V, George D. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. J Clin Endocrinol Metab. 2007;92(5):1971–4.
137. Aeberli I, Biebinger R, Lehmann R, L'Allemand D, Spinas GA, Zimmermann MB. Serum retinol-binding protein 4 concentration and its ratio to serum retinol are associated with obesity and metabolic syndrome components in children. J Clin Endocrinol Metab. 2007;92(11):4359–65.
138. Skop V, Kontrova K, Zidek V, Pravenec M, Kazdova L, Mikulik K, et al. Autocrine effects of visfatin on hepatocyte sensitivity to insulin action. Physiol Res. 2010;59(4):615–8.
139. Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A, et al. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab. 2007;6(5):363–75.
140. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science. 2005;307(5708):426–30.
141. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. Diabetologia. 2006;49(8):1909–14.
142. Nourbakhsh M, Nourbakhsh M, Gholinejad Z, Razzaghy-Azar M. Visfatin in obese children and adolescents and its association with insulin resistance and metabolic syndrome. Scand J Clin Lab Invest. 2015;75(2):183–8.
143. Martos-Moreno GA, Kratzsch J, Korner A, Barrios V, Hawkins F, Kiess W, et al. Serum visfatin and vaspin levels in prepubertal children: effect of obesity and weight loss after behavior modifications on their secretion and relationship with glucose metabolism. Int J Obes (Lond). 2011;35(10):1355–62.
144. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860–7.
145. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011;121(6):2111–7.
146. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010;72:219–46.
147. Daniele G, Guardado Mendoza R, Winnier D, Fiorentino TV, Pengou Z, Cornell J, et al. The inflammatory status score including IL-6, TNF-alpha, osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin resistance and hyperglycemia in type 2 diabetes mellitus. Acta Diabetol. 2014;51(1):123–31.
148. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci. 2014;15(4):6184–223.
149. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. J Clin Invest. 2017;127(1):1–4.
150. Ruan H, Liu F. Regulation of energy metabolism and maintenance of metabolic homeostasis: the adiponectin story after 20 years. J Mol Cell Biol. 2016;8(2):91–2.
151. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating amp-activated protein kinase. Nat Med. 2002;8(11):1288–95.
152. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Front Endocrinol (Lausanne). 2013;4:71.
153. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of acrp30/adiponectin. Proc Natl Acad Sci U S A. 2004;101(28):10308–13.
154. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009;302(2):179–88.
155. Gabrielsen JS, Gao Y, Simcox JA, Huang J, Thorup D, Jones D, et al. Adipocyte iron regulates adiponectin and insulin sensitivity. J Clin Invest. 2012;122(10):3529–40.
156. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–56.
157. Bobbert T, Rochlitz H, Wegewitz U, Akpulat S, Mai K, Weickert MO, et al. Changes of adiponectin oligomer composition by moderate weight reduction. Diabetes. 2005;54(9):2712–9.
158. Graessler J, Gruber M, Radke RB, Kopprasch S, Schwarz PE, Kamke W, et al. Type 2 diabetes in octogenarians is associated with decreased low molecular weight adiponectin. Gerontology. 2011;57(4):316–26.
159. Leth H, Andersen KK, Frystyk J, Tarnow L, Rossing P, Parving HH, et al. Elevated levels of high-molecular-weight adiponectin in type 1 diabetes. J Clin Endocrinol Metab. 2008;93(8):3186–91.
160. Rubenstein JH, Kao JY, Madanick RD, Zhang M, Wang M, Spacek MB, et al. Association of adiponectin multimers with Barrett's oesophagus. Gut. 2009;58(12):1583–9.

161. Ohman-Hanson RA, Cree-Green M, Kelsey MM, Bessesen DH, Sharp TA, Pyle L, et al. Ethnic and sex differences in adiponectin: from childhood to adulthood. J Clin Endocrinol Metab. 2016;101(12):4808–15.
162. Woo YC, Tso AW, Xu A, Law LS, Fong CH, Lam TH, et al. Combined use of serum adiponectin and tumor necrosis factor-alpha receptor 2 levels was comparable to 2-hour post-load glucose in diabetes prediction. PLoS One. 2012;7(5):e36868.
163. Arslanian S, El Ghormli L, Bacha F, Caprio S, Goland R, Haymond MW, et al. Adiponectin, insulin sensitivity, beta-cell function, and racial/ethnic disparity in treatment failure rates in today. Diabetes Care. 2017;40(1):85–93.
164. Martos-Moreno GA, Barrios V, Martinez G, Hawkins F, Argente J. Effect of weight loss on high-molecular weight adiponectin in obese children. Obesity (Silver Spring). 2010;18(12):2288–94.
165. Oh KJ, Lee DS, Kim WK, Han BS, Lee SC, Bae KH. Metabolic adaptation in obesity and type II diabetes: myokines, adipokines and hepatokines. Int J Mol Sci. 2016;18(1):8.
166. Ouchi N, Higuchi A, Ohashi K, Oshima Y, Gokce N, Shibata R, et al. Sfrp5 is an anti-inflammatory adipokine that modulates metabolic dysfunction in obesity. Science. 2010;329(5990):454–7.
167. Catalan V, Gomez-Ambrosi J, Rodriguez A, Perez-Hernandez AI, Gurbindo J, Ramirez B, et al. Activation of noncanonical wnt signaling through wnt5a in visceral adipose tissue of obese subjects is related to inflammation. J Clin Endocrinol Metab. 2014;99(8):E1407–17.
168. Zhao Y, Zhang C, Huang Y, Yu Y, Li R, Li M, et al. Up-regulated expression of WNT5a increases inflammation and oxidative stress via PI3K/AKT/NF-κB signaling in the granulosa cells of PCOS patients. J Clin Endocrinol Metab. 2015;100(1):201–11.
169. Bovolenta P, Esteve P, Ruiz JM, Cisneros E, Lopez-Rios J. Beyond wnt inhibition: new functions of secreted frizzled-related proteins in development and disease. J Cell Sci. 2008;121(Pt 6):737–46.
170. Mori H, Prestwich TC, Reid MA, Longo KA, Gerin I, Cawthorn WP, et al. Secreted frizzled-related protein 5 suppresses adipocyte mitochondrial metabolism through wnt inhibition. J Clin Invest. 2012;122(7):2405–16.
171. Koza RA, Nikonova L, Hogan J, Rim JS, Mendoza T, Faulk C, et al. Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. PLoS Genet. 2006;2(5):e81.
172. Lagathu C, Christodoulides C, Virtue S, Cawthorn WP, Franzin C, Kimber WA, et al. Dact1, a nutritionally regulated preadipocyte gene, controls adipogenesis by coordinating the wnt/beta-catenin signaling network. Diabetes. 2009;58(3):609–19.
173. Okada Y, Sakaue H, Nagare T, Kasuga M. Diet-induced up-regulation of gene expression in adipocytes without changes in DNA methylation. Kobe J Med Sci. 2009;54(5):E241–9.
174. Carstensen M, Herder C, Kempf K, Erlund I, Martin S, Koenig W, et al. Sfrp5 correlates with insulin resistance and oxidative stress. Eur J Clin Invest. 2013;43(4):350–7.
175. Hu W, Li L, Yang M, Luo X, Ran W, Liu D, et al. Circulating sfrp5 is a signature of obesity-related metabolic disorders and is regulated by glucose and liraglutide in humans. J Clin Endocrinol Metab. 2013;98(1):290–8.
176. Hu Z, Deng H, Qu H. Plasma sfrp5 levels are decreased in chinese subjects with obesity and type 2 diabetes and negatively correlated with parameters of insulin resistance. Diabetes Res Clin Pract. 2013;99(3):391–5.
177. Schulte DM, Muller N, Neumann K, Oberhauser F, Faust M, Gudelhofer H, et al. Pro-inflammatory wnt5a and anti-inflammatory sfrp5 are differentially regulated by nutritional factors in obese human subjects. PLoS One. 2012;7(2):e32437.
178. Canivell S, Rebuffat S, Ruano EG, Kostov B, Siso-Almirall A, Novials A, et al. Circulating sfrp5 levels are elevated in drug-naive recently diagnosed type 2 diabetic patients as compared with prediabetic subjects and controls. Diabetes Metab Res Rev. 2015;31(2):212–9.
179. Prats-Puig A, Soriano-Rodriguez P, Carreras-Badosa G, Riera-Perez E, Ros-Miquel M, Gomila-Borja A, et al. Balanced duo of anti-inflammatory sfrp5 and proinflammatory wnt5a in children. Pediatr Res. 2014;75(6):793–7.
180. Pachori AS, Madan M, Nunez Lopez YO, Yi F, Meyer C, Seyhan AA. Reduced skeletal muscle secreted frizzled-related protein 3 is associated with inflammation and insulin resistance. Obesity (Silver Spring). 2017;25(4):697–703.
181. Gauger KJ, Bassa LM, Henchey EM, Wyman J, Bentley B, Brown M, et al. Mice deficient in sfrp1 exhibit increased adiposity, dysregulated glucose metabolism, and enhanced macrophage infiltration. PLoS One. 2013;8(12):e78320.
182. Mahdi T, Hanzelmann S, Salehi A, Muhammed SJ, Reinbothe TM, Tang Y, et al. Secreted frizzled-related protein 4 reduces insulin secretion and is overexpressed in type 2 diabetes. Cell Metab. 2012;16(5):625–33.
183. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med. 2012;18(3):363–74.
184. Akdis CA, Blaser K. Mechanisms of interleukin-10-mediated immune suppression. Immunology. 2001;103(2):131–6.
185. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol. 2004;22:929–79.
186. Fillatreau S, Gray D, Anderton SM. Not always the bad guys: B cells as regulators of autoimmune pathology. Nat Rev Immunol. 2008;8(5):391–7.
187. Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science. 2013;339(6116):172–7.

188. Han JM, Patterson SJ, Speck M, Ehses JA, Levings MK. Insulin inhibits il-10-mediated regulatory t cell function: implications for obesity. J Immunol. 2014;192(2):623–9.
189. Dagdeviren S, Jung DY, Lee E, Friedline RH, Noh HL, Kim JH, et al. Altered interleukin-10 signaling in skeletal muscle regulates obesity-mediated inflammation and insulin resistance. Mol Cell Biol. 2016;36(23):2956–66.
190. Nakata M, Yamamoto S, Okada T, Gantulga D, Okano H, Ozawa K, et al. Il-10 gene transfer upregulates arcuate pomc and ameliorates hyperphagia, obesity and diabetes by substituting for leptin. Int J Obes (Lond). 2016;40(3):425–33.
191. Kim HJ, Higashimori T, Park SY, Choi H, Dong JY, Kim YJ, et al. Differential effects of interleukin-6 and-10 on skeletal muscle and liver insulin action in vivo. Diabetes. 2004;53(4):1060–7.
192. Charles BA, Doumatey A, Huang H, Zhou J, Chen G, Shriner D, et al. The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. J Clin Endocrinol Metab. 2011;96(12):E2018–22.
193. van Exel E, Gussekloo J, de Craen AJM, Frolich M, Bootsma-van der Wiel A, RGJ W. Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes – the Leiden 85-plus study. Diabetes. 2002;51(4):1088–92.
194. Esposito K, Pontillo A, Giugliano F, Giugliano G, Marfella R, Nicoletti G, et al. Association of low interleukin-10 levels with the metabolic syndrome in obese women. J Clin Endocrinol Metab. 2003;88(3):1055–8.
195. Straczkowski M, Kowalska I, Nikolajuk A, Krukowska A, Gorska M. Plasma interleukin-10 concentration is positively related to insulin sensitivity in young healthy individuals. Diabetes Care. 2005;28(8):2036–7.
196. Tam CS, Garnett SP, Cowell CT, Heilbronn LK, Lee JW, Wong M, et al. IL-6, Il-8 and Il-10 levels in healthy weight and overweight children. Horm Res Paediatr. 2010;73(2):128–34.
197. Chang JS, Bai CH, Huang ZC, Owaga E, Chao KC, Chang CC, et al. Interleukin 10 and clustering of metabolic syndrome components in pediatrics. Eur J Clin Invest. 2014;44(4):384–94.
198. Eckardt K, Gorgens SW, Raschke S, Eckel J. Myokines in insulin resistance and type 2 diabetes. Diabetologia. 2014;57(6):1087–99.
199. Febbraio MA, Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? Exerc Sport Sci Rev. 2005;33(3):114–9.
200. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012;8(8):457–65.
201. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund PB. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J Physiol. 2000;529(Pt 1):237–42.
202. Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B, et al. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J Physiol. 2001;537(Pt 2):633–9.
203. Nieto-Vazquez I, Fernandez-Veledo S, de Alvaro C, Lorenzo M. Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle. Diabetes. 2008;57(12):3211–21.
204. Jiang LQ, Duque-Guimaraes DE, Machado UF, Zierath JR, Krook A. Altered response of skeletal muscle to IL-6 in type 2 diabetic patients. Diabetes. 2013;62(2):355–61.
205. Ellingsgaard H, Ehses JA, Hammar EB, Van Lommel L, Quintens R, Martens G, et al. Interleukin-6 regulates pancreatic alpha-cell mass expansion. Proc Natl Acad Sci U S A. 2008;105(35):13163–8.
206. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from l cells and alpha cells. Nat Med. 2011;17(11):1481–9.
207. Hosick P, McMurray R, Hackney AC, Battaglini C, Combs T, Harrell J. Resting IL-6 and TNF-alpha level in children of different weight and fitness status. Pediatr Exerc Sci. 2013;25(2):238–47.
208. Jiang LQ, Franck N, Egan B, Sjogren RJ, Katayama M, Duque-Guimaraes D, et al. Autocrine role of interleukin-13 on skeletal muscle glucose metabolism in type 2 diabetic patients involves microrna let-7. Am J Physiol Endocrinol Metab. 2013;305(11):E1359–66.
209. Stanya KJ, Jacobi D, Liu S, Bhargava P, Dai L, Gangl MR, et al. Direct control of hepatic glucose production by interleukin-13 in mice. J Clin Invest. 2013;123(1):261–71.
210. Li F, Li Y, Duan Y, Hu CA, Tang Y, Yin Y. Myokines and adipokines: involvement in the crosstalk between skeletal muscle and adipose tissue. Cytokine Growth Factor Rev. 2017;33:73–82.
211. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A pgc1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463–8.
212. Crujeiras AB, Pardo M, Casanueva FF. Irisin: 'Fat' or artefact. Clin Endocrinol (Oxf). 2015;82(4):467–74.
213. Jedrychowski MP, Wrann CD, Paulo JA, Gerber KK, Szpyt J, Robinson MM, et al. Detection and quantitation of circulating human irisin by tandem mass spectrometry. Cell Metab. 2015;22(4):734–40.
214. Perakakis N, Triantafyllou GA, Fernandez-Real JM, Huh JY, Park KH, Seufert J, et al. Physiology and role of irisin in glucose homeostasis. Nat Rev Endocrinol. 2017;13(6):324–37.
215. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab. 2013;98(4):E769–78.

216. Rachid TL, Penna-de-Carvalho A, Bringhenti I, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. Fenofibrate (pparalpha agonist) induces beige cell formation in subcutaneous white adipose tissue from diet-induced male obese mice. Mol Cell Endocrinol. 2015;402:86–94.
217. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, et al. Irisin and fgf21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab. 2014;19(2):302–9.
218. Alis R, Sanchis-Gomar F, Pareja-Galeano H, Hernandez-Mijares A, Romagnoli M, Victor VM, et al. Association between irisin and homocysteine in euglycemic and diabetic subjects. Clin Biochem. 2014;47(18):333–5.
219. Choi YK, Bae KH, Jeong JY, Kim JG, Lee IK, Park KG, et al. Serum irisin levels in new-onset type 2 diabetes. Diabetes. 2013;62:A379.
220. Liu JJ, Wong MDS, Toy WC, Tan CSH, Liu S, Ng XW, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications. 2013;27(4):365–9.
221. Zhang M, Chen P, Chen S, Sun Q, Zeng QC, Chen JY, et al. The association of new inflammatory markers with type 2 diabetes mellitus and macrovascular complications: a preliminary study. Eur Rev Med Pharmacol Sci. 2014;18(11):1567–72.
222. Yan B, Shi X, Zhang H, Pan L, Ma Z, Liu S, et al. Association of serum irisin with metabolic syndrome in obese chinese adults. PLoS One. 2014;9(4):e94235.
223. Crujeiras AB, Pardo M, Arturo RR, Navas-Carretero S, Zulet MA, Martinez JA, et al. Longitudinal variation of circulating irisin after an energy restriction-induced weight loss and following weight regain in obese men and women. Am J Hum Biol. 2014;26(2):198–207.
224. Pardo M, Crujeiras AB, Amil M, Aguera Z, Jimenez-Murcia S, Banos R, et al. Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. Int J Endocrinol. 2014;2014:857270.
225. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity – correlation with body mass index. Peptides. 2013;39:125–30.
226. Binay C, Paketci C, Guzel S, Samanci N. Irisin and oxytocin as predictors of metabolic parameters in obese children. J Clin Res Pediatr Endocrinol. 2017;9(2):124–31.
227. Viitasalo A, Agren J, Venalainen T, Pihlajamaki J, Jaaskelainen J, Korkmaz A, et al. Association of plasma fatty acid composition with plasma irisin levels in normal weight and overweight/obese children. Pediatr Obes. 2016;11(4):299–305.
228. Al-Daghri NM, Alkharfy KM, Rahman S, Amer OE, Vinodson B, Sabico S, et al. Irisin as a predictor of glucose metabolism in children: sexually dimorphic effects. Eur J Clin Invest. 2014;44(2):119–24.
229. Izumiya Y, Bina HA, Ouchi N, Akasaki Y, Kharitonenkov A, Walsh K. Fgf21 is an akt-regulated myokine. FEBS Lett. 2008;582(27):3805–10.
230. Hojman P, Pedersen M, Nielsen AR, Krogh-Madsen R, Yfanti C, Akerstrom T, et al. Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. Diabetes. 2009;58(12):2797–801.
231. Camporez JP, Jornayvaz FR, Petersen MC, Pesta D, Guigni BA, Serr J, et al. Cellular mechanisms by which fgf21 improves insulin sensitivity in male mice. Endocrinology. 2013;154(9):3099–109.
232. Ji K, Zheng J, Lv J, Xu J, Ji X, Luo YB, et al. Skeletal muscle increases fgf21 expression in mitochondrial disorders to compensate for energy metabolic insufficiency by activating the mtor-yy1-pgc1alpha pathway. Free Radic Biol Med. 2015;84:161–70.
233. Stefan N, Haring HU. The role of hepatokines in metabolism. Nat Rev Endocrinol. 2013;9(3):144–52.
234. Gomez-Samano MA, Grajales-Gomez M, Zuarth-Vazquez JM, Navarro-Flores MF, Martinez-Saavedra M, Juarez-Leon OA, et al. Fibroblast growth factor 21 and its novel association with oxidative stress. Redox Biol. 2017;11:335–41.
235. Murata Y, Konishi M, Itoh N. Fgf21 as an endocrine regulator in lipid metabolism: from molecular evolution to physiology and pathophysiology. J Nutr Metab. 2011;2011:981315.
236. Sarruf DA, Thaler JP, Morton GJ, German J, Fischer JD, Ogimoto K, et al. Fibroblast growth factor 21 action in the brain increases energy expenditure and insulin sensitivity in obese rats. Diabetes. 2010;59(7):1817–24.
237. Gimeno RE, Moller DE. Fgf21-based pharmacotherapy – potential utility for metabolic disorders. Trends Endocrinol Metab. 2014;25(6):303–11.
238. Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology. 2007;148(2):774–81.
239. Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab. 2013;18(3):333–40.
240. Reinehr T, Woelfle J, Wunsch R, Roth CL. Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. J Clin Endocrinol Metab. 2012;97(6):2143–50.
241. Li G, Yin J, Fu J, Li L, Grant SF, Li C, et al. Fgf21 deficiency is associated with childhood obesity, insulin resistance and hypoadiponectinaemia: the BCAMS study. Diabetes Metab. 2017;43(3):253–60.
242. Giannini C, Feldstein AE, Santoro N, Kim G, Kursawe R, Pierpont B, et al. Circulating levels of fgf-21 in obese youth: associations with liver fat con-

tent and markers of liver damage. J Clin Endocrinol Metab. 2013;98(7):2993–3000.
243. Korwutthikulrangsri M, Mahachoklertwattana P, Chanprasertyothin S, Pongratanakul S, Poomthavorn P. Serum fibroblast growth factor 21 in overweight and obese thai children and adolescents: its relation to glucose metabolism and its change after glucose loading. Clin Endocrinol (Oxf). 2015;83(6):820–7.
244. Yu H, Xia F, Lam KS, Wang Y, Bao Y, Zhang J, et al. Circadian rhythm of circulating fibroblast growth factor 21 is related to diurnal changes in fatty acids in humans. Clin Chem. 2011;57(5):691–700.
245. Shim YS, Kang MJ, Oh YJ, Baek JW, Yang S, Hwang IT. Fetuin-A as an alternative marker for insulin resistance and cardiovascular risk in prepubertal children. J Atheroscler Thromb. 2017;24(10):1031–8.
246. Kalabay L, Chavin K, Lebreton JP, Robinson KA, Buse MG, Arnaud P. Human recombinant alpha 2-hs glycoprotein is produced in insect cells as a full length inhibitor of the insulin receptor tyrosine kinase. Horm Metab Res. 1998;30(1):1–6.
247. Auberger P, Falquerho L, Contreres JO, Pages G, Le Cam G, Rossi B, et al. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. Cell. 1989;58(4):631–40.
248. Samocha-Bonet D, Tam CS, Campbell LV, Heilbronn LK. Raised circulating fetuin-A after 28-day overfeeding in healthy humans. Diabetes Care. 2014;37(1):e15–6.
249. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. 2012;18(8):1279–85.
250. Heinrichsdorff J, Olefsky JM. Fetuin-A: the missing link in lipid-induced inflammation. Nat Med. 2012;18(8):1182–3.
251. Cayatte AJ, Kumbla L, Subbiah MT. Marked acceleration of exogenous fatty acid incorporation into cellular triglycerides by fetuin. J Biol Chem. 1990;265(10):5883–8.
252. Erridge C, Samani NJ. Saturated fatty acids do not directly stimulate toll-like receptor signaling. Arterioscler Thromb Vasc Biol. 2009;29(11):1944–9.
253. Ismail NA, Ragab S, El Dayem SM, Elbaky AA, Salah N, Hamed M, et al. Fetuin-A levels in obesity: differences in relation to metabolic syndrome and correlation with clinical and laboratory variables. Arch Med Sci. 2012;8(5):826–33.
254. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the heart and soul study. Circulation. 2006;113(8):E344.
255. Kaushik SV, Plaisance EP, Kim T, Huang EY, Mahurin AJ, Grandjean PW, et al. Extended-release niacin decreases serum fetuin-A concentrations in individuals with metabolic syndrome. Diabetes Metab Res Rev. 2009;25(5):427–34.
256. Lebensztejn DM, Bialokoz-Kalinowska I, Klusek-Oksiuta M, Tarasow E, Wojtkowska M, Kaczmarski M. Serum fetuin a concentration is elevated in children with non-alcoholic fatty liver disease. Adv Med Sci-Poland. 2014;59(1):81–4.
257. Song A, Xu M, Bi Y, Xu Y, Huang Y, Li M, et al. Serum fetuin-a associates with type 2 diabetes and insulin resistance in chinese adults. PLoS One. 2011;6(4):e19228.
258. Stefan N, Haring HU. Circulating fetuin-A and free fatty acids interact to predict insulin resistance in humans. Nat Med. 2013;19(4):394–5.
259. Hizli S, Abaci A, Ozdemir O, Akelma Z, Akin O. Relation of fetuin a levels with cardiac, subcutaneous lipid accumulation and insulin resistance parameters in turkish obese children. J Pediatr Endocrinol Metab. 2016;29(6):669–73.
260. Reinehr T, Roth CL. Fetuin-a and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. J Clin Endocrinol Metab. 2008;93(11):4479–85.
261. Brix JM, Stingl H, Hollerl F, Schernthaner GH, Kopp HP, Schernthaner G. Elevated fetuin-A concentrations in morbid obesity decrease after dramatic weight loss. J Clin Endocrinol Metab. 2010;95(11):4877–81.
262. Chen HY, Chiu YL, Hsu SP, Pai MF, Lai CF, Peng YS, et al. Association of serum fetuin a with truncal obesity and dyslipidemia in non-diabetic hemodialysis patients. Eur J Endocrinol. 2009;160(5):777–83.
263. Quagliarini F, Wang Y, Kozlitina J, Grishin NV, Hyde R, Boerwinkle E, et al. Atypical angiopoietin-like protein that regulates ANGPTL3. Proc Natl Acad Sci U S A. 2012;109(48):19751–6.
264. Ren G, Kim JY, Smas CM. Identification of RIFL, a novel adipocyte-enriched insulin target gene with a role in lipid metabolism. Am J Physiol Endocrinol Metab. 2012;303(3):E334–51.
265. Espes D, Martinell M, Carlsson PO. Increased circulating betatrophin concentrations in patients with type 2 diabetes. Int J Endocrinol. 2014;2014:323407.
266. Fu ZY, Berhane F, Fite A, Seyoum B, Abou-Samra AB, Zhang R. Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity. Sci Rep. 2014;23:4.
267. Hu H, Sun W, Yu S, Hong X, Qian W, Tang B, et al. Increased circulating levels of betatrophin in newly diagnosed type 2 diabetic patients. Diabetes Care. 2014;37(10):2718–22.
268. Gomez-Ambrosi J, Pascual E, Catalan V, Rodriguez A, Ramirez B, Silva C, et al. Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes. J Clin Endocrinol Metab. 2014;99(10):E2004–9.
269. Abu-Farha M, Abubaker J, Al-Khairi I, Cherian P, Noronha F, Hu FB, et al. Higher plasma betatrophin/angptl8 level in type 2 diabetes subjects does not

correlate with blood glucose or insulin resistance. Sci Rep. 2015;5:10949.
270. Cox AR, Barrandon O, Cai EP, Rios JS, Chavez J, Bonnyman CW, et al. Resolving discrepant findings on angptl8 in beta-cell proliferation: a collaborative approach to resolving the betatrophin controversy. PLoS One. 2016;11(7):e0159276.
271. Zhang R, Abou-Samra AB. A dual role of lipasin (betatrophin) in lipid metabolism and glucose homeostasis: consensus and controversy. Cardiovasc Diabetol. 2014;13:133.
272. Wu SM, Gao HJ, Ma YM, Fu LN, Zhang C, Luo XP. Characterisation of betatrophin concentrations in childhood and adolescent obesity and insulin resistance. Pediatr Diabetes. 2016;17(1):53–60.
273. Tuhan H, Abaci A, Anik A, Catli G, Kume T, Calan OG, et al. Circulating betatrophin concentration is negatively correlated with insulin resistance in obese children and adolescents. Diabetes Res Clin Pract. 2016;114:37–42.
274. Roth CL, Elfers C, Lass N, Reinehr T. Betatrophin: no relation to glucose metabolism or weight status in obese children before and after lifestyle intervention. Pediatr Diabetes. 2017;18(6):485–91.
275. Rayman MP. Selenium and human health. Lancet. 2012;379(9822):1256–68.
276. Akesson B, Bellew T, Burk RF. Purification of selenoprotein p from human plasma. Biochim Biophys Acta. 1994;1204(2):243–9.
277. Burk RF, Hill KE. Selenoprotein p: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. Annu Rev Nutr. 2005;25:215–35.
278. Burk RF, Hill KE. Selenoprotein p-expression, functions, and roles in mammals. Biochim Biophys Acta. 2009;1790(11):1441–7.
279. Steinbrenner H, Speckmann B, Pinto A, Sies H. High selenium intake and increased diabetes risk: experimental evidence for interplay between selenium and carbohydrate metabolism. J Clin Biochem Nutr. 2011;48(1):40–5.
280. Misu H, Takamura T, Matsuzawa N, Shimizu A, Ota T, Sakurai M, et al. Genes involved in oxidative phosphorylation are coordinately upregulated with fasting hyperglycaemia in livers of patients with type 2 diabetes. Diabetologia. 2007;50(2):268–77.
281. Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, et al. A liver-derived secretory protein, selenoprotein p, causes insulin resistance. Cell Metab. 2010;12(5):483–95.
282. Takamura T, Sakurai M, Ota T, Ando H, Honda M, Kaneko S. Genes for systemic vascular complications are differentially expressed in the livers of type 2 diabetic patients. Diabetologia. 2004;47(4):638–47.
283. Yang SJ, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, et al. Serum selenoprotein p levels in patients with type 2 diabetes and prediabetes: implications for insulin resistance, inflammation, and atherosclerosis. J Clin Endocrinol Metab. 2011;96(8):E1325–9.
284. Jung TW, Choi HY, Lee SY, Hong HC, Yang SJ, Yoo HJ, et al. Salsalate and adiponectin improve palmitate-induced insulin resistance via inhibition of selenoprotein p through the ampk-foxo1alpha pathway. PLoS One. 2013;8(6):e66529.
285. Ko BJ, Kim SM, Park KH, Park HS, Mantzoros CS. Levels of circulating selenoprotein p, fibroblast growth factor (fgf) 21 and fgf23 in relation to the metabolic syndrome in young children. Int J Obes (Lond). 2014;38(12):1497–502.
286. Zhang Y, Chen X. Reducing selenoprotein p expression suppresses adipocyte differentiation as a result of increased preadipocyte inflammation. Am J Physiol Endocrinol Metab. 2011;300(1):E77–85.
287. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006;295(11):1288–99.
288. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med. 2009;361(12):1152–63.
289. Perry JR, Weedon MN, Langenberg C, Jackson AU, Lyssenko V, Sparso T, et al. Genetic evidence that raised sex hormone binding globulin (shbg) levels reduce the risk of type 2 diabetes. Hum Mol Genet. 2010;19(3):535–44.
290. Wu TS, Hammond GL. Naturally occurring mutants inform shbg structure and function. Mol Endocrinol. 2014;28(7):1026–38.
291. Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA. Androgens, estrogens, and second messengers. Steroids. 1998;63(5-6):278–81.
292. Brand JS, Wareham NJ, Dowsett M, Folkerd E, van der Schouw YT, Luben RN, et al. Associations of endogenous testosterone and shbg with glycated haemoglobin in middle-aged and older men. Clin Endocrinol (Oxf). 2011;74(5):572–8.
293. Page-Wilson G, Goulart AC, Rexrode KM. Interrelation between sex hormones and plasma sex hormone-binding globulin and hemoglobin a1c in healthy postmenopausal women. Metab Syndr Relat Disord. 2009;7(3):249–54.
294. Wallace IR, McKinley MC, Bell PM, Hunter SJ. Sex hormone binding globulin and insulin resistance. Clin Endocrinol (Oxf). 2013;78(3):321–9.
295. Sorensen K, Aksglaede L, Munch-Andersen T, Aachmann-Andersen NJ, Petersen JH, Hilsted L, et al. Sex hormone-binding globulin levels predict insulin sensitivity, disposition index, and cardiovascular risk during puberty. Diabetes Care. 2009;32(5):909–14.
296. Aldhoon-Hainerova I, Zamrazilova H, Hill M, Hainer V. Insulin sensitivity and its relation to hormones in adolescent boys and girls. Metabolism. 2017;67:90–8.
297. Glueck CJ, Morrison JA, Daniels S, Wang P, Stroop D. Sex hormone-binding globulin, oligomenorrhea, polycystic ovary syndrome, and childhood

insulin at age 14 years predict metabolic syndrome and class III obesity at age 24 years. J Pediatr. 2011;159(2):308–313.e302.
298. Pinkney J, Streeter A, Hosking J, Mostazir M, Jeffery A, Wilkin T. Adiposity, chronic inflammation, and the prepubertal decline of sex hormone binding globulin in children: evidence for associations with the timing of puberty (earlybird 58). J Clin Endocrinol Metab. 2014;99(9):3224–32.
299. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. P Natl Acad Sci USA. 2004;101(44):15718–23.
300. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376–81.
301. Fiorucci S, Distrutti E. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. Trends Mol Med. 2015;21(11):702–14.
302. Heneghan HM, Miller N, Kerin MJ. Role of micrornas in obesity and the metabolic syndrome. Obes Rev. 2010;11(5):354–61.

12 Insulin Resistance and Cardiovascular Disease

Jessica E. Haley and Elaine M. Urbina

Introduction

The worldwide prevalence of obesity doubled from 1980 to 2008 [1]. Obesity is associated with the development of insulin resistance (IR) and the prevalence of IR among adults in the United States increased from 25.8% in 1988–1994 to 34.8% in 1999–2002—an increase of 35% [2]. A similar trend is occurring in the pediatric population, with the prevalence of IR in obese adolescents in the United States now reported as 52.1%, according to data collected from the National Health and Nutrition Examination Survey (NHANES) 1999–2002 [3]. Worldwide, the documented prevalence of IR in obese children ranges from 29.1% to 47% [4–8].

Epidemiology of Cardiovascular Disease in Adults with Insulin Resistance

It is well recognized that hypertension, dyslipidemia, and obesity increase the risk for hard cardiovascular (CV) events in adults. Hypertension is associated with a 1.6–2.8 times increased risk of cardiovascular disease (CVD) [9–11], dyslipidemia (low HDL or high triglycerides) increases the risk of CVD by 1.7 ($P \leq 0.001$), while obesity is associated with a 1.4 times increased risk (trend only, $P = 0.07$) [9]. However, risk factor clustering associated with IR in metabolic syndrome (MS) leads to a greater number of events than if only a single risk factor is present. Based on the World Health Organization (WHO) definition of metabolic syndrome, patients with metabolic syndrome have a threefold increased risk of cardiovascular disease compared to patients without the syndrome, [9] a risk that is above and beyond the risk imparted by individual cardiovascular risk factors (CVRFs).

Although clinical CVD is rare in the pediatric population, CVRFs can be identified in this age group that can predict future cardiovascular risk in adulthood. Obesity, dyslipidemia, hyperinsulinemia, and elevated blood pressure when found during childhood commonly persist into adulthood [12, 13]. More importantly, in addition to individual CVRFs, clusters of these risk factors track strongly into adulthood [12]. Specifically,

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clustering of hypertension, obesity, and dyslipidemia occurs more commonly in pediatric patients with persistently elevated serum insulin concentrations than in patients without hyperinsulinemia [13] and the prevalence of individual CVRFs in this population increases with decreasing insulin sensitivity [14]. Pediatric patients with insulin-resistant obesity are more likely to develop metabolic syndrome as adults than patients with insulin-sensitive obesity [15]. IR in youth is therefore an important risk factor for the development of CVD later in life.

Mechanisms Leading to Increased Cardiovascular Disease Risk with Insulin Resistance

There are numerous proposed mechanisms to explain the association between IR and early CVD. One such mechanism is at the level of the vascular endothelium. In the nondiseased state, vascular tone is determined by the balance of opposing forces of vasoconstriction and vasodilation mediated by endothelin and nitric oxide (NO) production, respectively [16, 17]. Insulin acts through insulin receptors on the vascular endothelium to stimulate both NO production (by stimulating insulin receptor substrate one-half/phosphatidylinositol 3-kinase [IRS½/PI3K] signaling) and endothelin (ET-1) production (via Ras/mitogen-activated protein kinase [MAPK]-dependent signaling). Under normal conditions, this results in net vasodilation, thus increasing delivery of insulin and glucose to skeletal muscle [18]. Animal models have shown that in the setting of IR there is reduced activation of the NO signaling pathway and enhanced activation of endothelin pathway, resulting in vasoconstriction instead of vasodilation (Fig. 12.1) [19, 20]. This vasoconstriction in IR is due to selective impairment of IRS½/PI3K signaling leading to an attenuated NO-mediated dilation, while the Ras/MAPK-dependent pathway remains unaffected [21, 22]. In a study of human umbilical vein endothelial cells, selective insulin resistance was mimicked by blocking PI3K. The cells were subsequently exposed to increasing concentrations of insulin. The resulting "selective insulin-resistant" state resulted in upregulation of

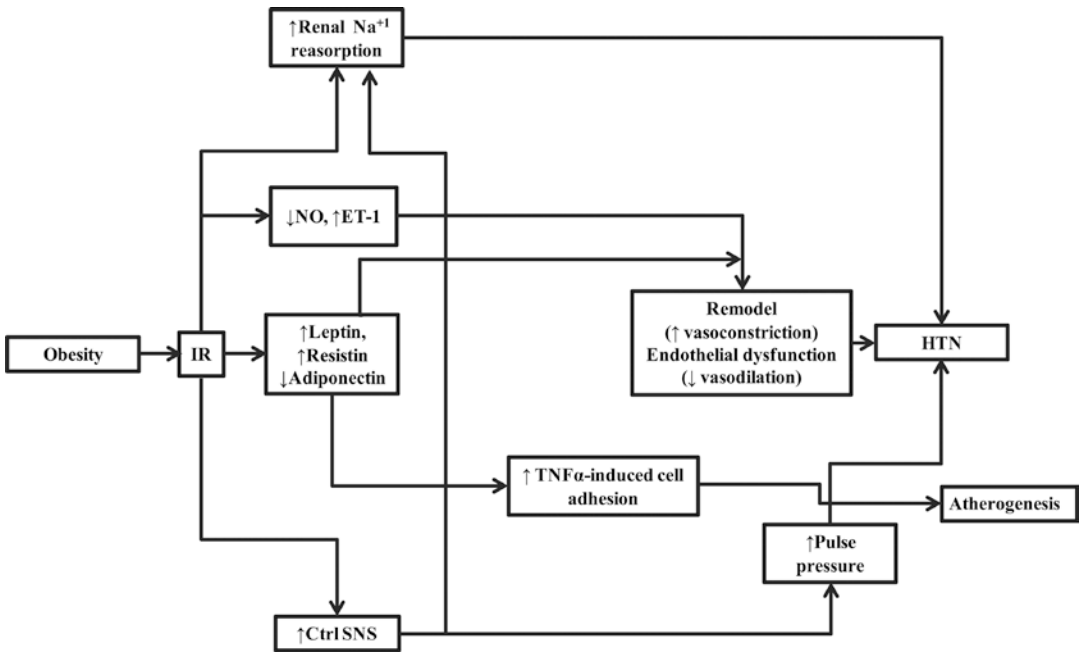

Fig. 12.1 Complex relationships among obesity, insulin resistance, atherogenesis, and hypertension. (Adapted from Feldstein et al. [20])

vascular cell adhesion molecule-1 and E-selectin, as well as increased adhesion of monocytes to endothelial cells [23], the actions of which begin the process of atherogenesis [24]. Similarly, the peptide resistin—found in macrophages, mononuclear leukocytes, and human bone marrow—has been found to promote vasoconstriction in the setting of IR, by stimulating ET-1 production, leading to vasoconstriction [25]. This imbalance in vasodilatory and vasoconstrictive factors is a key feature of IR [18] and results in systemic hypertension, a known risk factor for the development of CVD [26].

Resistin also upregulates adhesion molecules and chemokines, resulting in a proinflammatory state, while NO attenuates production of proinflammatory cytokines, decreases expression of vascular cell adhesion molecules, limits leukocyte recruitment, inhibits vascular smooth muscle cell proliferation, opposes apoptosis, attenuates platelet aggregation, and reduces monocyte adhesion to the vascular wall [27]. Therefore, in the setting of IR and decreased NO production, there is an increase in proinflammatory and prothrombotic factors that contribute to vascular dysfunction and atherosclerosis (Fig. 12.1) [20, 22].

The adipocyte has been found to be an important secretory organ for bioactive molecules [25, 28]. Many adipocyte-derived hormones have metabolic and vascular actions. Adiponectin is one such hormone with anti-inflammatory and vasodilatory actions similar to insulin. Specifically, adiponectin stimulates NO production, enhances NO bioavailability by upregulating endothelial nitric oxide synthase (eNOS) expression, and reduces reactive oxygen species production in endothelial cells [29]. Adiponectin accumulates in injured vessel walls and inhibits tissue necrosis factor alpha (TNF-α)-induced cell adhesion in human aortic endothelial cells [30], resulting in an antiatherogenic effect. Adiponectin inhibits the proliferation of myelomonocytic progenitors, as well as the phagocytic activity and TNF-α production by macrophages, both of which contribute to the anti-inflammatory effects [31]. Decreased adiponectin concentrations are observed in obesity, insulin resistance, and CVD [32], which may explain in part the increased incidence of vascular disease in these disease states (Fig. 12.1) [20]. Leptin, another adipocyte-derived hormone, has similar direct vascular action. Like insulin, under normal conditions, leptin induces vasodilation through a PI3K/eNOS pathway [33]. In insulin-resistant states, leptin levels are found to be chronically elevated and potentiate the pressor effects of hyperinsulinemia, resulting in hypertension (Fig. 12.1) [20, 34]. This further supports the concept of adipocyte–endothelial interaction in the setting of IR, resulting in endothelial dysfunction and CVD.

Hyperglycemia has also been implicated in endothelial dysfunction through several mechanisms, including inducing an increase in reactive oxygen species production [22]. This may occur through activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway, leakage of mitochondrial electron transport chain, or uncoupling of nitric oxide synthase [35]. The greater oxidative stress induced by these reactive oxygen species results in decreased bioavailability of the vasodilatory NO, which has been associated with cardiomyocyte hypertrophy [22].

Another mechanism by which elevated glucose affects vascular parameters is through the activation of the hexosamine biosynthetic pathway, which leads to increased production of glucosamine, a substance that impairs endothelial NO production [22]. Hyperglycemia also leads to the formation of advanced glycation end products (AGEs) that occur when excess glucose combines with free amino acids. Studies have shown specific procoagulatory changes occur when AGEs interact with the endothelial receptor for AGE (RAGE). Specifically, this interaction results in reduction of thrombomodulin activity, which prevents activation of the anticoagulatory protein C pathway [36]. This ligand–receptor interaction also results in increased tissue factor activity, which activates coagulation factors IX and X through the binding of factor VIIa [36], another procoagulatory effect. In both human and animal models, AGEs have also been found to inactivate NO in a dose-dependent fashion [36, 37]. Studies of diabetic animals have correlated defective vasodilatory response to NO with levels

of AGE. This defect was prevented in the same animal models by inhibiting AGE formation [37]. AGEs are also known to create abnormal cross-linking in collagen and elastin fibers [38], leading to increased arterial stiffness, a risk factor for development of left ventricular hypertrophy (LVH) [39]. LVH in turn is an independent risk factor for myocardial infarction in adults [40]. This may explain the observation that an increased pulse wave velocity (PWV) (measure of arterial stiffness) in adults in the Framingham Heart Study predicted incident CV events in only 7.8 years of follow-up [41].

Mechanisms for Effect of Insulin Resistance on Cardiac Structure and Function

Another proposed mechanism for insulin-induced cardiac hypertrophy is through the binding of insulin to insulin-like growth factor (IGF-1) receptor in the hyperinsulinemic state, which is possible due to structural similarity between insulin and IGF-1 [42]. Animal models have shown that increased IGF-1 binding stimulates cell proliferation and hypertrophy of neonatal cardiac myocytes [43].

Impact of Insulin Resistance on Renal and Neurologic Control of Blood Pressure

Insulin is known to affect renal sodium transport [44]. In healthy adults, urinary sodium excretion decreased significantly in subjects within 60 minutes of starting an insulin infusion secondary to increased sodium reabsorption in the distal nephron, despite maintenance of euglycemia [45]. The effect of insulin infusion on sodium reabsorption is preserved in the insulin-resistant state [44, 46]. One investigation found that subjects with metabolic syndrome had greater sodium reabsorption than the control subjects [47], resulting in a state of sodium overload and subsequent hypertension. In addition to increased sodium reabsorption, insulin has been found to increase levels of sympathetic nervous system activity [48, 49]. In a study by Rowe, nonobese male subjects receiving a constant infusion of insulin at varying rates were found to have dose-dependent increases in plasma norepinephrine, resulting in increased pulse pressure [48]—a measure that reflects increased arterial stiffness [50]. The sympathetic stimulant effects of insulin were also found in a group of insulin-resistant patients [51], suggesting a possible connection between chronic IR and hypertension, although the exact mechanism is unknown (Fig. 12.1) [20, 51].

Evidence for Target Organ Damage in Youth with Insulin Resistance

Despite the lack of hard CV events in children with IR, a growing body of evidence suggests that insulin resistance in youth results in target organ damage (TOD) in adulthood [52]. TOD is now being demonstrated at a much younger age in subjects with IR [39, 52–58].

Vascular Involvement

In addition to cardiac remodeling, IR has been associated with abnormal vascular structure and function in the pediatric population, including increased carotid intima-media thickness (cIMT). In a study of more than 250 adolescents (mean age 15 years), Ryder et al. found a graded increase in cIMT across tertiles of body mass index (BMI), visceral adipose tissue (measured by computed tomography [CT]), and IR from glucose clamp measurements [56]. Similarly, Akyol et al. found a progressive increase in carotid IMT in adolescents across the spectrum from lean to obese to obese with metabolic syndrome (Fig. 12.2) [59]. Endothelial dysfunction, as measured by brachial artery flow-mediated dilation (FMD), is also reduced in young subjects with IR [57]. In prepubertal obese children, IR (as measured by homeostatic model assessment of insulin resistance [HOMA-IR]) was also associated with increased PWV, a measure of arterial stiffness [58]. However, it is not clear

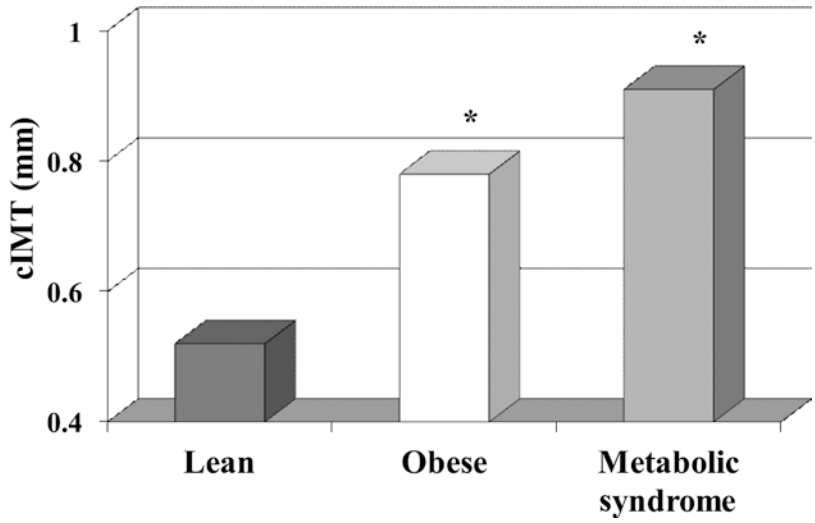

Fig. 12.2 Progressive increase in carotid intima-media thickness (cIMT) in youth across the spectrum from lean to obese to obese with metabolic syndrome. $P < 0.01$ versus control. (Adapted from Akyol et al. [59])

whether this association remains independent of obesity. Correia-Costa et al. [58] found the relationship between HOMA-IR and PWV remained significant in multivariable models adjusting for adiposity in prepubertal children while the relationship was lost after adjustment in adolescents [60]. The relationship between IR and arterial parameters in youth are relevant because adult studies have shown a direct association between increasing cIMT and rate of stroke or myocardial infarction [61], between PWV and incident CVD [41, 62], as well as increased CV event rates in adults with metabolic syndrome and decreased FMD [63]. Disturbingly, adolescents with increased arterial stiffness already demonstrate increased left ventricular mass (LVM) [39]. Our recent analyses also demonstrated a significant linear regression of global strain on common carotid artery cIMT [64], indicating worsening cardiac systolic function with increased arterial thickness (Fig. 12.3) and suggesting that vascular dysfunction may provide the link between IR and cardiac involvement.

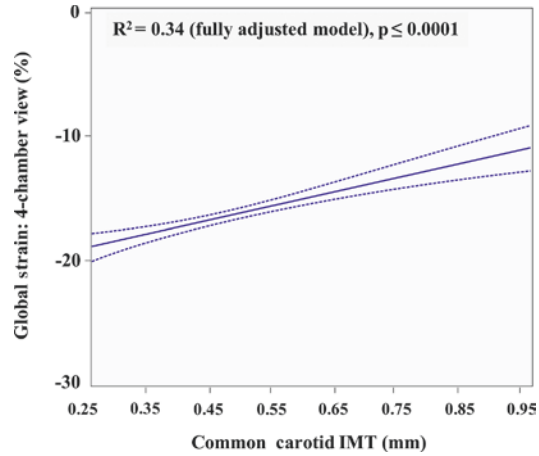

Fig. 12.3 Relationship between cardiac function and arterial thickness (regression mean and 95% CI; $R^2 = 0.34$ in model adjusted for age, sex, BMI, MAP, and HDL, P for slope differs from zero < 0.0001) [64]

Cardiac Involvement

In obese youth with metabolic syndrome, IR is associated with increased LVM. However, only obese youth were evaluated so whether this effect is independent of adiposity could not be tested [54, 55]. Investigators in the Bogalusa Heart Study also found that fasting insulin was related to LVM index, though only in obese adolescents, suggesting that insulin may be a permissive factor affecting heart mass, only active in the presence of obesity and its concomitant CV risk factors [53]. IR is also associated with left ventricular diastolic dysfunction in obese adolescent males, which is indicative of impaired myocardial relaxation [65].

Treatment of Insulin Resistance for Prevention of Cardiovascular Disease

In order to reduce CVD in adults, CV risk must be addressed in youth. The 2011 NIH guidelines on CV risk reduction in youth provides an evidence base for identifying and managing major CV risk factors from infancy to young adulthood [66]. Many of the interventions focusing on CVRF reduction have been shown to improve insulin sensitivity and, thus, decrease the CV risks associated with IR [67] and may have a beneficial effect on CV target organ damage.

Weight Loss

Weight loss is associated with a decrease in obesity-related comorbidities, including IR [67, 68]. Rocchini et al. found that the improvement in blood pressure in obese adolescents after an exercise program paralleled an improvement in insulin sensitivity, independent of weight loss [67]. Weight loss has also been shown to lead to improvement in cIMT [69], directly related to the magnitude of BMI reduction, and associated with reduction in BP [69]. This improvement in cIMT associated with weight loss is thought to be secondary to an associated improvement in IR that is commonly seen with decreased body weight. This is supported by the study in obese adolescents by de Lima Sanches, which demonstrated that a reduction in HOMA-IR predicted regression of cIMT independent of other CVRFs [70]. Similarly, Sanches found that after a 1-year weight loss program, subjects with IR at baseline had less regression of cIMT than a more insulin-sensitive group despite a larger drop in BMI in the IR group (Fig. 12.4) [71].

Physical Activity

There is growing evidence that exercise training alone, without calorie restriction or significant weight loss, improves insulin sensitivity in obese children and adolescents [72–76]. A few pediatric studies have examined the effect of physical activity on cardiac and vascular function. One study found that increased vigorous physical activity (as determined by accelerometry) over a 5-year period resulted in significantly smaller age-related increase in PWV (Urbina, unpublished data, 2019). Similarly, Watts et al. studied a group of obese adolescents with baseline impaired FMD and found that FMD improved with circuit training [77]. Physical activity has also been shown to improve cardiac function in obese adolescents. Ingul et al. demonstrated improvement in systolic and diastolic cardiac function after 3 months of aerobic training in obese adolescents [78]. Unfortunately, none of these studies on physical activity evaluated the effect of change in IR on improvement in CV target organ damage.

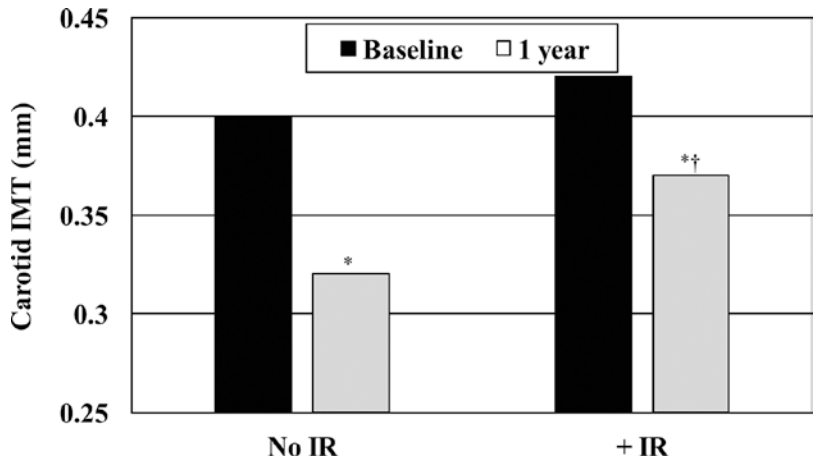

Fig. 12.4 Influence of insulin resistance (IR) on regression of carotid intima-media thickness (cIMT) after weight loss program. $P \leq 0.05$ for difference ∗from baseline, †by IR status. (Adapted from Sanches et al. [71])

Diet

Adherence to the Mediterranean diet has a well-documented effect of decreasing the incidence of CVD in adult subjects [79, 80]. The reduction in CVD provided by the Mediterranean diet may be due to a decrease in IR and improvement in endothelial function, as seen in a 2-year intervention in adults with metabolic syndrome where IR improved with a corresponding reduction in the levels of inflammation and endothelial function score (BP response to L-arginine infusion) [81]. A similar study in obese adolescents revealed improvement in BMI, glucose, and lipid profile after a 1-week diet intervention [82]. Studies are lacking in the adolescent population pertaining to changes in vascular function—an area requiring further study.

Insulin-Sensitizing Medications

The thiazolidinediones (TZD) have been used in the treatment of type 2 diabetes to improve insulin sensitivity [83]. These drugs target the peroxisome proliferator-activated receptor gamma (PPARγ) found on vascular muscle [84] and endothelium [85], and through direct binding to the receptor, improve insulin sensitivity [83]. Animal models have found that rosiglitazone leads to improved endothelial function, as shown by improved vasodilation compared to untreated mice [86]. The effects of rosiglitazone were evaluated in a group of obese adolescent females with polycystic ovary syndrome (PCOS). After 6 months of treatment, despite improved insulin sensitivity and decreased visceral adipose tissue, there was no significant change in cIMT or PWV [87]. However, such a short intervention may be inadequate to demonstrate a significant change in arterial stiffness. Ibáñez et al. treated adolescent females with PCOS with either estrogen or insulin-sensitizing medications (pioglitazone, metformin, and flutamide) for 18 months and saw cIMT regression in the group treated with insulin-sensitizing medication [88].

No studies have been conducted evaluating the effect of insulin-sensitizing treatment on cardiac structure and function. However, in bariatric surgery patients, after significant reduction in adiposity (as measured by BMI), there was an associated regression of left ventricular hypertrophy and improvement in diastolic function [89]. Improvement in IR may play a role since it is known that there is significant improvement in metabolic parameters in teens undergoing surgical weight loss [90].

Gaps in Knowledge

Although there are many proposed mechanisms linking IR to the cardiovascular system, it is unclear which of these pathways contribute more to overall cardiovascular risk across the life span. Therefore, designing more targeted treatment to prevent CVD is challenging. Another area that is in need of more study is in noninvasive vascular imaging in youth. Issues include the lack of normative data and standardization of protocols, making comparison of results difficult. Subsequently, vascular testing for risk stratification and monitoring of treatment response or progression of disease cannot be recommended clinically in pediatric patients at this time. Data is also lacking on the relative efficacy, risks, and economic burden of the various treatments for IR on both intermediate measures of target organ damage and eventual cardiovascular events in adulthood.

Summary

IR is associated with increased CV risk through a variety of mechanisms. There is evidence that IR in youth causes deleterious changes in target organ damage. Limited data suggest treatment of IR may improve these intermediate markers of CVD. Therefore, diagnosis and treatment of IR in youth is imperative to prevent future heart attack and stroke.

References

1. Paneni F, Costantino S, Cosentino F. Insulin resistance, diabetes, and cardiovascular risk. Curr Atheroscler Rep. 2014;16(7):419.
2. Liese AD, D'Agostino RB Jr, Hamman RF, Kilgo PD, Lawrence JM, Liu LL, et al. The burden of diabetes mellitus among US youth: prevalence estimates from the SEARCH for Diabetes in Youth Study. Pediatrics. [Multicenter Study Research Support, N.I.H., Extramural Research Support, U.S. Govt, P.H.S.]. 2006;118(4):1510–8.
3. Lee JM, Okumura MJ, Davis MM, Herman WH, Gurney JG. Prevalence and determinants of insulin resistance among U.S. adolescents: a population-based study. Diabetes Care. 2006;29(11):2427–32.
4. Caceres M, Teran CG, Rodriguez S, Medina M. Prevalence of insulin resistance and its association with metabolic syndrome criteria among Bolivian children and adolescents with obesity. BMC Pediatr. 2008;8:31.
5. Gobato AO, Vasques AC, Zambon MP, Barros Filho Ade A, Hessel G. Metabolic syndrome and insulin resistance in obese adolescents. Rev Paul Pediatr. 2014;32(1):55–62.
6. Holst-Schumacher I, Nunez-Rivas H, Monge-Rojas R, Barrantes-Santamaria M. Insulin resistance and impaired glucose tolerance in overweight and obese Costa Rican schoolchildren. Food Nutr Bull. 2008;29(2):123–31.
7. Manios Y, Moschonis G, Kourlaba G, Bouloubasi Z, Grammatikaki E, Spyridaki A, et al. Prevalence and independent predictors of insulin resistance in children from Crete, Greece: the Children Study. Diabet Med. 2008;25(1):65–72.
8. Tresaco B, Bueno G, Moreno LA, Garagorri JM, Bueno M. Insulin resistance and impaired glucose tolerance in obese children and adolescents. J Physiol Biochem. 2003;59(3):217–23.
9. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24(4):683–9.
10. Castelli WP. Epidemiology of coronary heart disease: the Framingham study. Am J Med. 1984;76(2A):4–12.
11. Kannel WB. Prevalence and implications of uncontrolled systolic hypertension. Drugs Aging. 2003;20(4):277–86.
12. Berenson GS, Srnivasan SR, Bogalusa Heart Study Group. Cardiovascular risk factors in youth with implications for aging: the Bogalusa Heart Study. Neurobiol Aging. 2005;26(3):303–7.
13. Bao W, Srinivasan SR, Berenson GS. Persistent elevation of plasma insulin levels is associated with increased cardiovascular risk in children and young adults. The Bogalusa Heart Study. Circulation. 1996;93(1):54–9.
14. Lee S, Gungor N, Bacha F, Arslanian S. Insulin resistance: link to the components of the metabolic syndrome and biomarkers of endothelial dysfunction in youth. Diabetes Care. 2007;30(8):2091–7.
15. Zhang H, Zhang T, Li S, Li Y, Hussain A, Fernandez C, et al. Long-term impact of childhood adiposity on adult metabolic syndrome is modified by insulin resistance: the Bogalusa Heart Study. Sci Rep. 2015;5:17885.
16. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med. 1990;323(1):27–36.
17. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest. 1994;94(6):2511–5.
18. Padilla J, Olver TD, Thyfault JP, Fadel PJ. Role of habitual physical activity in modulating vascular actions of insulin. Exp Physiol. 2015;100(7):759–71.
19. Eringa EC, Stehouwer CD, Roos MH, Westerhof N, Sipkema P. Selective resistance to vasoactive effects of insulin in muscle resistance arteries of obese Zucker (fa/fa) rats. Am J Physiol Endocrinol Metab. 2007;293(5):E1134–9.
20. Feldstein C, Julius S. The complex interaction between overweight, hypertension, and sympathetic overactivity. J Am Soc Hypertens. 2009;3(6):353–65.
21. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation. 2006;113(15):1888–904.
22. Muniyappa R, Iantorno M, Quon MJ. An integrated view of insulin resistance and endothelial dysfunction. Endocrinol Metab Clin North Am. 2008;37(3):685–711, ix–x.
23. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, et al. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. J Biol Chem. 2002;277(3):1794–9.
24. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362(6423):801–9.
25. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, et al. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation. 2003;108(6):736–40.
26. McFarlane SI, Banerji M, Sowers JR. Insulin resistance and cardiovascular disease. J Clin Endocrinol Metab. 2001;86(2):713–8.
27. Herman AG, Moncada S. Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. Eur Heart J. 2005;26(19):1945–55.
28. Shuldiner AR, Yang R, Gong DW. Resistin, obesity and insulin resistance--the emerging role of the adipocyte as an endocrine organ. N Engl J Med. 2001;345(18):1345–6.
29. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun. 2004;315(2):264–71.

30. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation. 1999;100(25):2473–6.
31. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood. 2000;96(5):1723–32.
32. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001;86(5):1930–5.
33. Vecchione C, Maffei A, Colella S, Aretini A, Poulet R, Frati G, et al. Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. Diabetes. 2002;51(1):168–73.
34. Skurk T, van Harmelen V, Blum WF, Hauner H. Angiotensin II promotes leptin production in cultured human fat cells by an ERK1/2-dependent pathway. Obes Res. 2005;13(6):969–73.
35. Faria A, Persaud SJ. Cardiac oxidative stress in diabetes: mechanisms and therapeutic potential. Pharmacol Ther. 2016;172:50–62.
36. Brownlee M. Lilly Lecture 1993. Glycation and diabetic complications. Diabetes. 1994;43(6):836–41.
37. Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest. 1991;87(2):432–8.
38. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J Hypertens. 2003;21(1):3–12.
39. Urbina EM, Dolan LM, McCoy CE, Khoury PR, Daniels SR, Kimball TR. Relationship between elevated arterial stiffness and increased left ventricular mass in adolescents and young adults. J Pediatr. [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Govt]. 2011;158(5):715–21.
40. Zile MR, Gaasch WH, Patel K, Aban IB, Ahmed A. Adverse left ventricular remodeling in community-dwelling older adults predicts incident heart failure and mortality. JACC Heart Fail. 2014;2(5):512–22.
41. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. 2010;121(4):505–11.
42. Straus DS. Growth-stimulatory actions of insulin in vitro and in vivo. Endocr Rev. 1984;5(2):356–69.
43. Ito H, Hiroe M, Hirata Y, Tsujino M, Adachi S, Shichiri M, et al. Insulin-like growth factor-I induces hypertrophy with enhanced expression of muscle specific genes in cultured rat cardiomyocytes. Circulation. 1993;87(5):1715–21.
44. Nakamura M, Yamazaki O, Shirai A, Horita S, Satoh N, Suzuki M, et al. Preserved Na/HCO3 cotransporter sensitivity to insulin may promote hypertension in metabolic syndrome. Kidney Int. 2015;87(3):535–42.
45. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J Clin Invest. 1975;55(4):845–55.
46. Rocchini AP, Katch V, Kveselis D, Moorehead C, Martin M, Lampman R, et al. Insulin and renal sodium retention in obese adolescents. Hypertension. 1989;14(4):367–74.
47. Strazzullo P, Barbato A, Galletti F, Barba G, Siani A, Iacone R, et al. Abnormalities of renal sodium handling in the metabolic syndrome. Results of the Olivetti Heart Study. J Hypertens. 2006;24(8):1633–9.
48. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. Diabetes. 1981;30(3):219–25.
49. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J Clin Invest. 1991;87(6):2246–52.
50. Mahfouz RA, Elawady W, Abdu M, Salem A. Associations of fractional pulse pressure to aortic stiffness and their impact on diastolic function and coronary flow reserve in asymptomatic diabetic patients with normal coronary angiography. Cardiol J. 2013;20(6):605–11.
51. Tack CJ, Smits P, Willemsen JJ, Lenders JW, Thien T, Lutterman JA. Effects of insulin on vascular tone and sympathetic nervous system in NIDDM. Diabetes. 1996;45(1):15–22.
52. Li S, Chen W, Srinivasan SR, Berenson GS. Influence of metabolic syndrome on arterial stiffness and its age-related change in young adults: the Bogalusa Heart Study. Atherosclerosis. 2005;180(2):349–54.
53. Urbina EM, Gidding SS, Bao W, Elkasabany A, Berenson GS. Association of fasting blood sugar level, insulin level, and obesity with left ventricular mass in healthy children and adolescents: the Bogalusa Heart Study. Am Heart J. 1999;138(1 Pt 1):122–7.
54. Davis CL, Kapuku G, Snieder H, Kumar M, Treiber FA. Insulin resistance syndrome and left ventricular mass in healthy young people. Am J Med Sci. 2002;324(2):72–5.
55. Bostanci BK, Civilibal M, Elevli M, Duru NS. Ambulatory blood pressure monitoring and cardiac hypertrophy in children with metabolic syndrome. Pediatr Nephrol. 2012;27(10):1929–35.
56. Ryder JR, Dengel DR, Jacobs DR Jr, Sinaiko AR, Kelly AS, Steinberger J. Relations among adiposity and insulin resistance with flow-mediated dilation, carotid intima-media thickness, and arterial stiffness in children. J Pediatr. 2016;168:205–11.
57. Miniello VL, Faienza MF, Scicchitano P, Cortese F, Gesualdo M, Zito A, et al. Insulin resistance and endothelial function in children and adolescents. Int J Cardiol. 2014;174(2):343–7.

58. Correia-Costa A, Correia-Costa L, Caldas Afonso A, Schaefer F, Guerra A, Moura C, et al. Determinants of carotid-femoral pulse wave velocity in prepubertal children. Int J Cardiol. 2016;218:37–42.
59. Akyol B, Boyraz M, Aysoy C. Relationship of epicardial adipose tissue thickness with early indicators of atherosclerosis and cardiac functional changes in obese adolescents with metabolic syndrome. J Clin Res Pediatr Endocrinol. 2013;5(3):156–63.
60. Urbina EM, Gao Z, Khoury PR, Martin LJ, Dolan LM. Insulin resistance and arterial stiffness in healthy adolescents and young adults. Diabetologia. 2012;55(3):625–31.
61. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999;340(1):14–22.
62. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318–27.
63. Suzuki T, Hirata K, Elkind MS, Jin Z, Rundek T, Miyake Y, et al. Metabolic syndrome, endothelial dysfunction, and risk of cardiovascular events: the Northern Manhattan Study (NOMAS). Am Heart J. 2008;156(2):405–10.
64. Mehta S, Khoury PR, Madsen NL, Dolan LM, Kimball TR, Urbina EM. Arterial thickness and stiffness are independently associated with left ventricular strain. J Am Soc Echocardiogr. 2018;31:99–104. PMC5756686.
65. Sahasrabuddhe AV, Pitale SU, Dhoble, Shivalkar J, Sagdeo MM. Cardiac diastolic dysfunction and regional body fat distribution in insulin resistant peripubertal obese males. J Assoc Physicians India. 2016;64(2):20–6.
66. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics. 2011;128(Suppl 5):S213–56.
67. Rocchini AP, Katch V, Schork A, Kelch RP. Insulin and blood pressure during weight loss in obese adolescents. Hypertension. 1987;10(3):267–73.
68. Su HY, Sheu WH, Chin HM, Jeng CY, Chen YD, Reaven GM. Effect of weight loss on blood pressure and insulin resistance in normotensive and hypertensive obese individuals. Am J Hypertens. 1995;8(11):1067–71.
69. Wunsch R, de Sousa G, Toschke AM, Reinehr T. Intima-media thickness in obese children before and after weight loss. Pediatrics. 2006;118(6):2334–40.
70. de Lima Sanches P, de Mello MT, Elias N, Fonseca FA, de Piano A, Carnier J, et al. Improvement in HOMA-IR is an independent predictor of reduced carotid intima-media thickness in obese adolescents participating in an interdisciplinary weight-loss program. Hypertens Res. 2011;34(2):232–8.
71. Sanches Pde L, Mello MT, Fonseca FA, Elias N, Piano A, Carnier J, et al. Insulin resistance can impair reduction on carotid intima-media thickness in obese adolescents. Arq Bras Cardiol. 2012;99(4):892–8.
72. Kim Y, Park H. Does regular exercise without weight loss reduce insulin resistance in children and adolescents? Int J Endocrinol. 2013;2013:402592.
73. Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW. Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. Diabetes Care. 2003;26(3):557–62.
74. Savoye M, Shaw M, Dziura J, Tamborlane WV, Rose P, Guandalini C, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. JAMA. 2007;297(24):2697–704.
75. Davis CL, Pollock NK, Waller JL, Allison JD, Dennis BA, Bassali R, et al. Exercise dose and diabetes risk in overweight and obese children: a randomized controlled trial. JAMA. 2012;308(11):1103–12.
76. Schmitz KH, Jacobs DR Jr, Hong CP, Steinberger J, Moran A, Sinaiko AR. Association of physical activity with insulin sensitivity in children. Int J Obes Relat Metab Disord. 2002;26(10):1310–6.
77. Watts K, Beye P, Siafarikas A, Davis EA, Jones TW, O'Driscoll G, et al. Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. J Am Coll Cardiol. 2004;43(10):1823–7.
78. Ingul CB, Tjonna AE, Stolen TO, Stoylen A, Wisloff U. Impaired cardiac function among obese adolescents: effect of aerobic interval training. Arch Pediatr Adolesc Med. 2010;164(9):852–9.
79. Kastorini CM, Panagiotakos DB, Chrysohoou C, Georgousopoulou E, Pitaraki E, Puddu PE, et al. Metabolic syndrome, adherence to the Mediterranean diet and 10-year cardiovascular disease incidence: the ATTICA study. Atherosclerosis. 2016;246:87–93.
80. Kris-Etherton P, Eckel RH, Howard BV, St Jeor S, Bazzarre TL. AHA science advisory: Lyon diet heart study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association step I dietary pattern on cardiovascular disease. Circulation. 2001;103(13):1823–5.
81. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA. 2004;292(12):1440–6.
82. Velazquez-Lopez L, Santiago-Diaz G, Nava-Hernandez J, Munoz-Torres AV, Medina-Bravo P, Torres-Tamayo M. Mediterranean-style diet reduces metabolic syndrome components in obese children and adolescents with obesity. BMC Pediatr. 2014;14:175.

83. Ryan MJ, Didion SP, Mathur S, Faraci FM, Sigmund CD. PPAR(gamma) agonist rosiglitazone improves vascular function and lowers blood pressure in hypertensive transgenic mice. Hypertension. 2004;43(3):661–6.
84. Marx N, Schonbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res. 1998;83(11):1097–103.
85. Marx N, Bourcier T, Sukhova GK, Libby P, Plutzky J. PPARgamma activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPARgamma as a potential mediator in vascular disease. Arterioscler Thromb Vasc Biol. 1999;19(3):546–51.
86. Walker AB, Chattington PD, Buckingham RE, Williams G. The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats. Diabetes. 1999;48(7):1448–53.
87. Tfayli H, Ulnach JW, Lee S, Sutton-Tyrrell K, Arslanian S. Drospirenone/ethinyl estradiol versus rosiglitazone treatment in overweight adolescents with polycystic ovary syndrome: comparison of metabolic, hormonal, and cardiovascular risk factors. J Clin Endocrinol Metab. 2011;96(5):1311–9.
88. Ibanez L, Diaz M, Sebastiani G, Marcos MV, Lopez-Bermejo A, de Zegher F. Oral contraception vs insulin sensitization for 18 months in nonobese adolescents with androgen excess: posttreatment differences in C-reactive protein, intima-media thickness, visceral adiposity, insulin sensitivity, and menstrual regularity. J Clin Endocrinol Metab. 2013;98(5):E902–7.
89. Ippisch HM, Inge TH, Daniels SR, Wang B, Khoury PR, Witt SA, et al. Reversibility of cardiac abnormalities in morbidly obese adolescents. J Am Coll Cardiol. 2008;51(14):1342–8.
90. Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Helmrath MA, Brandt ML, et al. Weight loss and health status 3 years after bariatric surgery in adolescents. N Engl J Med. 2016;374(2):113–23.

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

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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

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endocrinologist's knowledge of insulin signaling and the 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(beta)-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(alpha)-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 2 extracellular α(alpha)-units bound to 2 membrane-spanning β(beta)-units (Fig. 13.1). Activation begins with binding of insulin to the α(alpha)-subunit, which then triggers autophosphorylation of the β(beta)-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

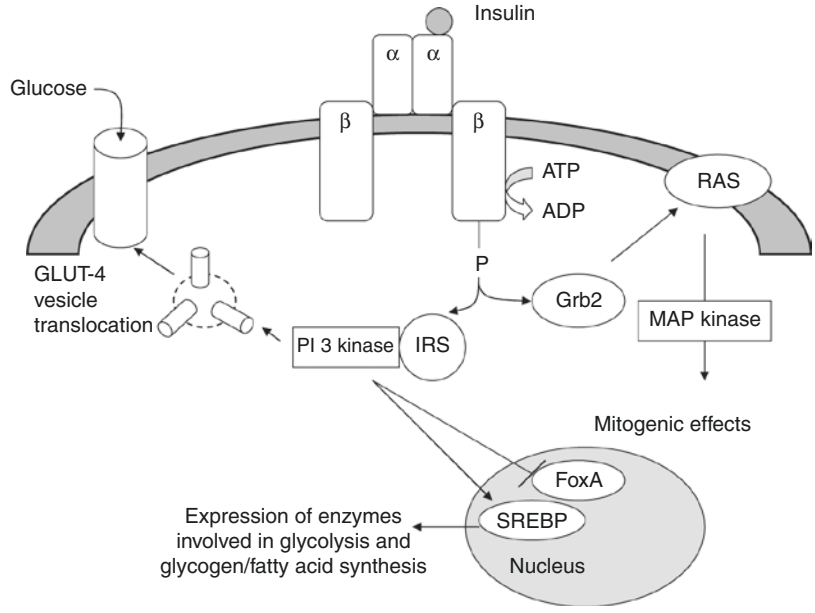

Fig. 13.1 Insulin receptor signaling pathway. Insulin binds to a four-subunit membrane-spanning receptor, triggering tyrosine phosphorylation of the beta subunit, which results in a signaling cascade with combined endpoints: (a) translocation of the GLUT-4 glucose transporter; (b) metabolic effects mediated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

different types of IRS proteins have been identified: IRS 1 (skeletal muscle), IRS 2 (liver), IRS 3 (adipose, β[beta]-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 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 nondependence 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(beta)-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 3 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 the 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 nondiabetic 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].

Insulin Resistance 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(beta)-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(beta)-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 an 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 pro-inflammatory 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 tumor necrosis factor-alpha (TNF-α[alpha]) synthesis. TNF-α(alpha) 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 enzymes in gluconeogenesis, and by increasing the activity of glucose-6-phosphatase, the enzyme responsible for the 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[alpha]) 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(gamma) 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(alpha) are derived mostly from macrophages in adipose tissue. Increased TNF-α(alpha) levels are found in obese individuals owing to increased macrophage infiltration in adipose tissue [69–71] and overexpression of TNF-α(alpha) by enlarged adipocytes [72]. Levels are elevated in individuals with NAFLD compared to controls matched for age, BMI, and sex [73]. TNF-α(alpha) 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 high-density lipoprotein (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 (gamma) (PPARγ[gamma])—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 child-

hood 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(beta)-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(alpha) and CPT-1, which therefore inhibit fatty acid oxidation. Other mechanisms may be simultaneously at work, including the indirect effects of increased TNFα(alpha) 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.

References

1. Der NB. Diabetes mellitus. Wien: Nothangels Handbuch. A. Holder; 1906.
2. Bajaj M, Defronzo RA. Metabolic and molecular basis of insulin resistance. J Nucl Cardiol. 2003;10(3):311–23.
3. Weickert MO, Pfeiffer AF. Signalling mechanisms linking hepatic glucose and lipid metabolism. Diabetologia. 2006;49(8):1732–41.
4. Pessin JE, Saltiel AR. Signaling pathways in insulin action: molecular targets of insulin resistance. J Clin Invest. 2000;106(2):165–9.
5. Roden M, Bernroider E. Hepatic glucose metabolism in humans – its role in health and disease. Best Pract Res Clin Endocrinol Metab. 2003;17(3):365–83.
6. Choudhury J, Sanyal AJ. Insulin resistance and the pathogenesis of nonalcoholic fatty liver disease. Clin Liver Dis. 2004;8(3):575–94, ix.
7. White MF. Insulin signaling in health and disease. Science. 2003;302(5651):1710–1.
8. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19–39.
9. Kahn CR. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism. 1978;27(12 Suppl 2):1893–902.
10. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Phys. 1979;237(3):E214–23.
11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
12. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402–10.

13. Chen H, Sullivan G, Yue LQ, Katz A, Quon MJ. QUICKI is a useful index of insulin sensitivity in subjects with hypertension. Am J Physiol Endocrinol Metab. 2003;284(4):E804–12.
14. Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. J Clin Endocrinol Metab. 2001;86(10):4776–81.
15. Quon MJ. Limitations of the fasting glucose to insulin ratio as an index of insulin sensitivity. J Clin Endocrinol Metab. 2001;86(10):4615–7.
16. Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am. 2004;33(2):283–303.
17. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes. 1997;46(10):1579–85.
18. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 2001;414(6865):799–806.
19. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med. 1999;107(5):450–5.
20. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183–92.
21. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology. 2002;36(2):403–9.
22. Angelico F, Del Ben M, Conti R, Francioso S, Feole K, Fiorello S, et al. Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2005;90(3):1578–82.
23. Wanless IR, Bargman JM, Oreopoulos DG, Vas SI. Subcapsular steatonecrosis in response to peritoneal insulin delivery: a clue to the pathogenesis of steatonecrosis in obesity. Mod Pathol. 1989;2(2):69–74.
24. Bhargava R, Senior PA, Ackerman TE, Ryan EA, Paty BW, Lakey JR, et al. Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. Diabetes. 2004;53(5):1311–7.
25. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115(5):1343–51.
26. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci USA. 2003;100(21):12027–32.
27. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia. 2005;48(4):634–42.
28. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem. 2004;279(31):32345–53.
29. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab. 2002;87(7):3023–8.
30. Cotrim HP, Carvalho F, Siqueira AC, Lordelo M, Rocha R, De Freitas LA. Nonalcoholic fatty liver and insulin resistance among petrochemical workers. JAMA. 2005;294(13):1618–20.
31. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, et al. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. J Gastroenterol Hepatol. 2004;19(8):854–8.
32. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol. 2006;45(4):600–6.
33. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. Gastroenterology. 2007;132(6):2087–102.
34. Schwimmer JB. Definitive diagnosis and assessment of risk for nonalcoholic fatty liver disease in children and adolescents. Semin Liver Dis. 2007;27(3):312–8.
35. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis. 2001;21(1):3–16.
36. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut. 2004;53(5):750–5.
37. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44(4):865–73.
38. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998;114(4):842–5.
39. Parekh S, Anania FA. Abnormal lipid and glucose metabolism in obesity: implications for nonalcoholic fatty liver disease. Gastroenterology. 2007;132(6):2191–207.
40. Sanyal AJ. Mechanisms of disease: pathogenesis of nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol. 2005;2(1):46–53.
41. Mater MK, Thelen AP, Pan DA, Jump DB. Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription. J Biol Chem. 1999;274(46):32725–32.
42. Schaffler A, Scholmerich J, Buchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue – emerging role in nonalcoholic fatty liver

disease. Nat Clin Pract Gastroenterol Hepatol. 2005;2(6):273–80.
43. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007;132(6):2169–80.
44. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care. 1994;17(9):961–9.
45. Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. Ann Intern Med. 1995;122(7):481–6.
46. Silverman JF, O'Brien KF, Long S, Leggett N, Khazanie PG, Pories WJ, et al. Liver pathology in morbidly obese patients with and without diabetes. Am J Gastroenterol. 1990;85(10):1349–55.
47. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology. 1990;12(5):1106–10.
48. Gordon ES. Lipid metabolism, diabetes mellitus, and obesity. Adv Intern Med. 1964;12:66–102.
49. Bjorntorp P, Bergman H, Varnauskas E. Plasma free fatty acid turnover rate in obesity. Acta Med Scand. 1969;185(4):351–6.
50. Boden G, Chen X. Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. J Clin Invest. 1995;96(3):1261–8.
51. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest. 1999;103(2):253–9.
52. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest. 2000;106(2):171–6.
53. Petersen KF, Shulman GI. Etiology of insulin resistance. Am J Med. 2006;119(5 Suppl 1):S10–6.
54. Bahl JJ, Matsuda M, DeFronzo RA, Bressler R. In vitro and in vivo suppression of gluconeogenesis by inhibition of pyruvate carboxylase. Biochem Pharmacol. 1997;53(1):67–74.
55. Massillon D, Barzilai N, Hawkins M, Prus-Wertheimer D, Rossetti L. Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. Diabetes. 1997;46(1):153–7.
56. Boden G, Cheung P, Stein TP, Kresge K, Mozzoli M. FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am J Physiol Endocrinol Metab. 2002;283(1):E12–9.
57. Roden M, Stingl H, Chandramouli V, Schumann WC, Hofer A, Landau BR, et al. Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. Diabetes. 2000;49(5):701–7.
58. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003;423(6941):762–9.
59. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116(7):1784–92.
60. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257(1):79–83.
61. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol. 2002;147(2):173–80.
62. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, et al. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. Diabetologia. 2004;47(8):1360–9.
63. Bajaj M, Suraamornkul S, Piper P, Hardies LJ, Glass L, Cersosimo E, et al. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. J Clin Endocrinol Metab. 2004;89(1):200–6.
64. Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab. 2005;90(6):3498–504.
65. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med. 2001;7(8):941–6.
66. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J Clin Invest. 2002;109(10):1345–50.
67. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, et al. Serum leptin levels in patients with nonalcoholic steatohepatitis. Am J Gastroenterol. 2000;95(12):3584–9.
68. Chalasani N, Crabb DW, Cummings OW, Kwo PY, Asghar A, Pandya PK, et al. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? Am J Gastroenterol. 2003;98(12):2771–6.
69. Permana PA, Menge C, Reaven PD. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochem Biophys Res Commun. 2006;341(2):507–14.
70. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112(12):1796–808.
71. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112(12):1821–30.
72. Peraldi P, Spiegelman B. TNF-alpha and insulin resistance: summary and future prospects. Mol Cell Biochem. 1998;182(1–2):169–75.

73. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology. 2004;40(1):46–54.
74. Hotamisligil GS. The role of TNFalpha and TNF receptors in obesity and insulin resistance. J Intern Med. 1999;245(6):621–5.
75. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS. 1998;12(7):F51–8.
76. Lo JC, Mulligan K, Tai VW, Algren H, Schambelan M. Body shape changes in HIV-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol. 1998;19(3):307–8.
77. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet. 1999;353(9170):2093–9.
78. Dube MP, Johnson DL, Currier JS, Leedom JM. Protease inhibitor-associated hyperglycaemia. Lancet. 1997;350(9079):713–4.
79. Sutinen J, Hakkinen AM, Westerbacka J, Seppala-Lindroos A, Vehkavaara S, Halavaara J, et al. Increased fat accumulation in the liver in HIV-infected patients with antiretroviral therapy-associated lipodystrophy. AIDS. 2002;16(16):2183–93.
80. Dowell P, Flexner C, Kwiterovich PO, Lane MD. Suppression of preadipocyte differentiation and promotion of adipocyte death by HIV protease inhibitors. J Biol Chem. 2000;275(52):41325–32.
81. Domingo P, Matias-Guiu X, Pujol RM, Francia E, Lagarda E, Sambeat MA, et al. Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. AIDS. 1999;13(16):2261–7.
82. Bastard JP, Caron M, Vidal H, Jan V, Auclair M, Vigouroux C, et al. Association between altered expression of adipogenic factor SREBP1 in lipoatrophic adipose tissue from HIV-1-infected patients and abnormal adipocyte differentiation and insulin resistance. Lancet. 2002;359(9311):1026–31.
83. Murata H, Hruz PW, Mueckler M. The mechanism of insulin resistance caused by HIV protease inhibitor therapy. J Biol Chem. 2000;275(27):20251–4.
84. Garg A. Lipodystrophies. Am J Med. 2000;108(2):143–52.
85. Garg A, Chandalia M, Vuitch F. Severe islet amyloidosis in congenital generalized lipodystrophy. Diabetes Care. 1996;19(1):28–31.
86. Chandalia M, Garg A, Vuitch F, Nizzi F. Postmortem findings in congenital generalized lipodystrophy. J Clin Endocrinol Metab. 1995;80(10):3077–81.
87. Haque WA, Vuitch F, Garg A. Post-mortem findings in familial partial lipodystrophy, Dunnigan variety. Diabet Med. 2002;19(12):1022–5.
88. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Phys. 1999;276(5 Pt 1):E977–89.
89. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840–6.
90. Kelley DE, Goodpaster BH, Storlien L. Muscle triglyceride and insulin resistance. Annu Rev Nutr. 2002;22:325–46.
91. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev. 2007;87(2):507–20.
92. Caldwell SH, Ikura Y, Iezzoni JC, Liu Z. Has natural selection in human populations produced two types of metabolic syndrome (with and without fatty liver)? J Gastroenterol Hepatol. 2007;22(Suppl 1):S11–9.
93. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, et al. Life without white fat: a transgenic mouse. Genes Dev. 1998;12(20):3168–81.
94. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. J Biol Chem. 2000;275(12):8456–60.
95. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature. 1999;401(6748):73–6.
96. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, et al. Leptin-replacement therapy for lipodystrophy. N Engl J Med. 2002;346(8):570–8.
97. Simha V, Szczepaniak LS, Wagner AJ, DePaoli AM, Garg A. Effect of leptin replacement on intrahepatic and intramyocellular lipid content in patients with generalized lipodystrophy. Diabetes Care. 2003;26(1):30–5.
98. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med. 2000;133(8):592–9.
99. Fartoux L, Poujol-Robert A, Guechot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut. 2005;54(7):1003–8.
100. Ortiz V, Berenguer M, Rayon JM, Carrasco D, Berenguer J. Contribution of obesity to hepatitis C-related fibrosis progression. Am J Gastroenterol. 2002;97(9):2408–14.
101. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001;33(6):1358–64.
102. Clouston AD, Jonsson JR, Purdie DM, Macdonald GA, Pandeya N, Shorthouse C, et al. Steatosis and chronic hepatitis C: analysis of fibrosis and stellate cell activation. J Hepatol. 2001;34(2):314–20.
103. Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk fac-

tor for nonresponse to antiviral treatment in chronic hepatitis C. Hepatology. 2003;38(3):639–44.
104. Hu KQ, Currie SL, Shen H, Cheung RC, Ho SB, Bini EJ, et al. Clinical implications of hepatic steatosis in patients with chronic hepatitis C: a multicenter study of US veterans. Dig Dis Sci. 2007;52(2):570–8.
105. Lok AS, Everhart JE, Chung RT, Padmanabhan L, Greenson JK, Shiffman ML, et al. Hepatic steatosis in hepatitis C: comparison of diabetic and nondiabetic patients in the hepatitis C antiviral long-term treatment against cirrhosis trial. Clin Gastroenterol Hepatol. 2007;5(2):245–54.
106. Sanyal AJ. Review article: non-alcoholic fatty liver disease and hepatitis C – risk factors and clinical implications. Aliment Pharmacol Ther. 2005;22(Suppl 2):48–51.
107. Patton HM, Patel K, Behling C, Bylund D, Blatt LM, Vallee M, et al. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J Hepatol. 2004;40(3):484–90.
108. Dharancy S, Malapel M, Perlemuter G, Roskams T, Cheng Y, Dubuquoy L, et al. Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection. Gastroenterology. 2005;128(2):334–42.
109. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology. 2004;126(3):840–8.
110. Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology. 2003;38(6):1384–92.
111. Lonardo A, Loria P, Bertolotti M, Carulli N. Statins and HCV: a complex issue. Hepatology. 2007;45(1):257.

14 Insulin Resistance and the Kidney in Youth

Petter Bjornstad and David Z. Cherney

Introduction

Diabetic kidney disease (DKD) remains a leading cause of morbidity and mortality in people with type 1 (T1D) and type 2 diabetes (T2D) [1–3]. In the United States, almost half of patients with renal failure have DKD, and most of these individuals have T2D [4]. DKD is also an important risk factor for the development of future cardiovascular disease. Current treatments are beneficial, but only partially protect against DKD. Therefore, identifying new therapeutic targets to impede DKD remains a public health priority. Youth-onset T2D carries a particularly high risk of progressive DKD [5–14]. Hyperfiltration (glomerular filtration rate [GFR] ≥135 mL/min/1.73 m^2) is common in youth with diabetes mellitus (DM) and predicts progressive DKD [15–18]. Hyperfiltration is associated with early changes in intrarenal hemodynamics beyond elevated GFR, including increased renal blood flow and increased glomerular pressure. With the current obesity epidemic and the rising incidence of pediatric T2D [14, 19], understanding the mechanisms that initiate hyperfiltration and the subsequent progression of kidney disease is needed to improve outcomes in youth with obesity, prediabetes, and T2D.

More recent insights have implicated renal hypoxia, stemming from a mismatch between renal oxygen delivery and demand, as a unifying pathway that may link hyperfiltration and the development of DKD [20]. In the setting of diabetes, the kidneys are highly metabolically active and have an increased energy requirement to sustain hyperfiltration and characteristic changes in intrarenal hemodynamic function [21]. However, there is emerging animal data suggesting that renal response to increased demand for energy substrate is insufficient, leading to hypoxia. In addition, patients with DM may exhibit a less oxygen-efficient fuel profile secondary to insulin resistance (IR) and the resulting elevation in serum free fatty acids (FFA) [21]. In this chapter, we will review the data linking IR with DKD in children and adults with DM, and summarize proposed pathways underlying the relationship between IR and DKD.

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The Natural History of Early Diabetic Kidney Disease

The natural history of DKD is characterized by a long silent period without clinical signs or symptoms of kidney disease. While this period is clinically silent, renal parenchymal damage progresses [22]. Early markers of DKD prior to the loss of renal function, such as hyperfiltration (GFR ≥135 mL/min/1.73 m^2) and albuminuria, can manifest in adolescents with T1D [23–25] and T2D [3, 18, 26, 27] and are also associated with early cardiovascular abnormalities [25, 28]. The appearance of albuminuria is usually the earliest clinical sign of DKD, but our understanding of early DKD has evolved over the last decade based on the demonstration that albuminuria does not necessarily imply progressive nephropathy and may, in fact, regress to normoalbuminuria, at least in the setting of T1D [29, 30]. In addition, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/*EDIC*) study reported incident impaired GFR (<60 mL/min/1.73 m^2) in 24% of patients without prior evidence of albuminuria—a phenotype that is also common in patients with T2D [31–33]. Furthermore, it has recently been proposed that hyperfiltration and albuminuria are distinct phenotypes of DKD [3], each with their own unique pathogenic mechanisms. For those reasons, changes in GFR may be the most clinically relevant measure of early DKD.

Conventional understanding is that hyperfiltration is a maladaptive glomerular response that precedes increased albumin excretion and subsequent decline of renal function in diabetic nephropathy [34]. In 1984, Mogensen et al. suggested that hyperfiltration might be an early phenotype of DKD [35, 36], and a number of studies have since affirmed this association, especially in adults with T2D [37]. More recently, we demonstrated that in adults with T1D, rapid GFR decline over 6 years was associated with baseline hyperfiltration and incident GFR impairment (<60 mL/min/1.73 m^2) [24]. In normal physiology, GFR declines with age and an annual loss of ~1 mL/min/1.73 m^2 has been reported in studies of normal aging [38]. Rapid GFR decline, defined as an annual GFR loss greater than 3 mL/min/1.73 m^2, represents a magnitude of change that is three times the rate of what is expected in normal physiology and corresponds to the 25 percentile of the cohort of the Cardiovascular Health Study with the largest decline in GFR [39, 40]. The cutoff is important to distinguish it from slow changes in GFR associated with aging, mild changes in hydration status, and beyond the range of noise expected when estimating GFR [39]. Rapid GFR decline has been shown to predict end-stage renal disease and cardiovascular outcomes [41, 42].

Youth-onset T2D carries the highest risk of DKD—significantly higher than in T1D or those with adult-onset T2D of similar disease duration [43]. In fact, adolescents with youth-onset T2D have twofold increased risk of microalbuminuria compared to youth with T1D [6, 9, 13]. In the TODAY study (Treatment Options for type 2 *Diabetes* in Adolescents and Youth), with an average follow-up of only 3.9 years, the prevalence of microalbuminuria among youth with T2D almost tripled (from 6.3% to 16.6%) [9]. Furthermore, up to 45% of youth with T2D progress to renal failure as adults, which is significantly greater than youth with T1D and individuals with adult-onset T2D [44, 45]. T2D remains the leading cause of end-stage renal disease (ESRD) in the Western world [46], and most patients with type T2D develop some degree of renal dysfunction during their lifetime [44]. While patients with T2D are significantly more insulin resistant than those with T1D [47, 48], youth and adults with T1D are substantially more insulin resistant than their peers without diabetes [47, 48]. A substantial amount of data support insulin sensitivity (IS) as an important risk factor of developing DKD in both T1D and T2D, but the mechanisms underlying these relationships remain unclear. Understanding the mechanisms underlying the relationship between insulin sensitivity and DKD progression will direct development of therapies to prevent or delay DKD.

Diabetic Kidney Disease and Insulin Resistance in Type 1 Diabetes

While the relationship between insulin sensitivity and vascular complications is increasingly recognized, it is not a recent discovery. In 1968, Martin et al. [49] demonstrated that reduced insulin sensitivity related to microvascular complications in adults with long-standing T1D. Almost three decades later, Yip et al. [50] linked reduced insulin sensitivity to microalbuminuria, and Orchard et al. [51] later demonstrated that estimated glucose disposal rate (eGDR) (Pittsburgh eGDR equation) predicted proteinuria in adults with T1D in the Pittsburgh Epidemiology of *Diabetes* Complications (*EDC*) study cohort.

Duca et al. developed and validated an improved method for estimating insulin sensitivity in people with T1D [52]. Compared to hyperinsulinemic–euglycemic clamp-measured insulin sensitivity, the estimated insulin sensitivity equation (eIS) performed better than previous equations for estimating IS in individuals both with and without T1D [52]. We used this equation to demonstrate that higher eIS at baseline predicted lower odds of developing diabetic nephropathy, diabetic retinopathy, proliferative diabetic retinopathy, and coronary atherosclerosis independent of other established risk factors [53, 54]. The same group also demonstrated that reduced estimated insulin sensitivity predicted incident microalbuminuria and rapid glomerular filtration decline estimated by cystatin C over 6 years [55] and that increased insulin sensitivity at baseline predicted regression of albuminuria over 6 years [54] in adults with T1D, similar to data in the EDC study.

Diabetic Kidney Disease and Insulin Resistance in Type 2 Diabetes

A growing body of literature has implicated insulin resistance in the progression of DKD in T2D [56, 57]. We demonstrated in a small cross-sectional study of youth with T2D, where 34% of the participants had albuminuria and 24% had hyperfiltration, that reduced insulin sensitivity, measured by hyperinsulinemic–euglycemic clamp technique, was associated with albuminuria and hyperfiltration [18]. Stratifying eGFR into tertiles, adolescents with T2D in the highest tertile of eGFR (Fig. 14.1) had lower IS than those in the mid and low tertiles, after adjusting for age, sex, pubertal stage, body mass index (BMI), and HbA1c (p = 0.02 and p = 0.04). IS was also inversely associated with eGFR after adjusting for sex and pubertal stage (β[beta] ± SE: −2.23 ± 0.87, p = 0.02); these associations were not observed for measures of HbA1c, LDL-C, or SBP [18]. These findings demonstrate that a significant proportion of adolescents with T2D showed evidence of early DKD and that IS, rather than traditional risk factors, was the strongest determinant of future renal disease risk [18]. In the TODAY study (n = 532), a large diverse national multicenter cohort, we observed hyperfiltration in 7.0% of participants at baseline and in 13.3% by 5 years, with a cumulative incidence of 5.0% over 5 years. There was an annual increase in eGFR (Fig. 14.2a) and an average decrease in eIS (Fig. 14.2b) [58]. Lower estimated insulin sensitivity was associated with a 2.1-fold increased risk of hyperfiltration (HR: 2.12, p = 0.008) in a univariable model, corresponding to an 8% increase in risk of hyperfiltration per 10% lower estimated insulin sensitivity. This asso-

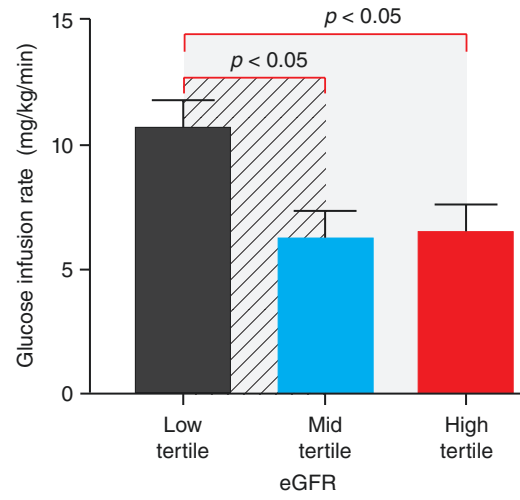

Fig. 14.1 Insulin sensitivity across tertiles of estimated glomerular filtration rate (eGFR) in youth with type 2 diabetes. (Adapted from [18])

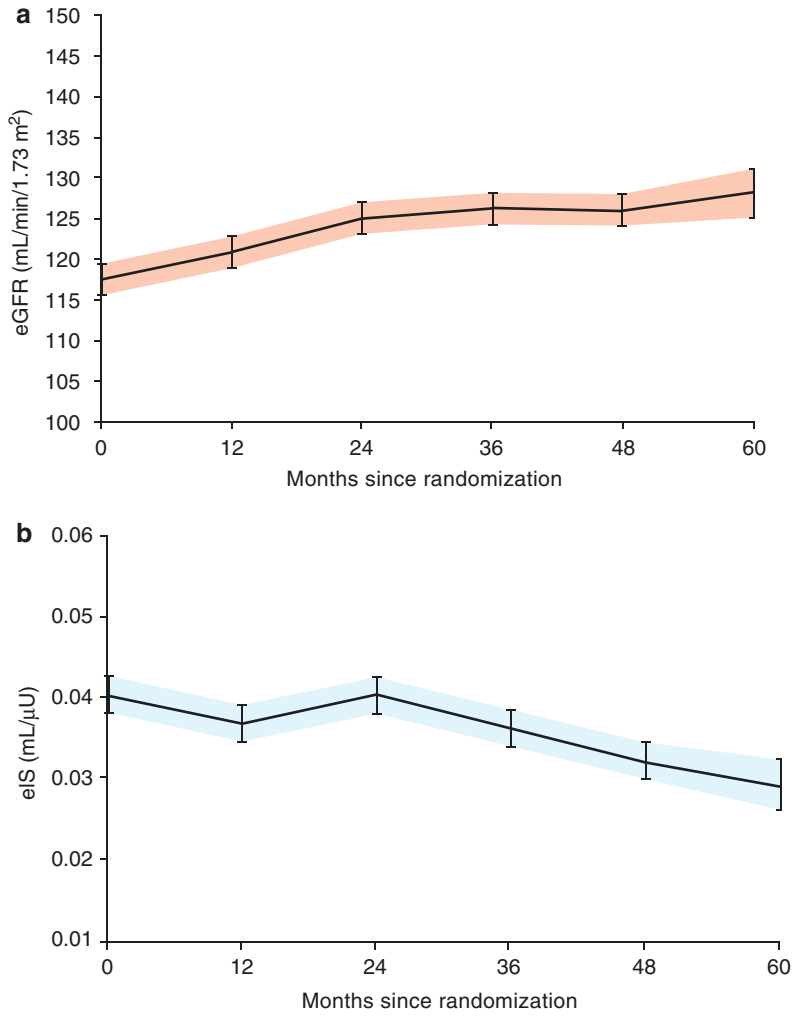

Fig. 14.2 (**a**) Changes in estimated glomerular filtration rate (eGFR) and (**b**) estimated insulin sensitivity (eIS) over 5 years in adolescents with type 2 diabetes. *p*-value for trend: unadjusted model $p < 0.0001$, adjusted model $p < 0.0001$. (Adapted from [58])

ciation remained significant in models adjusting for age, sex, race-ethnicity, BMI, HbA1c, treatment group, loss of glycemic control, and hypertension (HR: 2.15, $p = 0.01$). Neither HbA1c, BMI, age, loss of glycemic control nor development of hypertension were associated with increased risk of developing hyperfiltration in the adjusted model [59]. Overall, these findings support the hypothesis that IS is strongly and independently associated with hyperfiltration.

Aside from hyperfiltration, albuminuria is an early marker of nephropathy risk. Findings from SEARCH for Diabetes in Youth Study demonstrated a cross-sectional relationship between ACR and estimated insulin sensitivity [16]. The association between insulin resistance and progression of DKD has also been increasingly recognized in adults with T2D. Parvanova et al. [60] reported a significant cross-sectional association between measured insulin resistance and albuminuria in adults with T2D, while De Cosmo et al. [61] showed that adult males with the highest quartile of HOMA-IR were more likely to have increased albumin excretion than those in the lowest quartile. Others have demonstrated a longitudinal relationship between insulin resistance and incident albuminuria over time in adults (Table 14.1) [16, 18, 49–51, 53–55, 57, 60, 61].

Table 14.1 Selected publications demonstrating the association between insulin sensitivity (IS) and diabetic kidney disease (DKD)

Authors	Population	Findings	Reference
Type 1 diabetes			
Martin et al. (1968)	Adults	Estimated IS associated with DKD	[49]
Yip et al. (1993)	Adults	IS associated with microalbuminuria	[50]
Orchard et al. (2002)	Adults	Estimated IS predicted proteinuria	[51]
Bjornstad et al. (2013)	Adults	Estimated IS predicted early DKD, including albuminuria and rapid glomerular filtration rate (GFR) decline	[53, 55]
Bjornstad et al. (2014)	Adults	Improvement in estimated IS over time predicted regression of albuminuria	[54]
Type 2 diabetes			
Bjornstad et al. (2014)	Youth	Measured IS associated with albuminuria and hyperfiltration	[18]
Mottl et al. (2016)	Youth	Estimated IS associated with albuminuria	[16]
De Cosmo et al. (2005)	Adults	Estimated IS associated with albuminuria	[61]
Parvanova et al. (2006)	Adults	Estimated IS associated with albuminuria	[60]
Hsu et al. (2011)	Adults	Estimated IS associated with development of albuminuria	[57]

Proposed Mechanisms Linking Insulin Resistance and Diabetic Kidney Disease

The association between insulin resistance and hemodynamic changes in the kidney is increasingly recognized, but the underlying mechanisms are not completely understood. Hyperfiltration develops secondary to an increase in intraglomerular pressure, leading to structural changes over time, such as mesangial expansion and glomerular basement membrane thickening [62]. The mechanisms believed to underlie these pathological changes are complex and involve growth factors, neurohormonal activation, and changes in renal tubuloglomerular feedback [63]. Insulin resistance is also associated with an elevation of glomerular hydrostatic pressure causing increased renal vascular permeability and, ultimately, hyperfiltration [64–66]. Another possible mechanistic pathway linking insulin resistance to DKD is through effects on overall nonesterified fatty acid exposure and lipotoxicity, leading to the development of microangiopathy and arterial stiffness [67].

In addition to the above proposed pathways, renal hypoxia is increasingly being suggested as a central mechanism in the pathogenesis of DKD [20, 68, 69]. Animal models of diabetes suggest that glucosuria enhances sodium–glucose reabsorption through sodium–glucose cotransporter 2 (SGLT2), with resultant increased intracellular Na^+ concentration at the proximal tubule and increased activation of basolateral Na^+/K^+ ATPase, leading to net increased renal oxygen consumption compared to animals without diabetes [21] (Fig. 14.3). Diabetes-related sodium avidity at the proximal tubule also leads to diminished Na^+ delivery to the macula densa, thereby decreasing afferent arteriolar resistance via tubuloglomerular feedback mechanisms. Afferent dilation increases renal blood flow, glomerular pressure, and GFR. This mechanism sets up a vicious cycle, since [70, 71] the largest contributor to the increase in renal Na^+ reabsorption and oxygen consumption in diabetes is the augmented GFR, with consequent increased tubular Na^+ and glucose load. With increased renal oxygen consumption in DKD, additional energy efficient fuel is required to meet the increased demand and prevent renal parenchymal hypoxia.

There are emerging animal data that organs prone to complications are not able to sufficiently compensate for the effects of diabetes on fuel

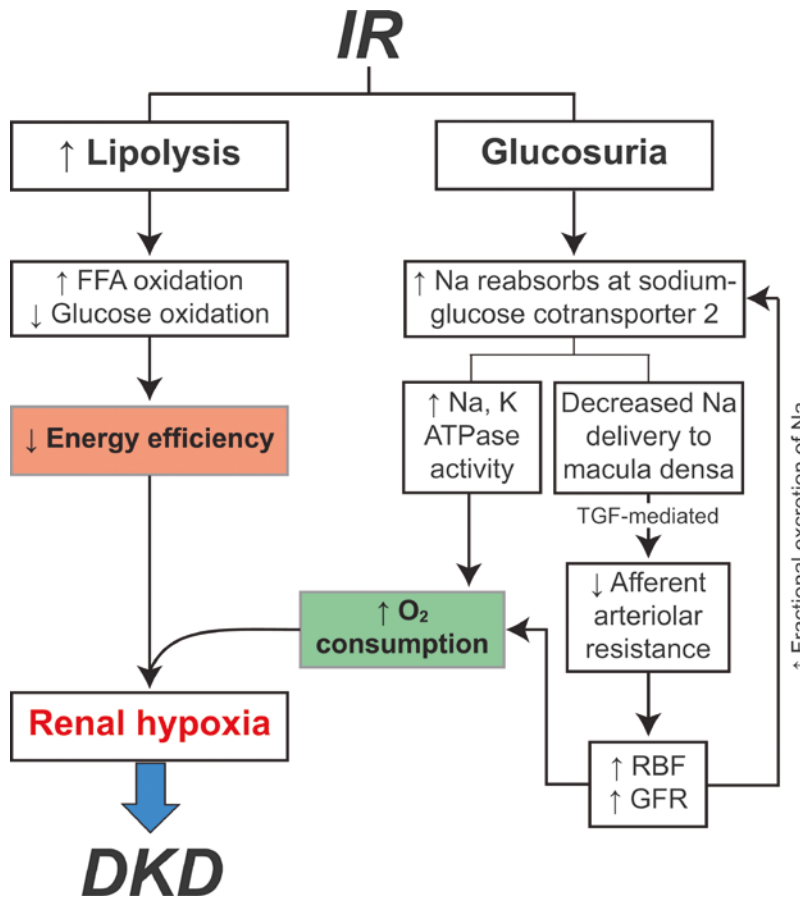

Fig. 14.3 The proposed role of renal energetics and intrarenal hemodynamics in diabetic kidney disease (DKD). IR insulin resistance, Na sodium, K potassium

generation. The unfavorable renal energy profile of T2D is likely driven by IR, impaired suppression of lipolysis, and increased circulating FFAs. FFAs impair insulin action by inhibiting insulin signaling pathways, leading to decreased renal cellular glucose uptake and reduction in glucose oxidation [72, 73]. For those reasons, the postulated changes associated with renal energetics in T2D include increased FFA oxidation and reduced glucose oxidation (Fig. 14.3). Because FFA oxidation produces ATP less efficiently than glucose oxidation, the altered energy utilization of the diabetic kidney is characterized by decreased renal oxygen delivery and increased oxygen consumption [21, 72–74]. This mismatch between renal oxygen delivery and demand could lead to renal hypoxia and progression of DKD (Fig. 14.3). On the therapeutic side, agents that alter energy substrate utilization, such as SGLT2 inhibitors, may exert favorable clinical effects in part through improvements in fuel delivery. For example, the substantial renal benefits recently demonstrated with SGLT2 inhibition in adults with T2D in the EMPA-REG OUTCOME trial have been hypothesized to be due to a shift toward increased ketone body production leading to improved cardiac contractility [21, 74, 75]. Whether or not this type of effect has an impact on renal hypoxia or energy production has not yet been studied but has been suggested by others [76].

In addition to the effects of tubuloglomerular feedback and renal hypoxia, neurohormones, such as the renin angiotensin aldosterone system, have been implicated as mediators of IS-related DKD risk. While the relationship between renin angiotensin aldosterone system (RAAS) and insulin sensitivity has also been extensively studied, recent discoveries have led to an improved understanding of how activated

tissue RAAS influences the development of insulin resistance [77]. Increased angiotensin II activity leads to impaired insulin secretion, insulin signaling and glucose uptake, and inflammation, which promotes the development of insulin resistance [78]. Large-scale clinical trials have evaluated the effect of RAAS blockade on the development of diabetes, but with conflicting results. The Nateglinide and Valsartan in Impaired Glucose Tolerance Outcome Research (NAVIGATOR) study compared the effect of valsartan and placebo for a median of 5 years on the development of diabetes mellitus and found that the valsartan-treated group had lower incidence of diabetes [79–81]. Conversely, the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study found no difference in the risk of developing diabetes between the ramipril and placebo groups [82]. Animal models have further suggested that angiotensin 1–7 improves insulin sensitivity, and prolonged administration of angiotensin 1–7 in fructose-fed rats was associated with improved insulin sensitivity [83].

Aside from RAAS neurohormones, arginine vasopressin (AVP) plays an essential role in the regulation of IS and volume status and may increase the risk of renal and vascular injury [84–88]. AVP concentrations are higher in people with T1D and T2D compared to healthy nondiabetic counterparts [89, 90]. Furthermore, the peptide copeptin, co-secreted with AVP from the neurohypophysis, reflects changes in AVP, and elevated concentrations of copeptin appear to increase the risk of cardiovascular mortality [85, 91]. Recent data from CACTI (Coronary Artery Calcification in Type 1 *Diabetes*) [92], GENEDIAB (Genetique de la Nephropathie Diabetique), GENESIS (Genetics Nephropathy and Sib pair *Study*) [93], and ZODIAC-31 (Zwolle Outpatient *Diabetes* project Integrating Available Care) [91] implicate elevated concentrations of copeptin as a strong risk factor for DKD in adults with T1D and T2D. In fact, the administration of vasopressin induces glomerular hyperfiltration and albuminuria in laboratory animals and in humans [86, 94, 95]. Disruption of the AVP system has been linked to the development of insulin resistance, obesity, metabolic syndrome, and type 2 diabetes [96, 97]. Chronic psychosocial stress is associated with increased AVP levels, and several reports have suggested that vasopressin overactivity may have a role in the association of stress with insulin resistance and T2D. Enhorning et al. demonstrated that copeptin independently predicted development of T2D and abdominal obesity in the Malmo Diet and Cancer Study [98]. The same group also reported an association between copeptin and development of albuminuria, which was independent of T2D status or the presence of hypertension [98]. Similarly, Saleem et al. found a cross-sectional association between plasma copeptin and insulin resistance [99] in a smaller study. Enhorning and coauthors more recently demonstrated that a genetic variation of the human AVP receptor 1b gene (AVPR1B) is linked with increased risk of developing obesity and T2D [100]. Despite interesting epidemiologic data, it remains unclear whether the relationship between insulin resistance and DKD is driven by disruption of the AVP system.

Clinical Trials

Insulin sensitivity can be modified by both lifestyle changes (diet and exercise) and drugs, such as biguanides and thiazolidinediones. Insulin sensitivity therefore holds promise as a therapeutic target to delay and stop the development of DKD in patients with T1D and T2D. Metformin is an inexpensive and well-tolerated medication [101]. In T2D, metformin is widely considered to protect against cardiovascular complications [101, 102]. In contrast, metformin is not advocated in any major national or international guidelines for the management of T1D [102]. In a recent meta-analysis of randomized trials, Vella et al. found that metformin therapy in T1D was associated with reduced insulin-dose requirements though no clear evidence for an improvement in glycemic control was demonstrated [102]. Moreover, Nadeau et al. recently showed that low-dose metformin decreased total daily insulin doses in adolescents with T1D, likely

representing improvement in insulin sensitivity [103]. Metformin is also associated with reduced low-density lipoprotein (LDL) cholesterol [104], precursors of advanced glycation end production [105, 106], and a decrease in blood pressure [107, 108] in adults with T1D and T2D. However, there are currently insufficient data on vascular outcomes, and their surrogates to routinely recommend metformin therapy in adolescents and adults with T1D for improving cardiovascular outcomes. For that reason, there is a need for well-designed randomized clinical trials of sufficient size and duration to show clinical benefit of metformin. Another important consideration is the significant variation observed in adults with T2D in response to metformin [101]. The inter-individual variation in metformin may also apply to patients with T1D. Genetic variation may be one of the important determinants of this inter-individual variation, and improved understanding of underlying genes and pathways has the potential to improve the effect of metformin on insulin sensitivity [101].

In adults with coronary artery disease and type 2 diabetes [109], the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) study showed no benefit of insulin-sensitizing therapy on progression of DKD. However, in this study population most participants already had baseline hypertension and hyperlipidemia [110]. The kidney injury in long-standing DKD in older adults with multiple cardiovascular risk factors may be far less responsive to changes in IS than the early alterations in renal function seen in adolescents with T1D and T2D, arguing for separate studies in youth. One hypothesis is that early intervention prior to establishment of vascular lesions may result in significant delay of clinical pathology, as demonstrated by the concept of "metabolic memory" in the DCCT-EDIC study [111–115]. Also, clinical cardiovascular disease typically does not manifest until older ages; for example, it took 17 years of follow-up for the benefits of intensive management to manifest in DCCT [116]. Improvements in outcomes due to adjunctive therapy in the era of intensive management may be more subtle, because large clinical trials and epidemiological cohort studies in adults have had conflicting results [117]. Furthermore, long-term studies in youth are lacking [117].

The Effects of Metformin on Cardiovascular Function in Adolescents with Type 1 Diabetes (EMERALD, NCT01808690) is a recently completed double-blind randomized clinical trial with metformin designed to evaluate if metformin will improve tissue-specific insulin resistance in T1D adolescents using the hyperinsulinemic–euglycemic clamp technique, as well as improve vascular, cardiac, exercise, and muscle mitochondrial function. The trial demonstrated that metformin therapy improved insulin sensitivity compared to placebo in lean and overweight/obese adolescents with T1D. Additionally, metformin therapy improved markers of vascular health, including aortic pulse wave velocity and wall shear stress compared to placebo [118]. The REducing with MetfOrmin Vascular Adverse Lesions in type 1 diabetes (REMOVAL, NCT01483560) [119] study is another recent completed double-blind randomized clinical trial with metformin to improve insulin sensitivity in adults with T1D in an attempt to prevent vascular complications. The REMOVAL trial demonstrated reduction in progression of maximal carotid artery intima-media thickness (cIMT), but not the primary outcome of mean cIMT, metformin vs. placebo in adults with T1D [120]. The effect of Metformin on Vascular and Mitochondrial Function in Type 1 Diabetes (MeT1, NCT01813929) study is also underway and seeks to evaluate the effect of improving insulin sensitivity on vascular and mitochondrial function in adults with T1D. Metformin Therapy for Overweight Adolescents with Type 1 Diabetes (NCT01881828), being performed in the T1D Exchange Clinic Network, will evaluate the efficacy and safety of the use of metformin in addition to standard insulin therapy in overweight and obese children and adolescents, ages 12 to <20 years, with T1D for at least 1 year. Furthermore, the insulin Clamp Ancillary Study for Assessment of Insulin Resistance (CASAI, NCT02045290) is an associated study underway to evaluate if metformin will improve tissue-specific insulin resistance in obese T1D adolescents using hyperinsulinemic–euglycemic clamp technique.

Smaller metformin trials are also underway. While extensive research is underway evaluating the roles of metformin, insulin sensitivity, and vascular function, dedicated studies examining the effect of insulin-sensitizing therapies on DKD are needed. Additional studies are also needed to assess the impact of other drugs that influence insulin sensitivity in T1D and T2D, including glucagon-like peptide-1 (GLP-1) analogues, dipeptidyl peptidase-4 inhibitor (DPP4 inhibitors), sodium–glucose cotransporter 2 (SGLT2) inhibitors, thiazolidinediones, and bromocriptine. Bromocriptine, a dopamine agonist, has long been used for the treatment of Parkinson's disease and prolactinomas. Its recent approval in a quick release formulation (BCQR) for T2D represents a novel mechanism to improve hyperglycemia and IR-associated dysmetabolism [121]. Dopamine signaling is abnormal in IR [121], and morning BCQR restores the dopamine surge in T2D, resulting in decreases in sympathetic activity, glucagon/hepatic glucose output, lipolysis and circulating free fatty acids [121], and RAAS activation [121, 122]. There are also ongoing clinical trials investigating the efficacy and safety of SGLT2 inhibitors reducing weight in obese/overweight patients (NCT00650806; NCT02235298).

Future Studies

To gain additional information about the mechanisms underlying the relationship between insulin sensitivity and renal health, dedicated translation research studies are needed. Detailed assessment of renal hemodynamics paired with hyperinsulinemic–euglycemic clamp technique has the potential to improve researchers' ability to relate early changes in renal hemodynamics with worsening insulin sensitivity in the course of DKD. The Gomez equations also offer tremendous potential in translational and mechanistic human DKD research [123]. These equations were created by Gomez et al. and use measurements of GFR, renal blood flow, effective renal plasma flow, renal vascular resistance, hematocrit, and serum protein to calculate afferent and efferent arteriolar resistances, glomerular pressure, and filtration pressure [123]. As an example of the application of Gomez' equations, we recently demonstrated that women with type T1D and hyperfiltration had higher efferent arteriolar resistance and lower renal blood flow compared to men with T1D and hyperfiltration [124]. Although the specific mechanisms responsible for the relatively higher efferent arteriolar resistance and lower renal blood flow in women with T1D and hyperfiltration compared to their male counterparts are still unclear, intrarenal renin-angiotensin-aldosterone system (RAAS) activation is a plausible mediator [125]. These observations might explain the greater humoral, renal, and systemic responsiveness of RAAS inhibition in women compared to men (35) and illustrate the potential of applying Gomez' equations in mechanistic DKD research.

Advanced imaging techniques, including renal blood oxygen level dependent (BOLD), magnetic resonance imaging (MRI), arterial spin labeling (ASL) MRI, and phase-contrast MRI (PC-MRI), also hold promise in characterizing renal oxygen consumption and hypoxia in early DKD. In the COMBINE (CKD Optimal Management with BInders and NicotinamidE) study [124], a large multicenter trial, Prasad et al. reported that adults with CKD have lower renal cortical oxygenation compared to controls (18.0 ± 1.62 vs. 20.6 ± 3.4 s^{-1}, $p < 0.0001$) by BOLD MRI [124]. Furthermore, renal blood flow measured by ASL MRI correlated strongly with eGFR ($r = 0.67$, $p < 0.0001$) [126]. The kidneys are highly metabolically active and have a high-energy requirement to sustain their GFR and intrarenal hemodynamic function [21, 127], which is exacerbated in hyperfiltration. The elevated GFR characteristic of hyperfiltration and the concomitant elevation in renal blood flow augment renal oxygen consumption. Therefore, the consequent renal hypoxia may stem from a mismatch between renal oxygen delivery and demand, a unifying pathway in the development of DKD. Research combining advanced renal imaging with renal hemodynamic assessments and measures of insulin sensitivity are now needed to elucidate the mechanisms explaining the strong relationships observed between insulin sensitivity and renal health in DKD.

Summary

DKD remains a significant public health burden. The increasing prevalence of T2D and T1D worldwide has led to a concomitant rise in DKD [128, 129]. Left untreated, patients with DKD, especially those with youth-onset T2D, have a high risk of progressing to ESRD and dialysis [129]. Particularly worrisome is the manifestation of early DKD in youth with T1D and T2D. Worsening insulin sensitivity may represent an early risk factor for future progression of DKD. While data support strong independent relationships between insulin sensitivity and renal health in T1D and T2D, the mechanisms at play remain elusive. Dedicated mechanistic and translational human research is now needed to improve our understanding of what initiates and drives early DKD.

References

1. Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Johansen K, et al. United States Renal Data System 2011 Annual Data Report: atlas of chronic kidney disease & end-stage renal disease in the United States. Am J Kidney Dis. [Editorial Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 2012;59(1 Suppl 1):A7, e1–420.
2. Saran R, Li Y, Robinson B, Abbott KC, Agodoa LY, Ayanian J, et al. US Renal Data System 2015 Annual Data Report: epidemiology of kidney disease in the United States. Am J Kidney Dis. [Editorial]. 2016;67(3 Suppl 1):A7–8.
3. Bjornstad P, Cherney DZ, Maahs DM, Nadeau KJ. Diabetic kidney disease in adolescents with type 2 diabetes: new insights and potential therapies. Curr Diab Rep. 2016;16(2):11.
4. Saran R, Li Y, Robinson B, Abbott KC, Agodoa LY, Ayanian J, et al. US Renal Data System 2015 Annual Data Report: epidemiology of kidney disease in the United States. Am J Kidney Dis. 2016;67(3 Suppl 1):Svii, S1–305.
5. Alleyn CR, Volkening LK, Wolfson J, Rodriguez-Ventura A, Wood JR, Laffel LM. Occurrence of microalbuminuria in young people with type 1 diabetes: importance of age and diabetes duration. Diabet Med. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010;27(5):532–7.
6. Eppens MC, Craig ME, Cusumano J, Hing S, Chan AK, Howard NJ, et al. Prevalence of diabetes complications in adolescents with type 2 compared with type 1 diabetes. Diabetes Care. [Comparative Study Multicenter Study]. 2006;29(6):1300–6.
7. Kiess W, Bottner A, Bluher S, Raile K, Galler A, Kapellen TM. Type 2 diabetes mellitus in children and adolescents--the beginning of a renal catastrophe? Nephrol Dial Transplant. [Editorial Research Support, Non-U.S. Gov't]. 2004;19(11):2693–6.
8. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the diabetes control and complications trial cohort. Diabetes Care. [Multicenter Study Research Support, U.S. Gov't, P.H.S.]. 1999;22(1):99–111.
9. TODAY Study Group. Rapid rise in hypertension and nephropathy in youth with type 2 diabetes: the TODAY clinical trial. Diabetes Care. [Randomized Controlled Trial Research Support, N.I.H., Extramural]. 2013;36(6):1735–41.
10. Yokoyama H, Okudaira M, Otani T, Takaike H, Miura J, Saeki A, et al. Existence of early-onset NIDDM Japanese demonstrating severe diabetic complications. Diabetes Care. 1997;20(5):844–7.
11. Yokoyama H, Okudaira M, Otani T, Watanabe C, Takaike H, Miuira J, et al. High incidence of diabetic nephropathy in early-onset Japanese NIDDM patients. Risk analysis. Diabetes Care. 1998;21(7):1080–5.
12. Rodriguez BL, Dabelea D, Liese AD, Fujimoto W, Waitzfelder B, Liu L, et al. Prevalence and correlates of elevated blood pressure in youth with diabetes mellitus: the SEARCH for diabetes in youth study. J Pediatr. [Multicenter Study Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.]. 2010;157(2):245–51 e1.
13. Maahs DM, Snively BM, Bell RA, Dolan L, Hirsch I, Imperatore G, et al. Higher prevalence of elevated albumin excretion in youth with type 2 than type 1 diabetes: the SEARCH for Diabetes in Youth study. Diabetes Care. [Multicenter Study Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.]. 2007;30(10):2593–8.
14. Al-Saeed AH, Constantino MI, Molyneaux L, D'Souza M, Limacher-Gisler F, Luo C, et al. An inverse relationship between age of type 2 diabetes onset and complication risk and mortality: the impact of youth-onset type 2 diabetes. Diabetes Care. 2016;39(5):823–9.
15. TODAY Study Group. Rapid rise in hypertension and nephropathy in youth with type 2 diabetes: the TODAY clinical trial. Diabetes Care. 2013;36(6):1735–41.
16. Mottl AK, Divers J, Dabelea D, Maahs DM, Dolan L, Pettitt D, et al. The dose-response effect of insulin sensitivity on albuminuria in children according to diabetes type. Pediatr Nephrol. 2016;31(6):933–40.
17. Mottl AK, Lauer A, Dabelea D, Maahs DM, D'Agostino RB Jr, Dolan LM, et al. Albuminuria according to status of autoimmunity and insulin sensitivity among youth with type 1 and type 2 diabetes. Diabetes Care. 2013;36(11):3633–8.

18. Bjornstad P, Maahs DM, Cherney DZ, Cree-Green M, West A, Pyle L, et al. Insulin sensitivity is an important determinant of renal health in adolescents with type 2 diabetes. Diabetes Care. 2014;37(11):3033–9.
19. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. JAMA. 2014;311(17):1778–86.
20. Neugarten J, Golestaneh L. Blood oxygenation level-dependent MRI for assessment of renal oxygenation. Int J Nephrol Renovasc Dis. 2014;7:421–35.
21. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care. 2016;39(7):1115–22.
22. Drummond K, Mauer M. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. Diabetes. [Multicenter Study Research Support, Non--U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2002;51(5):1580–7.
23. Bjornstad P, Roncal C, Milagres T, Pyle L, Lanaspa MA, Bishop FK, et al. Hyperfiltration and uricosuria in adolescents with type 1 diabetes. Pediatr Nephrol. 2016;31(5):787–93.
24. Bjornstad P, Cherney DZ, Snell-Bergeon JK, Pyle L, Rewers M, Johnson RJ, et al. Rapid GFR decline is associated with renal hyperfiltration and impaired GFR in adults with type 1 diabetes. Nephrol Dial Transplant. 2015;30(10):1706–11.
25. Cherney DZ, Miller JA, Scholey JW, Nasrallah R, Hebert RL, Dekker MG, et al. Renal hyperfiltration is a determinant of endothelial function responses to cyclooxygenase 2 inhibition in type 1 diabetes. Diabetes Care. 2010;33(6):1344–6.
26. Premaratne E, Macisaac RJ, Tsalamandris C, Panagiotopoulos S, Smith T, Jerums G. Renal hyperfiltration in type 2 diabetes: effect of age-related decline in glomerular filtration rate. Diabetologia. 2005;48(12):2486–93.
27. Troya MI, Bonet J, Salinas I, Torres F, Bonal J, Sanmarti A, et al. Early intensive treatment improves outcomes in patients with glomerular hyperfiltration and type 2 diabetes. Med Clin (Barc). 2016;146(2):55–60.
28. Cherney DZ, Reich HN, Jiang S, Har R, Nasrallah R, Hebert RL, et al. Hyperfiltration and the effect of nitric oxide inhibition on renal and endothelial function in humans with uncomplicated type 1 diabetes mellitus. Am J Physiol Regul Integr Comp Physiol. 2012;303(7):R710–8.
29. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. [Research Support, Non-U.S. Gov't Review]. 2005;28(1):164–76.
30. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS. Regression of microalbuminuria in type 1 diabetes. N Engl J Med. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2003;348(23):2285–93.
31. Molitch ME, Steffes M, Sun W, Rutledge B, Cleary P, de Boer IH, et al. Development and progression of renal insufficiency with and without albuminuria in adults with type 1 diabetes in the diabetes control and complications trial and the epidemiology of diabetes interventions and complications study. Diabetes Care. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010;33(7):1536–43.
32. Krolewski AS, Niewczas MA, Skupien J, Gohda T, Smiles A, Eckfeldt JH, et al. Early progressive renal decline precedes the onset of microalbuminuria and its progression to macroalbuminuria. Diabetes Care. 2014;37(1):226–34.
33. MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, Smith TJ, McNeil KJ, Jerums G. Nonalbuminuric renal insufficiency in type 2 diabetes. Diabetes Care. 2004;27(1):195–200.
34. Okada R, Yasuda Y, Tsushita K, Wakai K, Hamajima N, Matsuo S. Glomerular hyperfiltration in prediabetes and prehypertension. Nephrol Dial Transplant. [Comparative Study Research Support, Non--U.S. Gov't]. 2012;27(5):1821–5.
35. Mogensen CE. Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy. Scand J Clin Lab Invest. [Research Support, Non--U.S. Gov't]. 1986;46(3):201–6.
36. Mogensen CE. Glomerular hyperfiltration in human diabetes. Diabetes Care. [Review]. 1994;17(7):770–5.
37. Ruggenenti P, Porrini EL, Gaspari F, Motterlini N, Cannata A, Carrara F, et al. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. Diabetes Care. [Randomized Controlled Trial]. 2012;35(10):2061–8.
38. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. J Am Geriatr Soc. 1985;33(4):278–85.
39. Rifkin DE, Shlipak MG, Katz R, Fried LF, Siscovick D, Chonchol M, et al. Rapid kidney function decline and mortality risk in older adults. Arch Intern Med. [Research Support, N.I.H., Extramural]. 2008;168(20):2212–8.
40. Shlipak MG, Katz R, Kestenbaum B, Siscovick D, Fried L, Newman A, et al. Rapid decline of kidney function increases cardiovascular risk in the elderly. J Am Soc Nephrol. [Multicenter Study Research Support, N.I.H., Extramural Research Support, Non--U.S. Gov't]. 2009;20(12):2625–30.
41. Orchard TJ, Secrest AM, Miller RG, Costacou T. In the absence of renal disease, 20 year mortality risk in type 1 diabetes is comparable to that of the general population: a report from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia. [Research Support, N.I.H., Extramural]. 2010;53(11):2312–9.
42. Groop PH, Thomas MC, Moran JL, Waden J, Thorn LM, Makinen VP, et al. The presence and severity

of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes. [Multicenter Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2009;58(7):1651–8.
43. Solis-Herrera C, Triplitt CL, Lynch JL. Nephropathy in youth and young adults with type 2 diabetes. Curr Diab Rep. 2014;14(2):456.
44. Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR. Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. Diabetes. [Research Support, Non-U.S. Gov't]. 2006;55(6):1832–9.
45. Chen J, Muntner P, Hamm LL, Fonseca V, Batuman V, Whelton PK, et al. Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. J Am Soc Nephrol. [Research Support, Non--U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2003;14(2):469–77.
46. Ritz E, Orth SR. Nephropathy in patients with type 2 diabetes mellitus. N Engl J Med. [Review]. 1999;341(15):1127–33.
47. Nadeau KJ, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, Hull A, et al. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. J Clin Endocrinol Metab. 2010;95(2):513–21.
48. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. J Clin Endocrinol Metab. 2009;94(10):3687–95.
49. Martin FI, Stocks AE. Insulin sensitivity and vascular disease in insulin-dependent diabetics. Br Med J. 1968;2(5597):81–2.
50. Yip J, Mattock MB, Morocutti A, Sethi M, Trevisan R, Viberti G. Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. Lancet. [Research Support, Non-U.S. Gov't]. 1993;342(8876):883–7.
51. Orchard TJ, Chang YF, Ferrell RE, Petro N, Ellis DE. Nephropathy in type 1 diabetes: a manifestation of insulin resistance and multiple genetic susceptibilities? Further evidence from the Pittsburgh Epidemiology of Diabetes Complication Study. Kidney Int. 2002;62(3):963–70.
52. Duca LM, Maahs DM, Schauer IE, Bergman BC, Nadeau KJ, Bjornstad P, et al. Development and validation of a method to estimate insulin sensitivity in patients with and without type 1 diabetes. J Clin Endocrinol Metab. 2016;101(2):686–95.
53. Bjornstad P, Maahs DM, Duca LM, Pyle L, Rewers M, Johnson RJ, et al. Estimated insulin sensitivity predicts incident micro- and macrovascular complications in adults with type 1 diabetes over 6 years: the coronary artery calcification in type 1 diabetes study. J Diabetes Complications. 2016;30(4):586–90.
54. Bjornstad P, Maahs DM, Johnson RJ, Rewers M, Snell-Bergeon JK. Estimated insulin sensitivity predicts regression of albuminuria in type 1 diabetes. Diabet Med. 2015;32(2):257–61.
55. Bjornstad P, Snell-Bergeon JK, Rewers M, Jalal D, Chonchol MB, Johnson RJ, et al. Early diabetic nephropathy: a complication of reduced insulin sensitivity in type 1 diabetes. Diabetes Care. [Research Support, N.I.H., Extramural Research Support, Non--U.S. Gov't]. 2013;36(11):3678–83.
56. Dart AB, Sellers EA, Martens PJ, Rigatto C, Brownell MD, Dean HJ. High burden of kidney disease in youth-onset type 2 diabetes. Diabetes Care. [Comparative Study Research Support, Non--U.S. Gov't]. 2012;35(6):1265–71.
57. Hsu CC, Chang HY, Huang MC, Hwang SJ, Yang YC, Tai TY, et al. Association between insulin resistance and development of microalbuminuria in type 2 diabetes: a prospective cohort study. Diabetes Care. [Research Support, Non-U.S. Gov't]. 2011;34(4):982–7.
58. Bjornstad P, Nehus E, El Ghormli L, Bacha F, Libman IM, McKay S, et al. Insulin sensitivity and diabetic kidney disease in children and adolescents with type 2 diabetes: an observational analysis of data from the TODAY clinical trial. Am J Kidney Dis. 2018;71(1):65–74.
59. Bjornstad P, Laffel L, Arslanian S, Bacha F, El Ghormli L, Libman L, et al. Insulin sensitivity and hyperfiltration in adolescents with type 2 diabetes (T2D) in the TODAY clinical trial. E-theater oral presentation at American Diabetes Association Scientific Sessions 2016. New Orleans, 10–14 June 2016.
60. Parvanova AI, Trevisan R, Iliev IP, Dimitrov BD, Vedovato M, Tiengo A, et al. Insulin resistance and microalbuminuria: a cross-sectional, case-control study of 158 patients with type 2 diabetes and different degrees of urinary albumin excretion. Diabetes. [Multicenter Study]. 2006;55(5):1456–62.
61. De Cosmo S, Minenna A, Ludovico O, Mastroianno S, Di Giorgio A, Pirro L, et al. Increased urinary albumin excretion, insulin resistance, and related cardiovascular risk factors in patients with type 2 diabetes: evidence of a sex-specific association. Diabetes Care. [Multicenter Study Research Support, Non-U.S. Gov't]. 2005;28(4):910–5.
62. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest. [Review]. 2006;116(2):288–96.
63. Cherney DZ, Scholey JW, Miller JA. Insights into the regulation of renal hemodynamic function in diabetic mellitus. Curr Diabetes Rev. [Research Support, Non-U.S. Gov't Review]. 2008;4(4):280–90.
64. Catalano C, Muscelli E, Quinones Galvan A, Baldi S, Masoni A, Gibb I, et al. Effect of insulin on systemic and renal handling of albumin in nondiabetic and NIDDM subjects. Diabetes. 1997;46(5):868–75.
65. Cohen AJ, McCarthy DM, Stoff JS. Direct hemodynamic effect of insulin in the isolated perfused kidney. Am J Physiol. [In Vitro Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1989;257(4 Pt 2):F580–5.

66. Tucker BJ, Anderson CM, Thies RS, Collins RC, Blantz RC. Glomerular hemodynamic alterations during acute hyperinsulinemia in normal and diabetic rats. Kidney Int. [Research Support, U.S. Gov't, P.H.S.]. 1992;42(5):1160–8.
67. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2006;440(7086):944–8.
68. Fine LG, Norman JT. Chronic hypoxia as a mechanism of progression of chronic kidney diseases: from hypothesis to novel therapeutics. Kidney Int. 2008;74(7):867–72.
69. Fine LG, Orphanides C, Norman JT. Progressive renal disease: the chronic hypoxia hypothesis. Kidney Int Suppl. 1998;65:S74–8.
70. Sgouralis I, Layton AT. Mathematical modeling of renal hemodynamics in physiology and pathophysiology. Math Biosci. 2015;264:8–20.
71. Layton AT, Vallon V, Edwards A. Modeling oxygen consumption in the proximal tubule: effects of NHE and SGLT2 inhibition. Am J Physiol Renal Physiol. 2015;308(12):F1343–57.
72. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes. 2000;49(5):677–83.
73. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. Circulation. 2007;116(4):434–48.
74. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. N Engl J Med. 2016;375:323–34.
75. Cox PJ, Kirk T, Ashmore T, Willerton K, Evans R, Smith A, et al. Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. Cell Metab. 2016;24(2):256–68.
76. Gilbert RE. SGLT2 inhibitors: beta blockers for the kidney? Lancet Diabetes Endocrinol. 2016;4(10):814.
77. Leung PS. Mechanisms of protective effects induced by blockade of the renin-angiotensin system: novel role of the pancreatic islet angiotensin-generating system in type 2 diabetes. Diabet Med. 2007;24(2):110–6.
78. Underwood PC, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. Curr Hypertens Rep. 2013;15(1):59–70.
79. Group NS, Holman RR, Haffner SM, McMurray JJ, Bethel MA, Holzhauer B, et al. Effect of nateglinide on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010;362(16):1463–76.
80. Group NS, McMurray JJ, Holman RR, Haffner SM, Bethel MA, Holzhauer B, et al. Effect of valsartan on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010;362(16):1477–90.
81. Preiss D, Thomas LE, Wojdyla DM, Haffner SM, Gill JM, Yates T, et al. Prospective relationships between body weight and physical activity: an observational analysis from the NAVIGATOR study. BMJ Open. 2015;5(8):e007e901.
82. Lonn EM, Gerstein HC, Sheridan P, Smith S, Diaz R, Mohan V, et al. Effect of ramipril and of rosiglitazone on carotid intima-media thickness in people with impaired glucose tolerance or impaired fasting glucose: STARR (STudy of Atherosclerosis with Ramipril and Rosiglitazone). J Am Coll Cardiol. 2009;53(22):2028–35.
83. Giani JF, Mayer MA, Munoz MC, Silberman EA, Hocht C, Taira CA, et al. Chronic infusion of angiotensin-(1-7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. Am J Physiol Endocrinol Metab. 2009;296(2):E262–71.
84. Bankir L, Bouby N, Ritz E. Vasopressin: a novel target for the prevention and retardation of kidney disease? Nat Rev Nephrol. [Review]. 2013;9(4):223–39.
85. Clark WF, Sontrop JM, Huang SH, Moist L, Bouby N, Bankir L. Hydration and chronic kidney disease progression: a critical review of the evidence. Am J Nephrol. 2016;43(4):281–92.
86. Bardoux P, Martin H, Ahloulay M, Schmitt F, Bouby N, Trinh-Trang-Tan MM, et al. Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient Brattleboro rats. Proc Natl Acad Sci U S A. 1999;96(18):10397–402.
87. Enhorning S, Hedblad B, Nilsson PM, Engstrom G, Melander O. Copeptin is an independent predictor of diabetic heart disease and death. Am Heart J. 2015;169(4):549–56.e1.
88. Tasevska I, Enhorning S, Persson M, Nilsson PM, Melander O. Copeptin predicts coronary artery disease cardiovascular and total mortality. Heart. 2016;102(2):127–32.
89. Zerbe RL, Vinicor F, Robertson GL. Plasma vasopressin in uncontrolled diabetes mellitus. Diabetes. [Research Support, U.S. Gov't, P.H.S.]. 1979;28(5):503–8.
90. Roussel R, El Boustany R, Bouby N, Potier L, Fumeron F, Mohammedi K, et al. Plasma copeptin, AVP gene variants, and incidence of type 2 diabetes in a cohort from the community. J Clin Endocrinol Metab. 2016;101(6):2432–9.
91. Riphagen IJ, Boertien WE, Alkhalaf A, Kleefstra N, Gansevoort RT, Groenier KH, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with cardiovascular and all-cause mortality in patients with type 2 diabetes (ZODIAC-31). Diabetes Care. 2013;36(10):3201–7.
92. Bjornstad P, Maahs DM, Jensen T, Lanaspa MA, Johnson RJ, Rewers M, et al. Elevated copeptin is associated with atherosclerosis and diabetic kidney disease in adults with type 1 diabetes. J Diabetes Complications. 2016;30(6):1093–6.
93. Velho G, El Boustany R, Lefevre G, Mohammedi K, Fumeron F, Potier L, et al. Plasma copeptin, kidney outcomes, ischemic heart disease, and all-cause mortality in people with long-standing type 1 diabetes. Diabetes Care. 2016;39:2288–95.

94. Bouby N, Ahloulay M, Nsegbe E, Dechaux M, Schmitt F, Bankir L. Vasopressin increases glomerular filtration rate in conscious rats through its antidiuretic action. J Am Soc Nephrol. 1996;7(6):842–51.
95. Bardoux P, Bichet DG, Martin H, Gallois Y, Marre M, Arthus MF, et al. Vasopressin increases urinary albumin excretion in rats and humans: involvement of V2 receptors and the renin-angiotensin system. Nephrol Dial Transplant. 2003;18(3):497–506.
96. Enhorning S, Struck J, Wirfalt E, Hedblad B, Morgenthaler NG, Melander O. Plasma copeptin, a unifying factor behind the metabolic syndrome. J Clin Endocrinol Metab. 2011;96(7):E1065–72.
97. Enhorning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G, et al. Plasma copeptin and the risk of diabetes mellitus. Circulation. 2010;121(19):2102–8.
98. Enhorning S, Bankir L, Bouby N, Struck J, Hedblad B, Persson M, et al. Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmo Diet and Cancer Study cardiovascular cohort. Int J Obes (Lond). 2013;37(4):598–603.
99. Saleem U, Khaleghi M, Morgenthaler NG, Bergmann A, Struck J, Mosley TH Jr, et al. Plasma carboxy-terminal provasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. J Clin Endocrinol Metab. 2009;94(7):2558–64.
100. Enhorning S, Sjogren M, Hedblad B, Nilsson PM, Struck J, Melander O. Genetic vasopressin 1b receptor variance in overweight and diabetes mellitus. Eur J Endocrinol. 2016;174(1):69–75.
101. Pawlyk AC, Giacomini KM, McKeon C, Shuldiner AR, Florez JC. Metformin pharmacogenomics: current status and future directions. Diabetes. 2014;63(8):2590–9.
102. Vella S, Buetow L, Royle P, Livingstone S, Colhoun HM, Petrie JR. The use of metformin in type 1 diabetes: a systematic review of efficacy. Diabetologia. [Research Support, Non-U.S. Gov't Review]. 2010;53(5):809–20.
103. Nadeau KJ, Chow K, Alam S, Lindquist K, Campbell S, McFann K, et al. Effects of low dose metformin in adolescents with type I diabetes mellitus: a randomized, double-blinded placebo-controlled study. Pediatr Diabetes. 2015;16:196–203.
104. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N Engl J Med. [Clinical Trial Clinical Trial, Phase I Clinical Trial, Phase II Clinical Trial, Phase III Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't]. 1995;333(9):541–9.
105. Beisswenger PJ, Howell SK, Touchette AD, Lal S, Szwergold BS. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. Diabetes. [Clinical Trial Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1999;48(1):198–202.
106. Nagi DK, Yudkin JS. Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects. A study of two ethnic groups. Diabetes Care. [Clinical Trial Comment Comparative Study Randomized Controlled Trial Research Support, Non-U.S. Gov't]. 1993;16(4):621–9.
107. Giugliano D, Quatraro A, Consoli G, Minei A, Ceriello A, De Rosa N, et al. Metformin for obese, insulin-treated diabetic patients: improvement in glycaemic control and reduction of metabolic risk factors. Eur J Clin Pharmacol. [Clinical Trial Randomized Controlled Trial]. 1993;44(2):107–12.
108. Landin K, Tengborn L, Smith U. Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. J Intern Med. [Research Support, Non-U.S. Gov't]. 1991;229(2):181–7.
109. August P, Hardison RM, Hage FG, Marroquin OC, McGill JB, Rosenberg Y, et al. Change in albuminuria and eGFR following insulin sensitization therapy versus insulin provision therapy in the BARI 2D study. Clin J Am Soc Nephrol. [Research Support, N.I.H., Extramural]. 2014;9(1):64–71.
110. Bypass Angioplasty Revascularization Investigation 2 Diabetes Study Group. Baseline characteristics of patients with diabetes and coronary artery disease enrolled in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial. Am Heart J. [Comparative Study Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;156(3):528–36, 536.e1–5.
111. Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group, Lachin JM, White NH, Hainsworth DP, Sun W, Cleary PA, Nathan DM. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. Diabetes. 2015;64:631–42.
112. de Boer IH, Rue TC, Cleary PA, Lachin JM, Molitch ME, Steffes MW, et al. Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort. Arch Intern Med. [Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural]. 2011;171(5):412–20.
113. de Boer IH, Sun W, Cleary PA, Lachin JM, Molitch ME, Steffes MW, et al. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med. [Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural]. 2011;365(25):2366–76.
114. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, et al. The effect of intensive treatment of diabetes on the development and progression of

long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977–86.
115. Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. Diabetes Control and Complications Trial. JAMA. [Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1998;280(2):140–6.
116. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. [Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural]. 2005;353(25):2643–53.
117. Maahs DM, Daniels SR, de Ferranti SD, Dichek HL, Flynn J, Goldstein BI, et al. Cardiovascular disease risk factors in youth with diabetes mellitus: a scientific statement from the American Heart Association. Circulation. 2014;130:1532–58.
118. Bjornstad P, Schäfer M, Truong U, Cree-Green M, Pyle L, Baumgartner A, Garcia Reyes Y, Maniatis A, Nayak S, Wadwa RP, Browne LP, Reusch JEB, Nadeau KJ. Metformin improves insulin sensitivity and vascular health in youth with type 1 diabetes mellitus. Circulation. 2018;138(25):2895–907.
119. Petrie JR, Chaturvedi N, Ford I, Hramiak I, Hughes AD, Jenkins AJ, E Klein B, Klein R, Ooi TC, Rossing P, Sattar N, Stehouwer CD, Colhoun HM, REMOVAL Trial Team. Metformin in adults with type 1 diabetes: design and methods of Reducing with MetfOrmin Vascular Adverse Lesions (REMOVAL): an international multicentre trial. Diabetes Obes Metab. 2017;19(4):509–16. https://doi.org/10.1111/dom.12840. Epub 2017 Feb 17.
120. Petrie JR, Chaturvedi N, Ford I, Brouwers M, Greenlaw N, Tillin T, et al. Cardiovascular and metabolic effects of metformin in patients with type 1 diabetes (REMOVAL): a double-blind, randomised, placebo-controlled trial. Lancet Diabetes Endocrinol. 2017;5(8):597–609.
121. Defronzo RA. Bromocriptine: a sympatholytic, d2-dopamine agonist for the treatment of type 2 diabetes. Diabetes Care. [Editorial Research Support, Non-U.S. Gov't Review]. 2011;34(4):789–94.
122. Bell DS. Why does quick-release bromocriptine decrease cardiac events? Diabetes Obes Metab. [Review]. 2011;13(10):880–4.
123. Bjornstad P, Skrtic M, Lytvyn Y, Maahs DM, Johnson RJ, Cherney DZ. The Gomez' equations and renal hemodynamic function in kidney disease research. Am J Physiol Renal Physiol. 2016;311(5):F967–75. https://doi.org/10.1152/ajprenal.00415.2016.
124. Prasad PV, Wei L, Dunkle E, Zhou Y, Sprague SM, Investigators C. Magnetic resonance imaging sensitive to hypoxia and fibrosis in a multi-center study of chronic kidney disease. Abstract accepted for American Society of Nephrology Kidney Week; 2016.
125. Skrtic M, Lytvyn Y, Bjornstad P, Reich H, Scholey JW, Yip P, et al. The influence of sex on hyperfiltration in patients with uncomplicated type 1 diabetes. Am J Physiol Renal Physiol. 2017;312:F599–606.
126. Li LP, Tan H, Thacker J, Li W, Zhou Y, Kohn O, et al. Evaluation of renal blood flow in chronic kidney disease using arterial spin labeling perfusion MRI. Kidney Int Rep. 2017;2:36–43.
127. Korner A, Eklof AC, Celsi G, Aperia A. Increased renal metabolism in diabetes. Mechanism and functional implications. Diabetes. 1994;43(5):629–33.
128. Bjornstad P, Cherney D, Maahs DM. Early diabetic nephropathy in type 1 diabetes: new insights. Curr Opin Endocrinol Diabetes Obes. 2014;21(4):279–86.
129. Collins AJ, Foley RN, Herzog C, Chavers B, Gilbertson D, Ishani A, et al. US Renal Data System 2010 Annual Data Report. Am J Kidney Dis. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;57(1 Suppl 1):A8, e1–526.

Part III

Unique Models of Insulin Resistance in Youth

Intrauterine Growth Restriction and Insulin Resistance

15

Sara E. Pinney and Rebecca A. Simmons

Introduction

Intrauterine growth restriction (IUGR) has been linked to the later development of diseases in adulthood, such as type 2 diabetes (T2D), obesity, insulin resistance (IR), and the metabolic syndrome [1]. An adverse intrauterine milieu can have long-term effects on the development of organs and tissues, including pancreatic β(beta)-cells, adipocytes, myocytes, and hepatocytes—all of which are important in maintaining glucose homeostasis. The incidence of T2D has rapidly increased over the past several decades and is now reaching epidemic proportions. In 2017, the US Centers for Disease Control (CDC) reported that 7.2% of the US population has been diagnosed with diabetes, with the vast majority having type 2 [2]. The same 2017 CDC report estimated that 33.9% of the US population has prediabetes (84.1 million people)—a condition diagnosed based on fasting glucose or hemoglobin A1c. Although the prediabetes assessment in the CDC survey was based on fasting glucose and hemoglobin A1c, it can be assumed that the vast majority of those with prediabetes also had insulin resistance [2]. Maintenance of glucose homeostasis is a direct reflection of the balance between the ability of the pancreatic β(beta)-cell to adequately secrete insulin in response to rising glucose levels and the ability of peripheral tissues—including skeletal muscle, liver, and adipose tissue—to respond to insulin appropriately. When tissues fail to respond to insulin appropriately, it is termed insulin resistance. Environmental contributions to the development of insulin resistance and T2D potentially include exposures such as a suboptimal in utero environment, low birth weight, obesity, inactivity, and advancing age [3].

Population-based studies have established a clear relationship between an adverse intrauterine milieu and the development of abnormalities in glucose homeostasis, insulin resistance, and, ultimately, T2D. Although the developmental origins of health and disease hypothesis are frequently attributed to David Barker and Nick Hales and their work describing the association between low birth weight and the outcomes of T2D, glucose intolerance, and cardiovascular disease, in 1994, Phillips, Barker, Hales et al. published one of the first descriptions showing an association between a lower ponderal index at birth, which is a marker of an adverse intrauterine environment, and insulin resistance in adult

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life [4]. The ponderal index is a calculated metric of birth weight (kg)/length (m)^3 and is a measure of leanness that is sensitive for both very short and very tall individuals. The authors selected 103 adults (53 men and 50 women, mean age 52 years) with no evidence of T2D from a previous study that assessed glucose tolerance with oral glucose tolerance testing (OGTT) and then performed insulin tolerance tests on those individuals. Insulin resistance was determined as the slope of the fall in blood glucose on a log scale between 3 and 15 minutes after insulin injection. Participants with a lower ponderal index at birth were found to have insulin resistance as adults and the insulin resistance worsened with increasing adult body mass index (BMI) (Fig. 15.1). It was noted that infants with low ponderal indices at birth also had reduced mid-arm circumference and lower muscle mass as infants. The authors hypothesized that the relationship between a low ponderal index at birth and insulin resistance later in life may be due to a change in skeletal muscle structure and the ability to transport insulin through peripheral muscle fibers to enable its action and that this effect persisted into adult life, leading to defects in glucose uptake and insulin signaling.

Studies from the population affected by the Dutch Hunger Winter provide another example

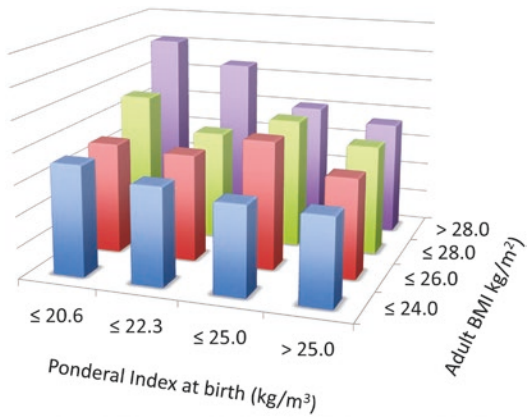

Fig. 15.1 Relationship between ponderal index at birth and adult body mass index (BMI), as described by Phillips et al. [4]. In the cohort of 103 adult men and women described by Phillips et al., those with the lowest ponderal index at birth had the highest BMI as adults

demonstrating the association between an aberrant intrauterine milieu and the development of insulin resistance in the offspring. Pregnant women exposed to the period in late World War II, during which daily caloric intake was limited to 400–800 kcal, delivered infants with lower birth weights. By age 50, these offspring had impaired glucose tolerance and insulin resistance compared to offspring who were in utero either the year before or after the famine [5, 6]. Ravelli et al. studied 702 men and women who were born between 1943 and 1947 in Amsterdam during World War II for whom they had access to detailed prenatal and postnatal health records at a mean age of 29 years [6]. Oral glucose tolerance testing was performed in participants who were divided into groups based on the gestational trimester during which their mothers were exposed to famine and were compared to offspring of women conceived before or after the famine period. Two-hour glucose concentrations were increased in offspring exposed to famine in the second and third trimesters of gestation. Offspring exposed to famine with lower birth weights had the highest glucose concentrations, and those who became obese later in life also had the most profound glucose intolerance. Prenatal exposure to famine was associated with increased fasting proinsulin and 2-hour insulin concentrations, suggesting that the offspring exposed to famine also had insulin resistance. The direct links between maternal malnutrition and the development of glucose intolerance and insulin resistance in the offspring provide strong evidence supporting the concept that poor nutrition in fetal life leads to permanent changes in the function of the pancreatic β(beta)-cell and in sensitivity of tissues to insulin, ultimately leading to insulin resistance later in life.

In addition to the landmark population-based studies from Hales, Barker, and Ravelli, there have been case-control cohort studies that aimed to better characterize the development of insulin resistance in offspring exposed to IUGR at different stages of postnatal life. Well-designed epidemiological studies characterizing the natural history of the metabolic consequences from IUGR are logistically challenging and expensive

to complete given the time period over which the participants must be followed in order to assess changes in glucose intolerance and insulin resistance throughout the lifespan.

Castanys-Munoz et al. performed a systematic review of population-based studies of infants born small for gestational age (SGA) published between 1994 and 2015, which investigated how postnatal weight gain and growth affected metabolic outcomes in offspring up to age 21 [7]. The authors reported that of 18 total studies published on 1119 children and adolescents, 12 studies found that postnatal weight gain was associated with insulin resistance and 6 studies did not detect such an association. There were no randomized control trials available for review. The 12 studies that reported an association between faster postnatal growth (measured as weight gain between birth and 8–21 years of age) and insulin resistance measured insulin resistance between ages 1 and 21 years of age using methods such as the homeostatic model assessment (HOMA), fasting insulin, OGTTs, intravenous glucose tolerance tests (IVGTTs), and clamp studies. Of the 13 studies that included both SGA participants and controls, 10 studies reported that the SGA offspring had significantly higher insulin resistance between the ages of 1 and 21 years. These results indicate that an adverse intrauterine environment followed by exposure to a nutrient-rich postnatal environment can lead to an increased rate of postnatal weight gain and result in the development of insulin resistance in the offspring.

Assessing insulin sensitivity can be more challenging in otherwise healthy children and adolescents than in adults since many of the techniques require multiple blood samples and intravenous sampling and are considered invasive. Studies of insulin sensitivity and resistance in children frequently use less sensitive proxies of insulin resistance/sensitivity rather than the gold standard techniques, such as the hyperinsulinemic–euglycemic clamp, due to the invasive nature of these procedures. The use of less-sensitive techniques, in addition to the fact that the metabolic abnormalities from intrauterine growth restriction are typically less profound at younger ages, contributes to the significance of the findings in these studies and provides additional evidence that there are underlying metabolic abnormalities in IUGR-exposed offspring that are due to in utero programming.

Jaquet et al. performed a case-control study investigating the effects of IUGR on glucose homeostasis in 24-year-old adult offspring using euglycemic–hyperinsulinemic clamp measurement [8]. In this study, IUGR subjects had decreased insulin-stimulated glucose uptake at 24 years of age without a major impairment of insulin secretion, and this relationship persisted after adjustment for BMI. IUGR offspring had 5% more body fat as young adults than their normal-birth-weight peers. In addition, low total body glucose uptake in adults born SGA was associated with a lesser degree of free fatty acid (FFA) suppression by insulin in adipose tissue, suggesting a functional role for adipose tissue at the early stages of the development of insulin resistance.

Another study by Leunissen et al. investigated the relationship between low birth weight and postnatal catchup growth and an increased risk of T2D in adult life. The authors studied 136 young adults aged 18–24 years who were divided into four groups: (1) individuals who were born SGA and had short stature as young adults, (2) individuals who were SGA and had catchup growth and had normal height as adults, (3) individuals born appropriate for gestational age (AGA) with idiopathic short stature as adults, and (4) individuals who were born AGA and had normal adult stature. Insulin sensitivity and disposition indices were measured using a frequently sampled IVGTT. They found that fat mass at birth was the only significant predictor of insulin sensitivity in young adulthood, whereas birth length and birth weight were not significant contributors to alterations in insulin sensitivity at age 18–24 years. After correction for age, sex, and adult body size, insulin sensitivity in adulthood was much lower in subjects born SGA with catchup growth compared with controls. None of the variables had a significant influence on disposition index, and there were no differences in disposition index between groups.

Another cohort study from Denmark studied the impact of early infant weight gain in SGA infants on glucose metabolism and cardiovascular risk factors in adolescence. Fabricius-Bjerre et al. studied 87 adolescents with a mean age of 17.6 years, including 30 former term SGA infants and 57 former term AGA infants [9]. The authors performed modified IVGTTs to measure glucose intolerance and insulin resistance and assessed body composition via dual-energy X-ray absorptiometry (DEXA). They found that accelerated growth during the first 3 months of age led to an increase in markers of insulin resistance in adolescence, particularly increased basal insulin levels and increased HOMA-IR.

There have been several studies looking for markers of insulin resistance detected in early childhood from former SGA infants. Reinehr et al. studied a cohort of 803 overweight children with a mean age of 11 years, of whom 35 were born SGA [10]. OGTTs were performed in all 35 SGA children, 147 AGA children, and 8 large for gestational age (LGA) children. For the purposes of this study, the authors defined pediatric features of the metabolic syndrome according to the definition of Weiss, including BMI >95th %ile for age plus 3 of the following: (blood pressure >95th % for height, age, and sex; triglycerides >95th %ile; and HDL <5th %ile). The authors found that after adjusting for age, sex, pubertal stage, and BMI-SDS score, former SGA status was significantly associated with increased blood pressure, insulin, and 2-hour glucose. Forty percent of the former SGA children met criteria for the metabolic syndrome, while only 17% of the AGA children met these criteria, leading to an odds ratio of developing metabolic syndrome in childhood in those born SGA of 4.08 (95% CI 1.48–11.22).

Another study that characterized the markers of glucose intolerance and insulin resistance in young children who were born SGA was published by Ibanez et al. [11]. The authors of this study sought to describe the timing of the development of central obesity and insulin resistance in a longitudinal cohort of children aged 2–4 years, of whom 22 were born with low birth weight and 29 had normal birth weight. They measured body composition by DEXA and insulin sensitivity was estimated by HOMA. There were no differences in mean height, weight, or BMI at ages 2, 3, and 4 years. At 2 years of age, the authors reported that SGA children were more insulin sensitive than AGA children. Between ages 2 and 4 years, despite similar gains in weight and BMI, SGA children gained more abdominal fat and body adiposity and less lean body mass than AGA children. At age 4 years, SGA children had greater adiposity, higher fasting insulin, and lower insulin sensitivity (as determined by HOMA-IR) than AGA children (p = 0.01–0.0004). The authors note that changes in body composition between ages 2 and 4 in SGA children were accompanied by an increase in insulin resistance and that accumulation of central and visceral adipose tissue can lead to insulin resistance, in part due to increased lipolysis and release of fatty acids. In addition, early hyperinsulinemia is a pancreatic β(beta)-cell compensatory response to muscle-specific insulin resistance, which establishes a positive feedback loop promoting additional central and peripheral adipose deposition, but further mechanistic studies are needed.

Finally, Soto and colleagues assessed insulin sensitivity and secretion in a cohort of 108 one-year-old infants, including 85 SGA and 23 AGA birth weights [12]. The SGA infants were further stratified according to catchup growth in weight or length during the first year of life. Measurements included serum lipids, fasting insulin levels, and calculated measurements of insulin sensitivity and insulin secretion during IVGTT. Fasting insulin was significantly higher in SGA infants who demonstrated catchup growth in weight compared to SGA infants who did not show evidence of weight catchup growth and AGA infants (mean ± SD, 32.6 ± 4.6 vs. 14.9 ± 2.3 vs. 21.4 ± 3.3 pM, respectively; p = 0.04). Insulin secretion and sensitivity were closely linked to patterns of rapid weight catchup growth and length catchup growth during early postnatal life. Fasting insulin sensitivity was directly related to weight catchup growth and current BMI, whereas insulin secretion was more directly related to length catchup growth, despite lower weight, BMI, and length (0.29 ± 0.19 nM vs. 0.40 V 0.07 nM; $p < 0.05$) compared with

AGA infants. These data suggest that the mechanisms linking adverse intrauterine environment and offspring insulin resistance are present and functioning as early as 1 year after birth.

The majority of the participants in the studies described above were from Northern Europe, but there are additional studies reporting alterations in glucose homeostasis and markers of insulin resistance in SGA children and adolescents in other populations around the world. Deng et al. aimed to determine the impact of height catchup growth and weight catchup growth on the development of insulin resistance in a Chinese population of children with ages ranging from 1.5 to 11 years with a history of SGA at birth [13]. They studied 30 children born SGA and catchup growth, 37 children born SGA and no catchup growth, and 42 children born AGA with normal heights. There were no differences in fasting glucose concentrations. HOMA-IR was significantly increased in SGA children with catchup growth compared with SGA children without catchup growth and AGA children (p = 0.002 and 0.036, respectively), and these differences were maintained after adjustment for sex, age, and BMI. SGA children with catchup growth had signs of insulin resistance, as indicated by increased fasting insulin concentrations and HOMA-IR, but did not have increases in β(beta)-cell function, as calculated by HOMA%, suggesting that although increased β(beta)-cell function can initially compensate for peripheral insulin resistance in SGA offspring, it may not be able to keep up with demand as the child ages. The authors conclude that Chinese SGA children who demonstrate catchup growth in height and high BMI are prone to the development of insulin resistance.

Bavdekar et al. studied a cohort of 8-year-old children (n = 477) to determine the relationships among birth weight, insulin resistance, and hyperlipidemia in childhood. After adjustment for current weight, age, and sex, a history of low birth weight in children was significantly associated with elevated fasting plasma insulin concentrations and HOMA-IR, increased stimulated glucose and insulin concentrations, and increased plasma total and low-density lipoprotein (LDL) cholesterol concentrations at 8 years of age. The most affected children were those with a history of low birth weight and high fat mass at age 8. The most insulin-resistant children were those with tall stature who had short parents. The authors note that, like other population-based studies in the United Kingdom, Europe, and India, they found no association between birth weight and insulin secretion at this age, indicating that IUGR appears to have more significant effects on peripheral insulin resistance at age 8 and that this effect precedes any effect on insulin secretion.

Although the epidemiological studies described above show clear associations between the adverse intrauterine milieu and the development of insulin resistance later in life, they provide little insight into the mechanisms responsible for the metabolic consequences in the adult offspring. The abnormal intrauterine milieu associated with IUGR limits the supply of critical substrates and hormones to the fetus and affects fetal development by permanently modifying gene expression and function of susceptible cells in the developing liver, visceral and subcutaneous fat tissue, and muscle.

Next, we will review the various animal models of IUGR and their specific effects on metabolic gene expression. In addition, we will review recent work aiming to better understand the molecular mechanisms in both animal models and human tissue samples that contribute directly to the malprogramming of gene expression during the critical fetal and neonatal periods, which may ultimately represent a critical time for intervention.

Experimental Models of Intrauterine Growth Restriction

Animal models based on an outbred genetic background offer an opportunity to examine the effects of environmental insults on gene expression during gestation or early postnatal life. Established models of IUGR have been developed in rodents, sheep, pigs, and nonhuman primates; however, rodents are often used for

models of fetal programming due to their shorter lifespan and shorter gestational periods, allowing for detailed study of the long-term effects resulting from an in utero exposure. The most common rodent models used for inducing IUGR to study the development of T2D are those that employ protein-calorie restriction, total calorie restriction, glucocorticoid exposure, or induction of uteroplacental insufficiency in the pregnant rodent. Although these models all have a specific pancreatic β(beta)-cell phenotype, for the purposes of this review we will focus on the effects in the liver, muscle, and adipose tissue and the outcome of insulin resistance in the offspring (Table 15.1).

Low-Protein Model

Initially established by Snoeck et al., offspring born to protein-restricted dams have lower birth weights and develop age-dependent glucose intolerance that progresses to overt diabetes in adult life [14]. In this model, dams are fed a diet containing 8% protein throughout gestation and lactation (LP), and offspring are compared to offspring of control dams fed an isocaloric diet containing 20% protein. There are no effects on conception rates or litter size, but placental and offspring birth weights are consistently reduced in this model. Pups of mothers on the LP diet have 5.5% lower birth weights than controls [14, 15].

In general, male offspring in the LP model have altered insulin secretory capacity and reduced β(beta)-cell mass mediated by a reduction in β(beta)-cell proliferation rate and an increase in apoptosis [14–20]. The 15-month-old male offspring of mothers fed an LP diet have hyperinsulinemia that presents prior to hyperglycemia [21]. At 17 months of age, male offspring have fasting hyperglycemia and postprandial hyperinsulinemia. Female offspring at 21 months of age have elevated fasting insulin concentrations compared to controls but no fasting hyperglycemia. Glucose tolerance during IVGTT is comparable

Table 15.1 Rodent models of intrauterine growth restriction (IUGR): molecular effects on insulin resistance

	Low protein	Total calorie restriction	Maternal glucocorticoid exposure	Uteroplacental insufficiency
IUGR-induced molecular changes leading to insulin resistance:	Reduced glucagon receptors in the liver Increased hepatic PEPCK activity and gluconeogenesis Increased expression of hepatic insulin receptors Reduced insulin-stimulated glucose uptake in skeletal muscle and adipose tissue Decreased expression of insulin-signaling proteins (PKCζ[zeta], p85α[alpha], p100β[beta]), and GLUT4 in skeletal muscle Decreased DNA methylation at GR and PPAR-α(alpha) promoters corresponding with increased hepatic mRNA expression persisting in F1 and F2 offspring	Peripheral insulin resistance related to aberrant glucose transport into insulin-sensitive tissues including skeletal muscle and adipose tissue Decreased GLUT4 expression: insulin-responsive glucose transporter in skeletal muscle Epigenetic change: decreased H3K14 acetylation; increased H3K9 at GLUT4 promoter	Increased PEPCK mRNA expression in liver Increased GR mRNA expression in liver Elevated postprandial insulin levels and impaired glucose tolerance	PEPCK and G6P mRNA levels are increased in the liver IRS2 and Akt-2 phosphorylation are blunted resulting in decreased hepatic insulin signaling Increased hepatic glucose production at baseline Decreased ability of insulin to suppress hepatic glucose production Impaired mitochondrial oxidative phosphorylation in the liver Treatment with Exendin-4, a GLP-1 agonist prevented the development of hepatic insulin resistance and oxidative stress in the UPI liver and reversed hepatic insulin-signaling defects

Abbreviations: *PEPCK* phosphoenolpyruvate carboxykinase, *UPI* uteroplacental insufficiency

in the female offspring and controls, but plasma insulin area under the curve is 1.9 times higher in the female offspring of LP fed dams [21].

Maternal protein restriction has profound long-term effects on glucose homeostasis and insulin signaling in offspring in the liver. At 3 months of age, there is a reduction in the expression of glucagon receptors [22], an associated increase in the activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK), and reduced activity of glycolytic glucokinase [23, 24], all resulting in increased hepatic glucose production. Increased hepatic glucose production persists at age 11 months despite the fact that the pups were fed a standard diet [23, 25]. At 3 months of age, livers from male offspring of protein-restricted dams displayed evidence of hepatic insulin resistance despite an increase in the expression of insulin receptors [22]. In addition, there is a decrease in circulating insulin-like growth factor 1 (IGF-1) concentration and a decrease in IGF-1 mRNA expression in hepatocytes derived from LP fetuses [26].

In the LP male offspring at 15 months, insulin-stimulated glucose uptake is reduced in both muscle and adipose tissue [27, 28]. Maternal protein restriction results in reduced skeletal muscle mass, but young adult LP offspring have improved insulin sensitivity at the level of the skeletal muscle [23], likely due to the increased number of insulin receptors expressed in growth-restricted skeletal muscle. At 15 months of age, insulin-stimulated glucose uptake was not detectable in muscle from LP-exposed offspring [27]. Similar findings were seen in adipocytes isolated from LP-exposed offspring at 15 months of age where insulin had little effect stimulating glucose uptake [28]. In both LP-exposed skeletal muscle and adipocytes, there were no changes in insulin receptor expression, suggesting that the defect was downstream of the insulin receptor. Ozanne et al. showed decreased expression of a number of insulin-signaling proteins in skeletal muscle in low-birth-weight human subjects and low-birth-weight rats born to LP dams [27, 29]. Low-birth-weight human males had decreased expression of protein kinase c (PKC)ζ(zeta), p85α(alpha), p110β(beta), and GLUT4 at age 19 years, while the 15-month-old male offspring of rats fed a low-protein diet showed decreased PKCζ(zeta), GLUT4, and p85 [27]. The similarity of the protein expression profile of the men with low birth weight and the rats suggests that the low-protein model of IUGR is appropriate for studying the development of peripheral insulin-signaling defects that may lead to insulin resistance [29].

Adipose tissue of LP-exposed offspring has reduced expression of the insulin-signaling protein p110β(beta) and insulin-responsive substrate 1 (IRS-1) and increased expression of pro-inflammatory cytokines interleukin (IL)-6 and IL1β(beta), which also compromise insulin signaling [30]. Tarry-Adkins et al. demonstrated that dietary supplementation with coenzyme Q_{10}, an anti-inflammatory agent, can prevent changes in insulin-signaling protein expression and mediate the development of insulin resistance in a model of IUGR [30].

Total Calorie Restriction Model

The total calorie restriction model of IUGR approximates a generalized poor nutritional state during pregnancy and the effects on the offspring. Three-month-old pregnant rats had food intake limited to 50% of ad-lib during pregnancy and lactation, which resulted in offspring with significantly lower birth weights who continued to remain small in adulthood [31]. In this model, there is no difference in litter size or in the male-to-female ratio between control and IUGR offspring. When calorie restriction is limited to 50% during gestation only, the offspring are born with reduced birth weights, but their body weights are increased and are greater than control offspring by postnatal day 20 [31]. When the time period during which the maternal nutritional deprivation (50% restriction) is limited to the 7–10 days of gestation, the pups were born small; some investigators found impaired β(beta)-cell development and reduced plasma glucose and insulin concentrations during the neonatal period [19, 32], while others found no statistical difference in pancreas weight, β(beta)-cell fractional area,

mass, replication rate, or apoptosis in the neonate [33]. At 21 days, the male pups had a 15% reduction in body weight and a 30% reduction in pancreatic weight, while there was no difference in female pups at this age [33]. There was a 50% reduction in absolute β(beta)-cell mass and a 30% reduction in β(beta)-cell replication but no change in rates of apoptosis in offspring at 21 days. At 3–4 months, the offspring continued to have reduced β(beta)-cell mass along with altered insulin response and sensitivity at 3–4 months of age [32, 34]. At 8 months of age, the reduction of β(beta)-cell mass was still apparent, and β(beta)-cells had a 40% lower insulin content compared to controls [32]. The offspring were not overtly hyperglycemic but did have decreased fasting insulin concentrations [32]. Offspring of dams exposed to total calorie restriction also develop peripheral insulin resistance related to aberrant glucose transport into insulin-sensitive tissues [35].

Maternal Glucocorticoid Exposure Model

Maternal treatment with high-dose glucocorticoids during the last trimester is used in obstetric practice to accelerate the rate of maturation of fetal lungs and other organs when there is concern about premature delivery. Daily treatment of pregnant rats with dexamethasone during the last week of pregnancy retarded fetal growth and led to a 10% decrease in birth weight and growth restriction that persisted through adulthood, while earlier gestational exposures to dexamethasone did not affect birth weight [36]. At 6 months of age, the offspring of rats exposed to dexamethasone in late gestation had impaired glucose tolerance and postprandial insulin resistance. Fasting insulin concentrations were not significantly different from controls [36]. At 8 months of age, hepatic expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucocorticoid receptor (GR) mRNA were increased. These observations suggest that excessive glucocorticoid exposure in late pregnancy predisposes the offspring to glucose intolerance and insulin resistance.

Uteroplacental Insufficiency Model

Uteroplacental insufficiency (UPI) is one of the most common causes of IUGR worldwide and is caused by disorders such as preeclampsia, maternal smoking, and abnormalities of uteroplacental development. UPI restricts the supply of crucial nutrients to the fetus, thereby limiting fetal growth. In one model of UPI in Sprague Dawley rats, pregnant dams undergo bilateral uterine artery ligation on gestational day 18 (term is 22) and deliver spontaneously [37]. UPI fetal rats have critical metabolic features characteristic of growth-retarded human fetuses: decreased glucose, insulin, IGF-1, amino acids, and oxygen [37–40]. Birth weights of UPI offspring are significantly lower than those of controls and UPI-affected offspring weigh less than control rats until approximately 7 weeks of age. Between 7 and 10 weeks of age, the growth rate of UPI offspring accelerates and weights of UPI rats surpass that of controls; by 26 weeks, UPI rats are obese [37]. There are no significant differences in blood glucose and insulin concentrations at 1 week of age. However, by 7–10 weeks of age, IUGR rats develop mild fasting hyperglycemia and hyperinsulinemia. UPI male offspring are glucose-intolerant and insulin-resistant by 15 weeks of age. First-phase insulin secretion in response to glucose is impaired by 7 weeks of age in UPI males, before the onset of hyperglycemia. By 6 months of age, UPI offspring developed diabetes, which was similar to type 2 diabetes seen in humans with progressive dysfunction of insulin secretion and insulin action [37].

Multiple studies have shown that intrauterine growth retardation is associated with increased oxidative stress in the human fetus [41, 42]. A key adaption enabling the fetus to survive in a limited energy environment may be the reprogramming of mitochondrial function, but this can have deleterious effects in cells with high energy requirements. Reactive oxygen species production and oxidative stress gradually increase in islets from offspring exposed to UPI [43]. ATP production is impaired and activities of complex I and II of the electron transport chain progressively declined in UPI islets [43]. There is decreased expression of

mitochondrially encoded genes in UPI islets, and mitochondrial dysfunction resulted in impaired insulin secretion [43]. In addition, UPI hepatocytes had lower rates of oxidation of pyruvate, glutamate, succinate, and alpha-ketoglutarate at 28 days of life and increased levels of manganese superoxide dismutase, suggesting that UPI impairs mitochondrial oxidative phosphorylation in the liver while also inducing an antioxidant response [44]. In UPI skeletal muscle, glycogen content and insulin-stimulated 2-deoxyglucose uptake were both reduced at 3–6 months of age. In addition, UPI skeletal muscle mitochondria have decreased pyruvate oxidation, leading to decreased ATP production and, eventually, decreased energy-dependent recruitment of GLUT4 to the cell surface of UPI myocytes. Decreased GLUT4 expression results in a decrease in skeletal muscle glucose uptake in the UPI rat [45]. The effects of UPI on mitochondria described above all contribute to hyperglycemia and insulin resistance that ultimately lead to the development of T2D.

Studies with the UPI model suggest that the aberrant intrauterine milieu permanently impairs insulin signaling in the liver, resulting in an augmentation gluconeogenesis [46]. At age 7–9 weeks, male offspring exposed to UPI have increased hepatic glucose production (HGP) at baseline, and insulin suppression of HGP was impaired. IRS2 and Akt-2 phosphorylation are significantly blunted in the UPI offspring, and PEPCK and glucose-6-phosphate mRNA levels are increased threefold [46]. These processes occur early in the UPI offspring's adult life, before the onset of hyperglycemia, indicating that UPI causes a primary defect leading to hepatic insulin resistance that contributes to the eventual development of overt hyperglycemia along with the β(beta)-cell defects described in this model [37, 43]. Follow-up studies demonstrated that treatment of the UPI offspring with exendin-4—a glucagon-like protein-1 agonist—during the first week of life permanently prevented the development of hepatic insulin resistance and oxidative stress in the UPI liver, while also permanently reversing hepatic insulin-signaling defects [47], preventing the development of T2D in the offspring.

Epigenetic Modifications

One mechanism by which environmental perturbations leading to IUGR can result in hyperinsulinemia and diabetes is by altering gene expression through epigenetic modifications. Epigenetic modifications are defined as mitotically heritable alterations in gene expression that are not related to changes in DNA sequence. Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene expression from one generation of cells to the next [48–52]. There are several distinct mechanisms through which epigenetic information can be inherited, including histone modifications, DNA methylation, and noncoding RNAs.

Histone Modifications

The amino termini of histone proteins can be modified by acetylation, methylation, sumoylation, phosphorylation, glycosylation, and ADP-ribosylation. Methylation of histones is associated with both transcription repression and activation. Lysine residues can be mono-, di-, or trimethylated in vivo, providing an additional level of regulation [48]. These modifications impact gene expression by altering chromatin structure or by recruiting histone modifiers.

DNA Methylation

DNA methylation occurs when DNA methyltransferase adds a methyl group at the C5 position of cytosine. Approximately 70% of CpG dinucleotides in human DNA are constitutively methylated, whereas most of the unmethylated CpGs are located in CpG islands, CG-rich sequences located near coding sequences that serve as promoters for their associated genes. Approximately half of mammalian genes have CpG islands. The methylation status of CpG islands within promoter sequences works as an essential regulatory element by modifying the binding affinity of transcription factors to

DNA-binding sites. In normal cells, most CpG islands remain unmethylated; however, under circumstances such as cancer and oxidative stress, they can become methylated de novo. This aberrant methylation is accompanied by local changes in histone modification and chromatin structure, such that the CpG island and its embedded promoter take on a repressed conformation that is incompatible with gene transcription. DNA methylation is commonly associated with gene silencing and contributes to X-chromosomal inactivation, genomic imprinting, and transcriptional regulation of tissue-specific genes during cellular differentiation [51].

Chromatin Remodeling in Liver and Skeletal Muscle in Intrauterine Growth Restriction Offspring

A reduction in glucose transport in muscle is a central basis for insulin resistance in IUGR offspring [29, 53]. Glucose transport, a rate-limiting step in glucose utilization under normal physiological circumstances, occurs by facilitated diffusion [54]. This process is mediated by a family of structurally related membrane-spanning glycoproteins, termed the facilitative glucose transporters (GLUT; Slc2 family of transport proteins; reviewed in [55]). Of the isoforms cloned to date, GLUT4 is the major insulin-responsive isoform expressed in insulin-sensitive tissues, such as skeletal muscle, adipose tissue, and cardiac muscle [55]. In the total calorie restriction model of IUGR, epigenetic marks associated with gene silencing have been described at the promoter of the glucose transporter 4 (*glut4*) in skeletal muscle [35]. The promoter region of *glut4* has been well characterized, and disruption of the myocyte enhancer factor 2 (MEF2)-binding site ablates tissue-specific *glut4* expression in transgenic mice [55]. Myogenic differentiation (MyoD) on the other hand is responsible for *glut4* expression in vitro during myoblast to myocyte differentiation [56]. MyoD binding with MEF2 and transcription-associated protein α(alpha)1 (TRα[alpha]1) spans the 502- to 420-bp region of the *glut4* gene in skeletal muscle. These two proteins synergistically enhance skeletal muscle *glut4* transcription and gene expression [56].

Raychaudhuri and colleagues demonstrated that IUGR is associated with an increase in MEF2D (a form of MEF2 that acts as an inhibitor) and a decrease in both MEF2A (a form of MEF2 that acts as an activator) and MyoD (a coactivator) binding to the *glut4* promoter in skeletal muscle [35]. Interestingly, no differential methylation of these three CpG clusters in the *glut4* promoter was found. Furthermore, they found increased DNA methyltransferase (DNMT) binding at the *glut4* gene at different ages: DNA methyltransferase 1 (DNMT1) postnatally and DNMT3a and DNMT3b in adults [35]. The increase in DNMT binding was associated with exposure to increased methyl CpG-binding protein 2 (MeCP2) concentrations. Covalent modifications of the histone code consisted of histone 3 lysine 14 (H3K14) deacetylation mediated by recruitment of HDAC1 and enhanced association of histone deacetylase 4 (HDAC4) enzymes. This set the stage for Suv39H1 methylase-mediated di-methylation of H3K9 and increased recruitment of heterochromatin protein 1, which partially inactivates postnatal and adult IUGR *glut4* gene transcription. These studies demonstrate that perinatal nutrient restriction resulting in IUGR leads to silencing histone modifications in skeletal muscle, which in turn directly decrease *glut4* gene expression. These events effectively create a metabolic knockdown of *glut4*, contributing to the T2D phenotype [35]. Hence, these studies show that histone modifications can be inherited stably in a calorie-restricted model of IUGR, mimicking the 1944 Dutch famine experience.

In the LP model of IUGR, Lillycrop et al. investigated the effect of unbalanced maternal nutrition on the methylation status of the peroxisomal proliferator-activated receptor (PPAR) and glucocorticoid receptor (GR) genes in the liver of IUGR rats and the relationship to hepatic gene expression. They found that in 1-month-old IUGR offspring, there was a 21% reduction in PPAR-α(alpha) promoter methylation in IUGR rat liver that corresponded to a tenfold increase in PPAR-α(alpha) gene expression [57]. In addi-

tion, they found that the GR promoter had a 23% decrease in methylation in livers from the protein-restricted IUGR offspring at 1 month of age, which was associated with a threefold increase in hepatic GR expression. The investigators further demonstrated that the decrease in PPAR-α(alpha) and GR promoter methylation in the protein-restricted offspring persisted into the F1 and F2 generations [58]. Finally, a subsequent study reported that the decreases in GC and PPAR-α(alpha) promoter methylation in the liver were preventable by providing folic acid supplementation to the protein-restricted dams [59].

Radford et al. sought to assess the role of imprinted genes in the developmental origins of health and disease [60]. It has been proposed that imprinted genes—a class of functionally monoallelic genes critical for growth and metabolic development—are uniquely susceptible to environmental change and that perturbations of the epigenetic regulation of the imprinting control regions of such genes may play a role the development of adult disease after an early life insult. The authors analyzed the hepatic expression of imprinted genes using microarray and quantitative polymerase chain reaction (PCR) in two affected generations of IUGR induced by total calorie restriction of the dam [60]. They found that imprinted genes as a class were not particularly susceptible to expression changes in the liver following in utero undernutrition. In addition, imprinted genes in the developing germline were not affected, and imprinted genes were largely stable in the second generation [60].

Brøns et al. studied men born with low birth weight (LBW) who were exposed to 5 days of a high-fat diet to investigate whether the presumed exposure to an altered intrauterine milieu programmed the ability to adapt to the effect of a high-fat diet later in life [61]. The authors showed that DNA methylation and gene expression of peroxisome proliferator-activated receptor γ(gamma), coactivator 1-α(alpha) (*PPARGC1A*) in human muscle were influenced by both exposure to a high-fat diet and low birth weight [61]. They studied 20 healthy young men with a history of LBW and 26 matched control men with normal birth weight after 5 days of high-fat, high-calorie diet (50% extra calories and 60% fat) and reported that LBW men had peripheral insulin resistance and reduced *PPARGC1A* and coregulated oxidative phosphorylation (OXPHOS) gene expression in muscle. *PPARGC1A* promoter methylation was significantly higher in LBW subjects during the control diet. After 5 days of high-fat feeding, the control subjects had a significant increase in *PPARGC1A* promoter methylation compared to baseline, but the LBW subjects did not show any difference [61]. The increase in *PPARGC1A* promoter methylation after high-fat feeding in the control men was reversible and did not correlate with mRNA expression. Although this study was the first to provide experimental support in humans that DNA methylation induced by overfeeding is reversible, the extent to which the persistent increased *PPARGC1A* promoter methylation in LBW subjects contributes to the decreases in mRNA expression of *PPARGC1A* and OXPHOS genes still needs to be defined.

Finally, Einstein et al. mapped genome-wide changes in DNA methylation in CD34 hematopoietic stem cells isolated from umbilical cord blood in IUGR (n = 5) and control offspring (n = 5) to determine if IUGR altered genome-wide DNA methylation [62]. Using a genome-wide DNA methylation assay, they identified 56 genetic loci with significant changes in DNA methylation between IUGR CD34 cells and controls. Functional relationships between genes containing loci with significant changes in DNA methylation were mapped using Ingenuity Pathway Analysis, and the resulting networks centered around *HNF4A*, a pancreatic transcription factor implicated in MODY1 and T2D. Additionally, four previously described imprinted genes associated with IUGR (IGF2 differentially methylated region [DMR], H19 DMR and promoter region, and KCNQ1 DMR) did not show any significant changes in DNA methylation in CD34 cells in this study. The authors conclude that the DNA methylation changes observed in the CD34 hematopoietic stem cells are indicative of the epigenetic dysregulation associated with intrauterine growth restriction and represent a form of cellular memory of the aberrant intrauterine environment.

Genome-Wide Association Studies Linking Birth Weight and Type 2 Diabetes

The studies and models described previously demonstrate a strong association between an aberrant intrauterine environment and the development of insulin resistance later in life. There is also evidence that specific genes may have effects on both birth weight and T2D. Genome-wide association studies (GWAS) have identified more than 100 genetic loci associated with T2D, suggesting that T2D is a complex genetic disorder influenced by interactions between multiple genetic susceptibility loci and environmental perturbations [63, 64]. Additionally, a recent GWAS identified 60 loci associated with birth weight and concluded that approximately 15% of birth weight variance is due to a genetic effect. The same study described a strong inverse correlation between birth weight and later life cardiometabolic disease, indicating that there are appreciable genetic components influencing the intrauterine environment [65, 66]. However, since the prevalence of T2D continues to increase worldwide, factors contributing to this increase cannot be attributed to genetics alone [2]. In addition, although there is a strong correlation between fetal and maternal genotype, it is not clear if the reported inverse correlation between birth weight and T2D actually reflects maternal genetic effects on birth weight that also influence the intrauterine environment [65].

Conclusion

The four rodent models of IUGR described previously demonstrate that exposure to an adverse environment in utero results in fetal adaptations that ultimately have consequences leading to abnormalities in glucose homeostasis and insulin metabolism in the adult animal. Such fetal adaptations lead to changes in gene expression, but the specific mechanisms that mediate these processes are still being investigated and likely involve a combination of environmental effects, genetic susceptibility, and epigenetic modifications (Fig. 15.2). IUGR-induced changes in the

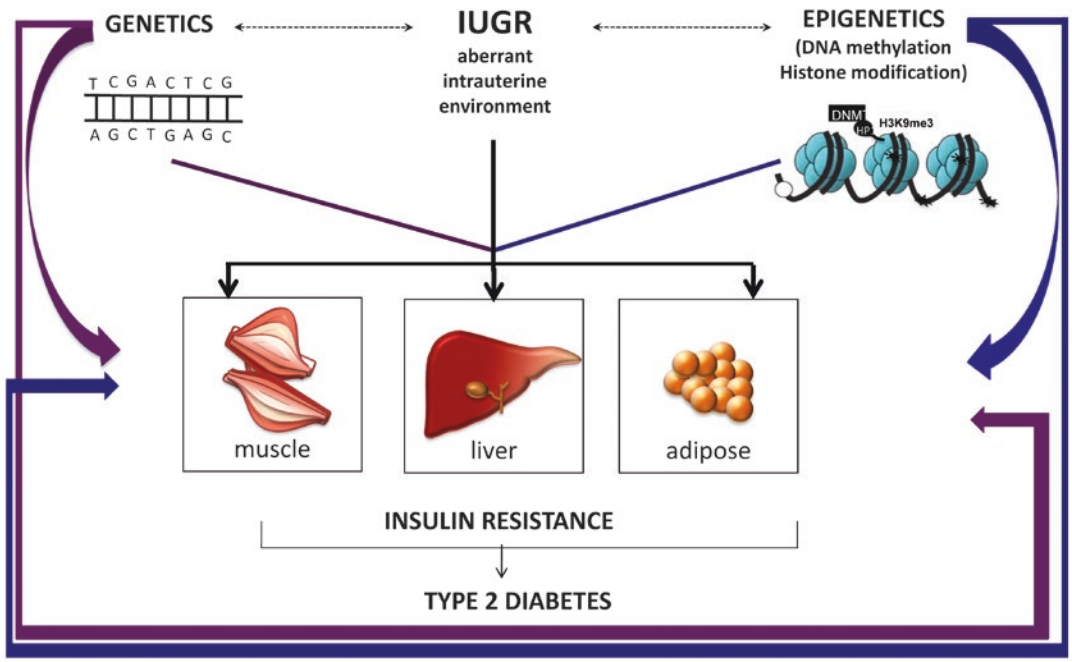

Fig. 15.2 There is a complex interaction between IUGR, genetics, and epigenetics resulting in insulin resistance in liver, skeletal muscle, and adipose tissue. IUGR in combination with epigenetic modifications (DNA methylation and histone modifications) and genetic susceptibility can lead to insulin resistance at peripheral tissues such as liver, muscle, and adipose tissue. A combination of impaired insulin secretion and insulin resistance underlies the pathophysiology of type 2 diabetes

epigenetic regulation of gene expression is one mechanism by which the in utero insult of IUGR can lead to the development of insulin resistance in adult offspring. *Hnf4α(alpha), PPAR-α(alpha)*, and the *GC* receptor all appear to be susceptible to epigenetic modifications induced by IUGR that can lead to abnormal expression. However, there remains a great deal of work to be done to determine additional mechanisms involved in altering gene expression at specific loci and on a genome-wide basis in IUGR that ultimately lead to the development of insulin resistance and T2D.

References

1. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia. 1993;36(1):62–7.
2. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017.
3. Jin W, Patti ME. Genetic determinants and molecular pathways in the pathogenesis of type 2 diabetes. Clin Sci (Lond). 2009;116(2):99–111.
4. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. Diabetologia. 1994;37(2):150–4.
5. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. Am J Clin Nutr. 1999;70(5):811–6.
6. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to famine. Lancet. 1998;351(9097):173–7.
7. Castanys-Munoz E, Kennedy K, Castaneda-Gutierrez E, Forsyth S, Godfrey KM, Koletzko B, et al. Systematic review indicates postnatal growth in term infants born small-for-gestational-age being associated with later neurocognitive and metabolic outcomes. Acta Paediatr. 2017;106(8):1230–8.
8. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C. Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab. 2000;85(4):1401–6.
9. Fabricius-Bjerre S, Jensen RB, Faerch K, Larsen T, Molgaard C, Michaelsen KF, et al. Impact of birth weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. PLoS One. 2011;6(6):e20595.
10. Reinehr T, Kleber M, Toschke AM. Small for gestational age status is associated with metabolic syndrome in overweight children. Eur J Endocrinol. 2009;160(4):579–84.
11. Ibanez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91(6):2153–8.
12. Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, et al. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. J Clin Endocrinol Metab. 2003;88(8):3645–50.
13. Deng HZ, Deng H, Su Z, Li YH, Ma HM, Chen HS, et al. Insulin resistance and adiponectin levels are associated with height catch-up growth in prepubertal Chinese individuals born small for gestational age. Nutr Metab (Lond). 2012;9(1):107.
14. Snoeck A, Remacle C, Reusens B, Hoet JJ. Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. Biol Neonate. 1990;57(2):107–18.
15. Dahri S, Snoeck A, Reusens-Billen B, Remacle C, Hoet JJ. Islet function in offspring of mothers on low-protein diet during gestation. Diabetes. 1991;40(Suppl 2):115–20.
16. Dahri S, Reusens B, Remacle C, Hoet JJ. Nutritional influences on pancreatic development and potential links with non-insulin-dependent diabetes. Proc Nutr Soc. 1995;54(2):345–56.
17. Wilson MR, Hughes SJ. The effect of maternal protein deficiency during pregnancy and lactation on glucose tolerance and pancreatic islet function in adult rat offspring. J Endocrinol. 1997;154(1):177–85.
18. Berney DM, Desai M, Palmer DJ, Greenwald S, Brown A, Hales CN, et al. The effects of maternal protein deprivation on the fetal rat pancreas: major structural changes and their recuperation. J Pathol. 1997;183(1):109–15.
19. Bertin E, Gangnerau MN, Bellon G, Bailbe D, Arbelot De Vacqueur A, Portha B. Development of beta-cell mass in fetuses of rats deprived of protein and/or energy in last trimester of pregnancy. Am J Physiol Regul Integr Comp Physiol. 2002;283(3):R623–30.
20. Boujendar S, Reusens B, Merezak S, Ahn MT, Arany E, Hill D, et al. Taurine supplementation to a low protein diet during foetal and early postnatal life restores a normal proliferation and apoptosis of rat pancreatic islets. Diabetologia. 2002;45(6):856–66.
21. Hales CN, Desai M, Ozanne SE, Crowther NJ. Fishing in the stream of diabetes: from measuring insulin to the control of fetal organogenesis. Biochem Soc Trans. 1996;24(2):341–50.
22. Ozanne SE, Smith GD, Tikerpae J, Hales CN. Altered regulation of hepatic glucose output in the male offspring of protein-malnourished rat dams. Am J Physiol. 1996;270(4 Pt 1):E559–64.
23. Desai M, Crowther NJ, Lucas A, Hales CN. Organ-selective growth in the offspring of protein-restricted mothers. Br J Nutr. 1996;76(4):591–603.
24. Rees WD, Hay SM, Brown DS, Antipatis C, Palmer RM. Maternal protein deficiency causes hypermethylation of DNA in the livers of rat fetuses. J Nutr. 2000;130(7):1821–6.
25. Desai M, Byrne CD, Meeran K, Martenz ND, Bloom SR, Hales CN. Regulation of hepatic enzymes and insulin levels in offspring of rat dams fed a reduced-protein diet. Am J Physiol. 1997;273(4 Pt 1):G899–904.

26. El-Khattabi I, Gregoire F, Remacle C, Reusens B. Isocaloric maternal low-protein diet alters IGF-I, IGFBPs, and hepatocyte proliferation in the fetal rat. Am J Physiol Endocrinol Metab. 2003;285(5):E991–E1000.
27. Ozanne SE, Olsen GS, Hansen LL, Tingey KJ, Nave BT, Wang CL, et al. Early growth restriction leads to down regulation of protein kinase C zeta and insulin resistance in skeletal muscle. J Endocrinol. 2003;177(2):235–41.
28. Ozanne SE, Dorling MW, Wang CL, Nave BT. Impaired PI 3-kinase activation in adipocytes from early growth-restricted male rats. Am J Physiol Endocrinol Metab. 2001;280(3):E534–9.
29. Ozanne SE, Jensen CB, Tingey KJ, Storgaard H, Madsbad S, Vaag AA. Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. Diabetologia. 2005;48(3):547–52.
30. Tarry-Adkins JL, Fernandez-Twinn DS, Madsen R, Chen JH, Carpenter A, Hargreaves IP, et al. Coenzyme Q10 prevents insulin signaling dysregulation and inflammation prior to development of insulin resistance in male offspring of a rat model of poor maternal nutrition and accelerated postnatal growth. Endocrinology. 2015;156(10):3528–37.
31. Holemans K, Verhaeghe J, Dequeker J, Van Assche FA. Insulin sensitivity in adult female rats subjected to malnutrition during the perinatal period. J Soc Gynecol Investig. 1996;3(2):71–7.
32. Garofano A, Czernichow P, Breant B. Beta-cell mass and proliferation following late fetal and early postnatal malnutrition in the rat. Diabetologia. 1998;41(9):1114–20.
33. Matveyenko AV, Singh I, Shin BC, Georgia S, Devaskar SU. Differential effects of prenatal and postnatal nutritional environment on ss-cell mass development and turnover in male and female rats. Endocrinology. 2010;151(12):5647–56.
34. Blondeau B, Garofano A, Czernichow P, Breant B. Age-dependent inability of the endocrine pancreas to adapt to pregnancy: a long-term consequence of perinatal malnutrition in the rat. Endocrinology. 1999;140(9):4208–13.
35. Raychaudhuri N, Raychaudhuri S, Thamotharan M, Devaskar SU. Histone code modifications repress glucose transporter 4 expression in the intrauterine growth-restricted offspring. J Biol Chem. 2008;283(20):13611–26.
36. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest. 1998;101(10):2174–81.
37. Simmons RA, Templeton LJ, Gertz SJ. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. Diabetes. 2001;50(10):2279–86.
38. Ogata ES, Bussey ME, Finley S. Altered gas exchange, limited glucose and branched chain amino acids, and hypoinsulinism retard fetal growth in the rat. Metabolism. 1986;35(10):970–7.
39. Simmons RA, Gounis AS, Bangalore SA, Ogata ES. Intrauterine growth retardation: fetal glucose transport is diminished in lung but spared in brain. Pediatr Res. 1992;31(1):59–63.
40. Unterman T, Lascon R, Gotway MB, Oehler D, Gounis A, Simmons RA, et al. Circulating levels of insulin-like growth factor binding protein-1 (IGFBP-1) and hepatic mRNA are increased in the small for gestational age (SGA) fetal rat. Endocrinology. 1990;127(4):2035–7.
41. Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. Diabetes. 2003;52(3):734–40.
42. Myatt L, Eis AL, Brockman DE, Kossenjans W, Greer IA, Lyall F. Differential localization of superoxide dismutase isoforms in placental villous tissue of normotensive, pre-eclamptic, and intrauterine growth-restricted pregnancies. J Histochem Cytochem. 1997;45(10):1433–8.
43. Simmons RA, Suponitsky-Kroyter I, Selak MA. Progressive accumulation of mitochondrial DNA mutations and decline in mitochondrial function lead to beta-cell failure. J Biol Chem. 2005;280(31):28785–91.
44. Peterside IE, Selak MA, Simmons RA. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. Am J Physiol Endocrinol Metab. 2003;285(6):E1258–66.
45. Selak MA, Storey BT, Peterside I, Simmons RA. Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. Am J Physiol Endocrinol Metab. 2003;285(1):E130–7.
46. Vuguin P, Raab E, Liu B, Barzilai N, Simmons R. Hepatic insulin resistance precedes the development of diabetes in a model of intrauterine growth retardation. Diabetes. 2004;53(10):2617–22.
47. Raab EL, Vuguin PM, Stoffers DA, Simmons RA. Neonatal exendin-4 treatment reduces oxidative stress and prevents hepatic insulin resistance in intrauterine growth-retarded rats. Am J Physiol Regul Integr Comp Physiol. 2009;297(6):R1785–94.
48. Bannister AJ, Kouzarides T. Reversing histone methylation. Nature. 2005;436(7054):1103–6.
49. Bernstein E, Allis CD. RNA meets chromatin. Genes Dev. 2005;19(14):1635–55.
50. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet. 2009;10(5):295–304.
51. Gopalakrishnan S, Van Emburgh BO, Robertson KD. DNA methylation in development and human disease. Mutat Res. 2008;647(1–2):30–8.
52. Mellor J. The dynamics of chromatin remodeling at promoters. Mol Cell. 2005;19(2):147–57.
53. Thamotharan M, Shin BC, Suddirikku DT, Thamotharan S, Garg M, Devaskar SU. GLUT4 expression and subcellular localization in the intrauterine growth-restricted adult rat female offspring. Am J Physiol Endocrinol Metab. 2005;288(5):E935–47.
54. Fueger PT, Shearer J, Bracy DP, Posey KA, Pencek RR, McGuinness OP, et al. Control of muscle glu-

cose uptake: test of the rate-limiting step paradigm in conscious, unrestrained mice. J Physiol. 2005;562(Pt 3):925–35.
55. Karnieli E, Armoni M. Transcriptional regulation of the insulin-responsive glucose transporter GLUT4 gene: from physiology to pathology. Am J Physiol Endocrinol Metab. 2008;295(1):E38–45.
56. Moreno H, Serrano AL, Santalucia T, Guma A, Canto C, Brand NJ, et al. Differential regulation of the muscle-specific GLUT4 enhancer in regenerating and adult skeletal muscle. J Biol Chem. 2003;278(42):40557–64.
57. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr. 2005;135(6):1382–6.
58. Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA. Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. Br J Nutr. 2007;97(3):435–9.
59. Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring. Br J Nutr. 2008;100(2):278–82.
60. Radford EJ, Isganaitis E, Jimenez-Chillaron J, Schroeder J, Molla M, Andrews S, et al. An unbiased assessment of the role of imprinted genes in an intergenerational model of developmental programming. PLoS Genet. 2012;8(4):e1002605.
61. Brons C, Jacobsen S, Nilsson E, Ronn T, Jensen CB, Storgaard H, et al. Deoxyribonucleic acid methylation and gene expression of PPARGC1A in human muscle is influenced by high-fat overfeeding in a birthweight-dependent manner. J Clin Endocrinol Metab. 2010;95(6):3048–56.
62. Einstein F, Thompson RF, Bhagat TD, Fazzari MJ, Verma A, Barzilai N, et al. Cytosine methylation dysregulation in neonates following intrauterine growth restriction. PLoS One. 2010;5(1):e8887.
63. Sanghera DK, Blackett PR. Type 2 diabetes genetics: beyond GWAS. J Diabetes Metab. 2012;3(198):pii: 6948.
64. Grotz AK, Gloyn AL, Thomsen SK. Prioritising causal genes at type 2 diabetes risk loci. Curr Diab Rep. 2017;17(9):76.
65. Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, et al. Genome-wide associations for birth weight and correlations with adult disease. Nature. 2016;538(7624):248–52.
66. Tyrrell J, Richmond RC, Palmer TM, Feenstra B, Rangarajan J, Metrustry S, et al. Genetic evidence for causal relationships between maternal obesity-related traits and birth weight. JAMA. 2016;315(11):1129–40.

16 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 the metabolic syndrome, putting them at increased risks for diabetes and other cardiometabolic comorbidities. In the past 30 years, the key role of insulin resistance 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 insulin resistance in the same way as the metabolic syndrome and women at risk for PCOS are also subject to the development of the metabolic syndrome. Thus, this chapter will explore the relationships between the metabolic syndrome and PCOS.

Overview

Polycystic ovary syndrome affects 8% to 12% of women of childbearing age and represents the most frequent medical cause of female infertility [1, 2]. 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 [2–4]. Other hyperandrogenic disorders must be excluded (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome).

The key role of insulin resistance and subsequent hyperinsulinemia in the pathogenesis of PCOS has been underscored with increasing evidence in the literature of the last decades. 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 clinical and pathophysiological connections between PCOS and the metabolic syndrome.

Insulin Resistance and Polycystic Ovary Syndrome

The literature of the last 30 years has demonstrated the presence of insulin resistance and compensatory hyperinsulinemia in the majority of women with PCOS (between 44% and 70%) [5]. Using the gold standard hyperinsulinemic–euglycemic clamp, it has been shown that women with PCOS have a lower peripheral insulin sensitivity, independently of obesity [6, 7]. The authors attributed these results to a form of insulin resistance related to adiposity, as well as another

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form that was intrinsic to the syndrome. Insulin resistance has been shown to arise early in the disease, as obese adolescents with PCOS are also significantly more insulin-resistant than obese adolescents without PCOS [8–10]. Furthermore, women with PCOS have been shown to present similar insulin resistance than age and body mass index (BMI)-matched individuals with type 2 diabetes (T2D) [11]. Several lines of evidence from insulin sensitization by drugs or physical activity have proven that hyperandrogenism and menstrual cyclicity improve in both lean and obese women with PCOS by decreasing insulin resistance [5, 12–15].

Accordingly, Dunaif et al. described an insulin receptor or post-receptor signal transduction defect in women with PCOS [6]. Later, they demonstrated increased insulin receptor serine phosphorylation in women with PCOS in vitro, which could account for the post-binding defect of insulin action [16]. Other in vitro studies using adipocytes from nonobese PCOS women showed insensitivity to inhibition of lipolysis by insulin and a decrease in adipocyte glucose uptake [17], two features consistent with an insulin receptor or post-receptor defect. More recently, the role of muscular mitochondrial dysfunction and impaired oxidative phosphorylation as a cause of peripheral insulin resistance in PCOS has been suggested in the literature [9, 11]. A decrease in the expression of genes implicated in mitochondrial oxidative metabolism has been shown in muscle of women with PCOS [18]. Furthermore, measurement of mitochondrial function in vivo in obese adolescent with PCOS has shown decreased postexercise oxidative phosphorylation, as compared to obese adolescents without PCOS [9].

It has also been shown that PCOS insulin resistance is not global, since PCOS women do not show resistance to insulin actions on ovarian androgen production or 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 cells studies in vitro [19–21]. Moreover, theca cells from PCOS women showed a greater androgen production in response to insulin in comparison to theca cells of women without PCOS [22, 23]. Insulin also inhibits sex hormone-binding globulin production by the liver, thus increasing free testosterone [24, 25]. Notably, insulin sensitizers [26] or insulin-lowering drugs [27, 28] increase sex hormone-binding globulin levels in PCOS women. Insulin may also increase ovarian responsiveness to luteinizing hormone (LH) stimulation [29] and pituitary release of LH [15]. By increasing ovarian responsiveness to LH and intraovarian androgen production, insulin could cause premature activation of preovulatory ovarian follicles and arrest of subsequent follicles. Arrested follicles provide a constant supply of androgens, since atretic follicles are deficient in aromatase. Taken together, all of these effects of abnormal insulin action lead to hyperandrogenism [30]. A summary of our proposed pathogenesis of PCOS is illustrated in Fig. 16.1.

It is important to note that, while many women present with hyperinsulinemic insulin resistance, including most obese women, only a minority 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 [31–33] and have been shown to normalize their hyperandrogenemia with diazoxide, a pure insulin-lowering medication (Fig. 16.2) [28], as well as insulin-sensitizing drugs (metformin and rosiglitazone) [34]. Therefore, insulin action plays a role in the pathogenesis of PCOS even in women with normal insulin levels and normal insulin sensitivity, suggesting hyperresponsiveness 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 [35].

However, in most PCOS women, this insulin sensitivity defect is probably not severe enough to manifest at normal insulin concentrations, and they must develop global insulin resistance with compensatory hyperinsulinemia before PCOS becomes clinically apparent. Therefore, the association of PCOS with insulin resistance may

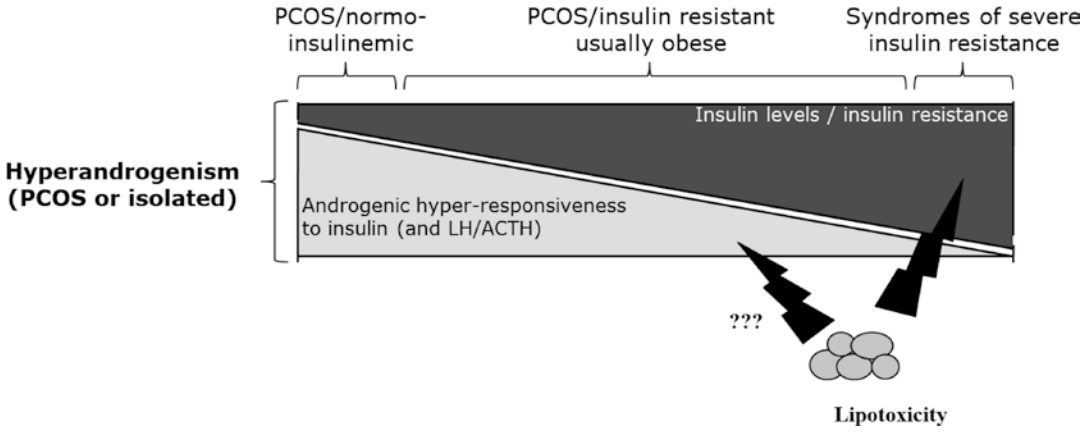

Fig. 16.1 Proposed pathophysiology of PCOS. PCOS is represented by a spectrum of clinical presentations, where PCOS hyperandrogenism symptoms result from the interaction between lipotoxicity and hyperresponsiveness of androgen-secreting organs to insulin. On one end of the spectrum, PCOS may manifest because of a high genetic predisposition to the effects of lipotoxicity on androgen-secreting tissue despite low degree of insulin resistance and hyperinsulinism. On the opposite end of the spectrum, women with high insulin resistance and hyperinsulinism may have hyperandrogenic manifestations despite a relatively low genetic predisposition

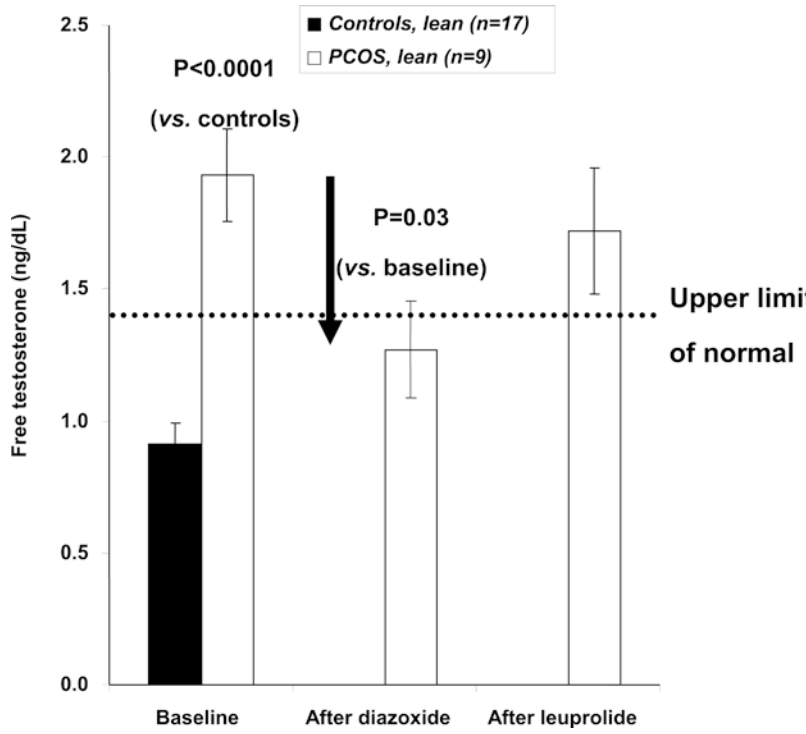

Fig. 16.2 Calculated free testosterone concentrations before and after treatment with diazoxide and leuprolide acetate in lean control (bar to the left, $n = 17$) and lean PCOS women (bars to the right, $n = 9$). Results are presented as means and standard error of the mean (SEM). (Adapted with permission from Baillargeon and Carpentier [28])

predominately reflect selection based on clinical presentation. According to this hypothesis, the insulin resistance characteristic of PCOS might not be specific but, instead, shares the same causes as other insulin-resistant states. These might be genetically or epigenetically determined or acquired following deleterious lifestyle and/or weight gain.

Overexposure of non-adipose tissues to free fatty acids (FFAs) leading to functional cellular

defects (lipotoxicity) is now considered a key mechanism in the development of muscular and hepatic insulin resistance, as well as pancreatic β(beta) cell dysfunction, that leads to T2D [36]. Deleterious cellular consequences of FFA are exacerbated by defective mitochondrial β(beta)-oxidation of FFA due to mitochondrial dysfunction, which was described in PCOS as mentioned above [9, 11, 18]. Non-adipose tissue can be exposed to FFA through either the uptake of circulating FFAs, which are higher during fasting, or the uptake of FFA coming from hydrolysis of triglycerides by endothelial lipoprotein lipase circulating in chylomicrons or VLDL (triglycerides–rich lipoproteins). Increased levels of circulating FFA may result from adipose tissue dysfunction, with decreased suppressibility of intracellular lipolysis by insulin [37, 38]. Although few studies have shown an increase in fasting circulating FFA in PCOS women and adolescents [10, 39], both adults and adolescents with PCOS were found to display higher postprandial levels of triglycerides [40, 41]. Recent evidence suggests that ovarian and adrenal cells may also be overexposed to circulating FFA in PCOS and that it may contribute directly to the overproduction of androgens [42–44].

Since insulin resistance is a key factor in the pathogenesis of PCOS and is highly inheritable [45], along with PCOS [2, 30, 46, 47], siblings of women with PCOS should be more affected by insulin resistance and the metabolic syndrome than the general population. Accordingly, our group 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 [48]. We also determined that girls aged between 8 and 14 years, who have a first-degree relative affected by PCOS, display insulin resistance and resistance to insulin-mediated lipolysis compared to age-matched control girls, suggesting this is an early inheritable event [37]. Other studies found decreased insulin sensitivity in relatives of women with PCOS [49–51]. Therefore, siblings of PCOS women might also inherit the insulin resistance and metabolic syndrome typical of PCOS.

Metabolic Syndrome and Polycystic Ovary Syndrome

Much overlap exists between PCOS and the metabolic syndrome, both clinically and biochemically (Fig. 16.3) [52]. According to the 2005 National Institutes of Health National Cholesterol Education Program–Adult Treatment Panel III (NCEP-ATPIII)[53], 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 ≥130/85 mm Hg or antihypertensive medication, and fasting serum glucose ≥100 mg/dL (5.6 mmol/L) or known T2D. Most of the studies cited herein used this definition,

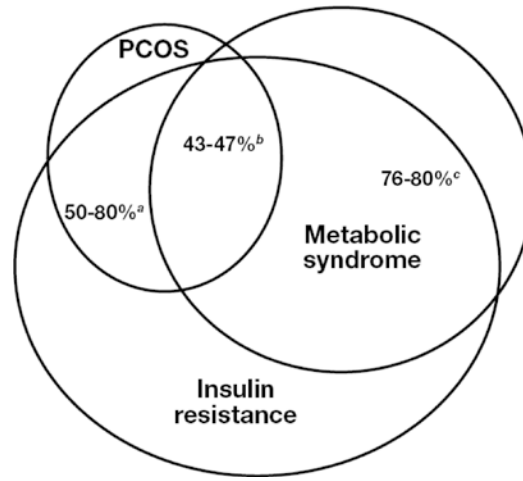

Fig. 16.3 Relationships among polycystic ovary syndrome, the metabolic syndrome, and insulin resistance. Approximately 50–80% of PCOS women are insulin-resistant and 43–47% have the metabolic syndrome. Similarly, approximately 68–74% of individuals with the metabolic syndrome have insulin resistance. (Adapted with permission from Essah and Nestler [52])

but a more recent definition established by the International Diabetes Federation is increasingly adopted worldwide: see [54] for details.

Assuming insulin resistance is a central factor in the pathogenesis of the two disorders [5], it is not surprising to find an increased prevalence of the metabolic syndrome in women with PCOS and a high rate of PCOS in women with the metabolic syndrome. Clinically, women presenting both with PCOS and the metabolic syndrome were found to have more pronounced hyperandrogenemia and increased frequency of acanthosis nigricans [55]. These findings suggest a greater degree of insulin resistance 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 16.1 summarizes the prevalence of these aspects in women with PCOS [55–61].

Overlap Between Metabolic Syndrome and Polycystic Ovary Syndrome

The metabolic syndrome is overrepresented among women with PCOS. A systematic review and meta-analysis published in 2010 reported the prevalence of metabolic syndrome in women with PCOS as compared to a control population from 18 studies, including 2256 women with PCOS and 4130 women without PCOS [62]. Overall, women with PCOS had higher prevalence of metabolic syndrome: 22.5% of women with PCOS versus 17.7% of the controls; OR 3.01 (2.06–4.41). Considering the subgroup of studies who included only BMI-matched controls [63–67], the odds ratio for metabolic syndrome was 2.20 in women with PCOS in comparison to controls.

The overrepresentation of the metabolic syndrome in PCOS, in comparison to same age general population, was shown to be present even in individuals under 20 years old [55]. Accordingly, Vural et al. found a prevalence of the metabolic syndrome of 11.6% in lean women with PCOS aged between 18 and 22 years compared to 0% in controls [59].

Obesity explains to a large extent the presence of the metabolic syndrome among women with PCOS who seek medical attention, and the prevalence of metabolic syndrome is much higher in women with PCOS with overweight and obesity [57, 60]. As described in more details later, the referral bias of obese/overweight women observed among women with PCOS may have overestimated the prevalence of metabolic syndrome among the general PCOS population [68] but remains significant in comparison to women without PCOS with the same BMI [62]. On the other hand, none of the women with a BMI <27 kg/m^2 (n = 52) had metabolic syndrome in the study from Ehrmann et al. [60], and a significantly lower proportion of women had metabolic syndrome in the lean PCOS population versus obese PCOS population (15.8% vs. 58%) in a study from Attaoua et al. [65]. Also, according to one study in adolescents with overweight or obesity, the prevalence of metabolic syndrome seems largely influenced by obesity at this age, and PCOS per se may not contribute to the same extent to metabolic syndrome early in the disease [69]. However, more studies will be needed to understand the risk of metabolic syndrome in the young, lean population with PCOS.

It has also been shown that the prevalence of the metabolic syndrome is increased among first-degree relatives of women with PCOS. As noted above, Sam et al. [50] found a higher prevalence of the metabolic syndrome in sisters presenting the phenotype of PCOS (52%) or hyperandrogenemia with normal menses (23%), as compared with unaffected sisters (7%). These authors also reported that the prevalence of metabolic syndrome was increased in mothers, brothers, and fathers of women with PCOS compared to population from the National Health and Nutrition Examination Survey III (NHANES III) [49, 70].

Obesity and Polycystic Ovary Syndrome

Approximately 80% of women with PCOS in the United States and 50% outside of the United States are obese [2]. However, this high

Table 16.1 Studies reporting prevalence of metabolic syndrome and individual components of metabolic syndrome in women with polycystic ovary syndrome [55–61]

Study	Type of study	Population and PCOS criteria	Mean/ median age (yr)	Mean BMI (kg/ m^2)	Prevalence MS PCOS (%)	Prevalence of MS controls (%)	OR/RR and p value	Glucose criteria of MS	Lipids criteria of MS	Abdominal obesity criteria of MS	Blood Pressure criteria of MS
Glueck 2003 [56]	Prospective	PCOS $n = 138$ 1990 NIH	31	N/A	46.4% (age-adjusted)	22.8% NHANES III	$p < 0.001$	5.1%	High-TG: 32.6% Low-HDL: 64.5%	85.5%	44.9%
Apridonidze 2005 [55]	Retrospective	PCOS $n = 106$ 1990 NIH	N/A	N/A	43%	NHANES III	8.0 (20–29 yr) 4.0 (30–39 yr)	3.8%	High-TG 35% Low-HDL: 68%	67%	45%
Dokras 2005 [57]	Retrospective	PCOS: $n = 129$ 1990 NIH Control: $n = 177$	PCOS: 27 Control: 43	N/A	34.9% Age-adjusted 47.3%	6.8% Age-adjusted 4.3%	20.18 (95% CI 7.45–54.69)	12.6%	High TG: 46.8% Low-HDL 63.7%	BMI >30: 72.3%	N/A
Vrbíková 2005 [58]	Prospective	PCOS $n = 69$ 2003 Rotterdam Control $n = 73$	PCOS: 24 Control: Age-matched	PCOS: 23.1 Control: 21.9	N/A	N/A	N/A	0%	High TG: 5.8% Low-HDL: 34.8%	11%	13%
Vural 2005 [59]	Prospective case-control	PCOS: $n = 43$ Control: $n = 43$	PCOS: 21 Control: Age-matched	PCOS: 23.4 Control: 21.5	2.3% NCPE 11.6% WHO	0%	N/A	N/A	N/A	N/A	N/A
Ehrmann 2006 [60]	Prospective multicentric	PCOS: $n = 394$ (multiethnic)	N/A	N/A	33.4%	N/A	N/A	5%	High TG: 32% Low HDL 66%	80%	21%
Sharma 2015 [61]	Prospective cross-sectional	PCOS: $n = 120$ 2003 Rotterdam Controls: $n = 80$	PCOS: 30 Control: 31	PCOS: 27.4 Control: 24.6	39.2%	23.8%	$p = 0.03$	17.5%	High TG: 15% Low HDL: 87.5%	46%	44%

MS metabolic syndrome, *PCOS* polycystic ovarian syndrome, *BMI* body mass index (kg/m^2), *TG* triglycerides, *HDL* high-density lipoprotein, *N/A* data nonavailable

percentage may have been overestimated secondary to a referral bias in PCOS clinical studies [68, 71]. In fact, Lizneva et al. showed that PCOS women with obesity in an unselected US population represented 28% of all PCOS women, the same prevalence as in controls (28.4%), but represented 63% of PCOS women referred to the reproductive endocrinology clinic [68]. This referral bias may have led to an overestimation of the occurrence of obesity in women with PCOS. Indeed, this group also showed that the risk of PCOS was only slightly elevated with obesity in women of reproductive age [72]. Still, obesity is associated with an exacerbation of the clinical manifestations of PCOS, which may cause some women to seek medical advice.

It was previously shown that the obesity pattern tended to be android in women with PCOS. These studies were using the waist-to-hip ratio or dual-energy X-ray absorptiometry (DEXA) scan to evaluate body fat distribution. However, more precise evaluation of abdominal fat and visceral fat using magnetic resonance imaging (MRI) or computed tomography (CT) scan did not confirm these observations [10, 73–76]. It is, therefore, still unclear whether women with PCOS are more prone to an android body fat distribution.

Dyslipidemia and Polycystic Ovary Syndrome

Low serum high-density lipoprotein (HDL) cholesterol is the most frequently occurring component of the metabolic syndrome among women with PCOS and occurs in 68% of the cases [52]. It has also been demonstrated that PCOS women present with a significant increase in low-density lipoprotein (LDL) cholesterol levels when compared with controls. Moreover, they frequently present with abnormally high concentrations of small, dense LDL particles and triglyceride–rich lipoproteins—a profile known to be atherogenic [77]. Notably, dyslipidemia occurs both in lean and overweight PCOS women and affects all age groups [77]. Lipoprotein (a) (Lp(a)), an atherogenic lipoprotein and independent risk factor for cardiovascular disease, has also been shown to be elevated in women with PCOS [78–80].

Hypertension and Polycystic Ovary Syndrome

Evidence regarding the risk of hypertension attributed to PCOS *per se* is still scarce and conflicting. A few cohort studies reported an increased prevalence of hypertension in women with PCOS [81, 82], and a systematic review and meta-analysis of 40 observational studies concluded that PCOS in pregnancy was associated with greater risk of pregnancy-induced hypertension [83]. However, these studies involved important methodological biases, as the presence of hypertension was self-reported and/or control populations were not matched or similar for BMI or other confounding risk factors. In two case-control studies of non-hypertensive young women, 24-hour ambulatory monitoring in small numbers of participants showed conflicting results. In the first study, daytime systolic and mean blood pressures were slightly higher in a group of 18 women with PCOS in comparison to 17 controls of similar age and BMI. The difference persisted after correction for confounding factors. However, nighttime systolic, diastolic, and office blood pressures did not differ between the groups [84]. In the second study, mean daytime systolic and diastolic blood pressures were not different between groups, but there was an absence of nighttime dip pattern in women with PCOS [85]. Of importance, in this study, hypertension was diagnosed in 33% of young women with PCOS and was better identified using 24-hour ambulatory monitoring than office blood pressure. On the other hand, a higher daytime blood pressure was more associated with obesity than PCOS itself.

It is also not clear whether age influences the risk of high blood pressure in women with PCOS. In a case-control study, Coviello et al. reported that obese adolescents with PCOS had a high prevalence (27%) of hypertension compared to the reference population from NHANES III (1%), as defined by seated blood pressure ≥95th percentile [86]. Furthermore, 41% had a blood

pressure ≥90th percentile, compared to only 2% in the reference population from NHANES III [86]. As the control group was nonobese, obesity per se may explain this high proportion of hypertension and prehypertension early in life. However, in a study of non-hypertensive obese adolescents with PCOS (n = 36) compared to obese controls (n = 17), inpatient 24-hour blood pressure monitoring did not show significant difference in systolic or diastolic blood pressure between groups [87]. Nevertheless, 16.7% of adolescent girls with PCOS were defined as prehypertensive, compared to 11.8% in the control group (p = 0.299), and the proportion of nighttime non-dippers was 25% (n = 9) in girls with PCOS versus 5.9% (n = 1) in obese controls (p = 0.082). These differences were not statistically different between groups, but this study may have been underpowered for this outcome [87].

In conclusion, there is a lack of large-scale data, using gold standard measurement for blood pressure, to conclude whether PCOS per se is related to high blood pressure. Still, obesity seems to be an important risk factor for this component of the metabolic syndrome, and a significant proportion of obese women and adolescents may have hypertension.

Diabetes and Polycystic Ovary Syndrome

With the implication of insulin resistance in the pathogenesis of PCOS, it is not surprising to find an increased prevalence of impaired glucose tolerance (IGT) and T2D among women with PCOS [62]. Indeed, cross-sectional and prospective studies have found that the prevalence of IGT and T2D were 20–37% and 7.5–15%, respectively, among women with PCOS [88–94]. These numbers are much higher than in the US female population of comparable age, where the prevalence of IGT and diabetes were estimated at 7% and 3%, respectively [95]. A systematic review and meta-analysis have found an OR 2.5 and 4.0 for IGT and T2D, respectively, in women with PCOS in comparison to BMI-matched controls [62]. In addition, both lean and obese women with PCOS have an increased risk of diabetes, although obesity and PCOS have an additive effect on glucose tolerance [89, 94]. Progression from IGT to diabetes is also increased twofold- to fivefold in the population with PCOS [90, 92]. In a prospective controlled study of 149 women with PCOS and 166 controls without diabetes at baseline, the relative risk of developing T2D after 8 years of follow-up was almost 4 times higher in women with PCOS in comparison to controls, after adjustment for age, and 2.4 times after further adjustment for BMI [94]. Moreover, women with PCOS tended to develop diabetes at an earlier age.

The prevalence of PCOS is also increased among women with T2D. Indeed, in two retrospective studies of premenopausal women with T2D, 27% were found to have PCOS [96] and 82% had polycystic ovary on transvaginal ultrasound [97]. Thus, PCOS is probably present in more than one-quarter of women with T2D.

Cardiovascular Risk and Polycystic Ovary Syndrome

As shown previously, PCOS is associated with several traditional cardiovascular risk factors, including hypertension, dyslipidemia, and abnormal glucose tolerance, which may be exacerbated by the high proportion of obesity associated with this syndrome. However, PCOS is also associated with endothelial dysfunction and altered coagulation parameters, implying increased vascular oxidative stress, increased hemostasis, and dysfibrinolysis [98, 99]. Insulin resistance is associated with increased levels of serum inflammatory mediators [100, 101], which are implicated in the pathogenesis of cardiovascular disease. Accordingly, women with PCOS display higher levels of markers of inflammation or hypercoagulability, such as tumor necrosis factor-alpha (TNF-alpha), C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), homocysteine, tissue plasminogen activator (t-PA), and endothelin-1 [78].

A current systematic review and meta-analysis of literature suggests that early endothelial dysfunction, assessed by flow-mediated dilation [102], and premature subclinical carotid atherosclerosis [103] are more common in women with PCOS, even if they are young and have a normal

weight. Premature subclinical carotid atherosclerosis, measured by increased carotid intima-media thickness, was also reported in obese [87, 104] and normal weight [59, 104] adolescents or young adults with PCOS, in comparison to age and BMI matched-controls.

It is therefore not surprising to find studies demonstrating early signs of subclinical coronary atherogenesis in premenopausal [105–108] and postmenopausal [107] women with PCOS. Furthermore, some small studies have reported left ventricular diastolic dysfunction or hypertrophy [109–111]. However, premature coronary atherosclerosis remains debated because coronary arterial calcification and aortic atherosclerosis plaque were not higher in PCOS premenopausal women in comparison to a large cohort from the Dallas Heart study [112].

Despite an increase in many surrogates of cardiovascular risk factors and evidence of precocious atherosclerosis deposition, the evidence supporting an association between PCOS and increased cardiovascular events or mortality is still unclear. This is due to the limited number of large, long-term, cohort studies able to adequately identify women with a history of PCOS. A systematic review and meta-analysis from de Groot et al. in 2011 included only five studies that reported the effect of PCOS on cardiovascular outcomes, of which only two were classified as having high methodological quality [113]. This meta-analysis reported a two-fold increased risk of combined cardiovascular events in women with PCOS, which remained increased by approximately 50% when adjusted for BMI. One of the two studies identified with high methodological quality, and weighting for 22.3% of the meta-analysis, was retracted recently by authors because of diagnostic coding errors and inability to replicate the original results [114], further limiting the interpretation of this literature. The best evidence at this time is probably from the prospective cohort of the Nurse Health Study that used the presence of very irregular menses as a surrogate of PCOS, which is considered an excellent proxy. This cohort included 82,439 women, the largest number to date, followed for 14 years up to a relatively young mean age of 48 years [115]. Women with very irregular menses displayed a risk of developing fatal or nonfatal myocardial infarct that was significantly increased by 50%. In addition, the risk of stroke also tended to be increased but did not reach statistical significance in this young cohort (RR 1.30; 95% IC 0.97–1.74).

Taken together, these results suggest that PCOS might indeed increase the risk for cardiovascular diseases, as suspected with its higher prevalence of individual components of the metabolic syndrome.

Hepatic Steatosis and Nonalcoholic Steatohepatitis (NASH)

Hepatic steatosis and nonalcoholic steatohepatitis (NASH)—steatosis with hepatic injury and inflammation—are strongly associated with the metabolic syndrome, insulin resistance, dyslipidemia, and cardiovascular disease. Evidence is growing that, for the same BMI, women with PCOS are at higher risk than women without PCOS of developing nonalcoholic fatty liver disease (NAFLD), including hepatic steatosis and NASH. In a systematic review and meta-analysis reporting 17 studies including 2734 women with PCOS and 2561 controls of similar age and BMI, women with PCOS were at significantly higher risk of NAFLD (OR [95% CI]: 2.54 [2.19–2.95]) [116]. In a recent study, Cree-Green et al. showed that NAFLD is also frequent at a young age in obese adolescents with PCOS in comparison to obese adolescent with the same BMI (49% vs. 14%) [10]. The accumulation of intrahepatic triglycerides (IHTG) may occur in PCOS due to increased delivery of fatty acids to the liver, increased hepatic fatty acid synthesis (de novo lipogenesis), decreased fatty acid oxidation, or relative saturation of lipoprotein triglyceride export [117, 118]. These hypothetical mechanisms derive from studies in the different population of NAFLD and have not yet been assessed in PCOS.

More importantly, IHTG accumulation leads to hepatic lipotoxicity that alters liver metabolism and may be central to the development of hepatic insulin resistance and T2D in PCOS. Belan et al. [119] showed that alanine aminotransferase (ALT) in PCOS women was correlated with hepatic insulin resistance (based on the fasting homeostatic model assessment of

insulin resistance [HOMA-IR]) and negatively correlated with whole-body insulin sensitivity (Matsuda index) and insulin secretion (based on the 2-hour 75 g oral glucose tolerance test [OGTT]). Furthermore, an ALT cutoff ≥24 IU/L (within the normal range) was a strong predictor of whole-body insulin resistance and could, therefore, help clinicians to identify women with PCOS at higher risk of metabolic alterations. Moreover, many studies consistently found an association between serum ALT and androgen concentrations, independent of BMI or insulin resistance [119–121]. Taken together, these studies underscore the relationship that exists between insulin resistance, hepatic lipotoxicity, and hyperandrogenism in women with PCOS.

Obstructive Sleep Apnea and Polycystic Ovary Syndrome

Since obstructive sleep apnea has been associated with the insulin resistance syndrome [122, 123], it is interesting to note that women with PCOS have a higher prevalence of obstructive sleep apnea than weight and age-matched controls [124–127]. Notably, it was demonstrated that insulin resistance and impairment in glucose tolerance are strongly correlated with the apnea–hypopnea index in PCOS women [126]. Sleep apnea is also associated with other features of PCOS—namely, increased levels of the inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF alpha), as well as visceral fat [122].

Metabolic Syndrome Screening in Polycystic Ovary Syndrome

Screening for T2D with an oral glucose tolerance test among women with PCOS is well accepted and is supported by the American College of Gynecology, the American Association of Clinical Endocrinologists, the Endocrine Society, and the Androgen Excess-PCOS Society [3, 128–130]. However, the American Diabetes Association recommends screening PCOS women with an OGTT only when their fasting plasma glucose (FPG) is over 5.6 mmol/L (100 mg/dL) [130]. We assessed the predictive value of the FPG cutoff point of 5.6 mmol/L for detection of abnormal glucose tolerance in PCOS women and found specificity of 98.7%, but a sensitivity of only 48% (Fig. 16.4) [131]. With this screening guideline,

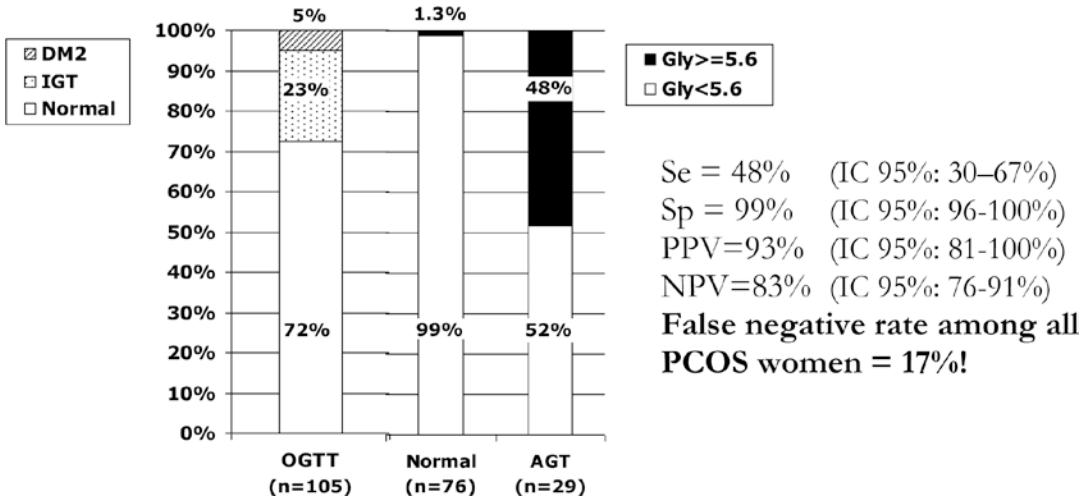

Fig. 16.4 Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the fasting plasma glucose cutoff value of 5.6 mmol/L for the screening of abnormal glucose tolerance (AGT). DM2 type 2 diabetes mellitus, IGT impaired glucose tolerance, and OGTT oral glucose tolerance test. (Adapted with permission from Gagnon and Baillargeon [131]. © Canadian Medical Association (2007). This work is protected by copyright, and the making of this copy was with the permission of the Canadian Medical Association Journal (www.cmaj.ca) and Access Copyright. Any alteration of its content or further copying in any form whatsoever is strictly prohibited unless otherwise permitted by law)

for every six PCOS women screened with only an FPG, one would be missed with abnormal glucose tolerance. Thus, it has been recommended to screen all women with PCOS using an OGTT [3, 4, 131, 132]. The new International 2018 PCOS guidelines support that screening should be done in all women with PCOS and in adolescents with additional risk factors at baseline (overweight/obese or from high-risk ethnicity group) [133]. These guidelines also recommend to perform an OGTT in those with higher risk at baseline or when a diagnosis of IGT would change clinical practice, such as lifestyle intervention or metformin use. Optimal testing is unclear in both adult and adolescent population and Hba1c, fasting glucose, or OGTT can be used depending of the clinical context, taking into consideration costs and convenience. In the past years, many authors have also recommended screening for other components of the metabolic syndrome in women with PCOS. Accordingly, it was suggested at the Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) meeting that all obese PCOS women should be screened for the different aspects of the metabolic syndrome [4, 132]. 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 the 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. It could also guide clinicians to avoid treatments that may lead to an aggravation of the metabolic syndrome, such as the use of oral contraceptives when contraception is not required (see below). If the results are normal, screening again at 2- to 5-year intervals is probably indicated, or more often for those at higher cardiovascular risk, since normal results do not preclude the future appearance of metabolic syndrome [3, 4, 133].

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

Treatment

In the context of increased metabolic risk and possible cardiovascular risk associated with PCOS, the mainstay of treatment should rely on prevention and treatment of metabolic and cardiovascular risk factors, in conjunction with the aim of controlling symptoms.

The basis of treatment for both metabolic syndrome and PCOS relies on lifestyle modification. Weight loss in obese or overweight women, exercise, as well as diet modification and avoidance of toxic substances (cigarette, alcohol, drugs) have direct effects on the risk for T2D and cardiovascular disease [134–137]. Moderate weight loss of 5% to 10% of total body weight should be encouraged because it is realistic and induces a significant decrease in visceral adipose tissue, with important benefits on insulin resistance and β(beta)-cell function [135].

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 assuring a reliable reversible contraceptive method [138]. However, long-term metabolic complications of the syndrome are receiving growing attention. Since OCs appear to decrease insulin sensitivity and glucose tolerance in the short term [139–141], 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 [142, 143]. Effectively, a large 15-year cohort study of >one million women from the general population found a doubling in the relative risks of myocardial infarction (RR = 1.3–2.3) and ischemic stroke (RR = 1.4–2.2) with current use of low-dose OCs (30–40 mcg ethinyl estradiol) in comparison to nonuse of OCs [142]. However, this finding is not specific to women with PCOS, who have higher risk factors of cardiovascular disease at baseline.

Much attention has been given to the use of insulin sensitizers in PCOS, mainly the biguanide metformin. In addition to treating oligoanovulation, fertility, menstrual irregularities, and hyperandrogenemia in a majority of women with PCOS, insulin sensitizers also improve glucose tolerance and other cardiovascular risk factors, such as triglycerides and LDL-C levels, blood pressure, and endothelial dysfunction [144–147]. Indeed, metformin has been shown to reduce cardiovascular events in obese individuals with T2D [148]. It is, however, important to note that metformin takes time to achieve all of its effects—at least 6 months for menstrual regularity [34, 149] and up to 1 year for hirsutism [150]. Thus, 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. Accordingly, most guidelines recommend using metformin in all PCOS women with IGT or T2D [3, 128]. In PCOS women with normal glucose tolerance, a simple way to assess their cardiometabolic risk is to determine the concomitant presence of the metabolic syndrome, as defined by NCEP-ATPIII or IDF guidelines [53, 54], for example.

The effects of insulin sensitizers and OCs as monotherapy for the management of PCOS are summarized in Table 16.2. Insulin-sensitizing drugs could 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. As mentioned, it is recommended that women with PCOS and abnormal glucose tolerance use metformin in order to reduce their risk of cardiometabolic complications [3, 128]. Although few studies directly assessing 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 [5, 147].

Table 16.2 Effects of insulin sensitizers and oral contraceptives on different aspects of polycystic ovary syndrome

	Insulin sensitizers	Oral contraceptives
Infertility	++	
Menstrual irregularity	++	+++
Hirsutism	+/++	++
Acne	+	++
Insulin resistance 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, – no positive effect, *DM* diabetes mellitus

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 [3].

Summary

PCOS and metabolic syndrome present many common features, and the implication of insulin resistance in the pathogenesis of both syndromes partly explains the overlap. Since, in most cases, insulin resistance and compensatory hyperinsulinemia is required for the appearance of PCOS, the majority of PCOS women are also at increased risk for development of the metabolic syndrome. Indeed, PCOS and the metabolic syndrome may be two alternate clinical presentations of the same underlying pathophysiology,

related to insulin resistance and/or lipotoxicity. Accordingly, PCOS should be considered as a female expression of the insulin resistance syndrome and a risk factor for metabolic and cardiovascular complications.

Since PCOS women are at increased risk of developing cardiovascular disease and T2D, 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 when contraception is not required, especially in the presence of abnormal glucose tolerance.

References

1. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25:544–51. https://doi.org/10.1093/humrep/dep399.
2. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev. 2015;36:487–525. https://doi.org/10.1210/er.2015-1018.
3. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2013;98:4565–92. https://doi.org/10.1210/jc.2013-2350.
4. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41–7.
5. Carreau A-M, Baillargeon J-P. PCOS in adolescence and type 2 diabetes. Curr Diab Rep. 2015;15:564. https://doi.org/10.1007/s11892-014-0564-3.
6. Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T. Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. Diabetes. 1992;41:1257–66.
7. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38:1165–74.
8. Lewy VD, Danadian K, Witchel SF, Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. J Pediatr. 2001;138:38–44. https://doi.org/10.1067/mpd.2001.109603.
9. Cree-Green M, Newcomer BR, Coe G, Newnes L, Baumgartner A, Brown MS, et al. Peripheral insulin resistance in obese girls with hyperandrogenism is related to oxidative phosphorylation and elevated serum free fatty acids. Am J Physiol Endocrinol Metab. 2015;308:E726–33.
10. Cree-Green M, Bergman BC, Coe GV, Newnes L, Baumgartner AD, Bacon S, et al. Hepatic steatosis is common in adolescents with obesity and PCOS and relates to De Novo lipogenesis but not insulin resistance. Obesity (Silver Spring). 2016;24:2399–406. https://doi.org/10.1002/oby.21651.
11. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33:981–1030. https://doi.org/10.1210/er.2011-1034.
12. Baillargeon J-P. Use of insulin sensitizers in polycystic ovarian syndrome. Curr Opin Investig Drugs. 2005;6:1012–22.
13. Baillargeon J-P, Iuorno MJ, Nestler JE. Insulin sensitizers for polycystic ovary syndrome. Clin Obstet Gynecol. 2003;46:325–40.
14. Huber-Buchholz MM, Carey DG, Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab. 1999;84:1470–4. https://doi.org/10.1210/jcem.84.4.5596.
15. Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab. 1997;82:4075–9. https://doi.org/10.1210/jcem.82.12.4431.
16. Dunaif A, Xia J, Book CB, Schenker E, Tang Z. Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest. 1995;96:801–10. https://doi.org/10.1172/JCI118126.
17. Ek I, Arner P, Bergqvist A, Carlström K, Wahrenberg H. Impaired adipocyte lipolysis in nonobese women with the polycystic ovary syndrome: a possible link to insulin resistance? J Clin Endocrinol Metab. 1997;82:1147–53. https://doi.org/10.1210/jcem.82.4.3899.
18. Skov V, Glintborg D, Knudsen S, Jensen T, Kruse TA, Tan Q, et al. Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. Diabetes. 2007;56:2349–55. https://doi.org/10.2337/db07-0275.
19. Willis D, Mason H, Gilling-Smith C, Franks S. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. J

Clin Endocrinol Metab. 1996;81:302–9. https://doi.org/10.1210/jcem.81.1.8550768.
20. Nahum R, Thong KJ, Hillier SG. Metabolic regulation of androgen production by human thecal cells in vitro. Hum Reprod. 1995;10:75–81.
21. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab. 1986;62:904–10. https://doi.org/10.1210/jcem-62-5-904.
22. Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab. 1998;83:2001–5. https://doi.org/10.1210/jcem.83.6.4886.
23. Nelson-Degrave VL, Wickenheisser JK, Hendricks KL, Asano T, Fujishiro M, Legro RS, et al. Alterations in mitogen-activated protein kinase kinase and extracellular regulated kinase signaling in theca cells contribute to excessive androgen production in polycystic ovary syndrome. Mol Endocrinol. 2005;19:379–90. https://doi.org/10.1210/me.2004-0178.
24. Yki-Järvinen H, Mäkimattila S, Utriainen T, Rutanen EM. Portal insulin concentrations rather than insulin sensitivity regulate serum sex hormone-binding globulin and insulin-like growth factor binding protein 1 in vivo. J Clin Endocrinol Metab. 1995;80:3227–32. https://doi.org/10.1210/jcem.80.11.7593430.
25. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72:83–9. https://doi.org/10.1210/jcem-72-1-83.
26. Ciotta L, De Leo V, Farina M, Pafumi C, La Marca A, Cianci A. Endocrine and metabolic effects of insulin sensitizers in the treatment of patients with polycystic ovary syndrome and hyperinsulinaemia. Gynecol Obstet Invest. 2001;51:44–50. doi:52890
27. Penna IAA, Canella PRB, Reis RM, Silva de Sá MF, Ferriani RA. Acarbose in obese patients with polycystic ovarian syndrome: a double-blind, randomized, placebo-controlled study. Hum Reprod. 2005;20:2396–401. https://doi.org/10.1093/humrep/dei104.
28. Baillargeon J-P, Carpentier A. Role of insulin in the hyperandrogenemia of lean women with polycystic ovary syndrome and normal insulin sensitivity. Fertil Steril. 2007;88:886–93. https://doi.org/10.1159/000052890.
29. Veldhuis JD, Strauss JF, Silavin SL, Kolp LA, Klase PA, Juchter D, et al. The role of cholesterol esterification in ovarian steroidogenesis: studies in cultured swine granulosa cells using a novel inhibitor of acyl coenzyme A: cholesterol acyltransferase. Endocrinology. 1985;116:25–30. https://doi.org/10.1210/endo-116-1-25.
30. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016;37:467–520. https://doi.org/10.1210/er.2015-1104.
31. Vrbíková J, Cibula D, Dvoráková K, Stanická S, Sindelka G, Hill M, et al. Insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2004;89:2942–5. https://doi.org/10.1210/jc.2003-031378.
32. Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Tapanainen JS. Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome. Hum Reprod. 2000;15:1266–74.
33. Ciampelli M, Fulghesu AM, Cucinelli F, Pavone V, Caruso A, Mancuso S, et al. Heterogeneity in beta cell activity, hepatic insulin clearance and peripheral insulin sensitivity in women with polycystic ovary syndrome. Hum Reprod. 1997;12:1897–901.
34. Baillargeon J-P, Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Nestler JE. Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. Fertil Steril. 2004;82:893–902. https://doi.org/10.1016/j.fertnstert.2004.02.127.
35. Baillargeon J-P, Nestler JE. Commentary: polycystic ovary syndrome: a syndrome of ovarian hypersensitivity to insulin? J Clin Endocrinol Metab. 2006;91:22–4. https://doi.org/10.1210/jc.2005-1804.
36. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev. 2002;23:201–29. https://doi.org/10.1210/edrv.23.2.0461.
37. Trottier A, Battista M-C, Geller DH, Moreau B, Carpentier AC, Simoneau-Roy J, et al. Adipose tissue insulin resistance in peripubertal girls with first-degree family history of polycystic ovary syndrome. Fertil Steril. 2012;98:1627–34. https://doi.org/10.1016/j.fertnstert.2012.08.025.
38. Villa J, Pratley RE. Adipose tissue dysfunction in polycystic ovary syndrome. Curr Diab Rep. 2011;11:179–84. https://doi.org/10.1007/s11892-011-0189-8.
39. Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K. Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertil Steril. 2009;91:213–9. https://doi.org/10.1016/j.fertnstert.2007.11.031.
40. Velázquez ME, Bellabarba GA, Mendoza S, Sánchez L. Postprandial triglyceride response in patients with polycystic ovary syndrome: relationship with waist-to-hip ratio and insulin. Fertil Steril. 2000;74:1159–63.

41. Vine DF, Wang Y, Jetha MM, Ball GD, Proctor SD. Impaired ApoB-Lipoprotein and Triglyceride Metabolism in Obese Adolescents With Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2017;102:970–82. https://doi.org/10.1210/jc.2016-2854.
42. Mai K, Bobbert T, Reinecke F, Andres J, Maser-Gluth C, Wudy SA, et al. Intravenous lipid and heparin infusion-induced elevation in free fatty acids and triglycerides modifies circulating androgen levels in women: a randomized, controlled trial. J Clin Endocrinol Metab. 2008;93:3900–6. https://doi.org/10.1210/jc.2008-0714.
43. Bellanger S, Battista M-C, Fink GD, Baillargeon J-P. Saturated fatty acid exposure induces androgen overproduction in bovine adrenal cells. Steroids. 2012;77:347–53. https://doi.org/10.1016/j.steroids.2011.12.017.
44. Leblanc S, Battista M-C, Noll C, Hallberg A, Gallo-Payet N, Carpentier AC, et al. Angiotensin II type 2 receptor stimulation improves fatty acid ovarian uptake and hyperandrogenemia in an obese rat model of polycystic ovary syndrome. Endocrinology. 2014;155:3684–93. https://doi.org/10.1210/en.2014-1185.
45. Kriketos AD, Greenfield JR, Peake PW, Furler SM, Denyer GS, Charlesworth JA, et al. Inflammation, insulin resistance, and adiposity: a study of first-degree relatives of type 2 diabetic subjects. Diabetes Care. 2004;27:2033–40.
46. Legro RS, Driscoll D, Strauss JF, Fox J, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci U S A. 1998;95:14956–60.
47. Zhao H, Lv Y, Li L, Chen Z-J. Genetic studies on polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol. 2016;37:56–65. https://doi.org/10.1016/j.bpobgyn.2016.04.002.
48. Baillargeon J-P, Carpentier AC. Brothers of women with polycystic ovary syndrome are characterised by impaired glucose tolerance, reduced insulin sensitivity and related metabolic defects. Diabetologia. 2007;50:2424–32. https://doi.org/10.1007/s00125-007-0831-9.
49. Sam S, Legro RS, Essah PA, Apridonidze T, Dunaif A. Evidence for metabolic and reproductive phenotypes in mothers of women with polycystic ovary syndrome. Proc Natl Acad Sci U S A. 2006;103:7030–5. https://doi.org/10.1073/pnas.0602025103.
50. Sam S, Legro RS, Bentley-Lewis R, Dunaif A. Dyslipidemia and metabolic syndrome in the sisters of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:4797–802. https://doi.org/10.1210/jc.2004-2217.
51. Yildiz BO, Yarali H, Oguz H, Bayraktar M. Glucose intolerance, insulin resistance, and hyperandrogenemia in first degree relatives of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88:2031–6. https://doi.org/10.1210/jc.2002-021499.
52. Essah PA, Nestler JE. The metabolic syndrome in polycystic ovary syndrome. J Endocrinol Invest. 2006;29:270–80. https://doi.org/10.1007/BF03345554.
53. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112:2735–52. https://doi.org/10.1161/CIRCULATIONAHA.105.169404.
54. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120:1640–5. https://doi.org/10.1161/CIRCULATIONAHA.109.192644.
55. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:1929–35. https://doi.org/10.1210/jc.2004-1045.
56. Glueck CJ, Papanna R, Wang P, Goldenberg N, Sieve-Smith L. Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. Metabolism. 2003;52(7):908–15.
57. Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening women with polycystic ovary syndrome for metabolic syndrome. Obstet Gynecol. 2005;106:131–7. https://doi.org/10.1097/01.AOG.0000167408.30893.6b.
58. Vrbíková J, Vondra K, Cibula D, Dvoráková K, Stanická S, Srámková D, et al. Metabolic syndrome in young Czech women with polycystic ovary syndrome. Hum Reprod. 2005;20(12):3328–32.
59. Vural B, Caliskan E, Turkoz E, Kilic T, Demirci A. Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome. Hum Reprod. 2005;20:2409–13. https://doi.org/10.1093/humrep/dei100.
60. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN, et al. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91:48–53. https://doi.org/10.1210/jc.2005-1329.
61. Sharma S, Majumdar A. Prevalence of metabolic syndrome in relation to body mass index and polycystic ovarian syndrome in Indian women. J Hum Reprod Sci. 2015;8(4):202–8.
62. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome:

a systematic review and meta-analysis. Hum Reprod Update. 2010;16:347–63. https://doi.org/10.1093/humupd/dmq001.
63. Álvarez-Blasco F, Botella-Carretero JI, San Millán JL, Escobar-Morreale HF. Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women. Arch Intern Med. 2006;166:2081. https://doi.org/10.1001/archinte.166.19.2081.
64. Faloia E, Canibus P, Gatti C, Frezza F, Santangelo M, Garrapa GGM, et al. Body composition, fat distribution and metabolic characteristics in lean and obese women with polycystic ovary syndrome. J Endocrinol Invest. 2004;27:424–9. https://doi.org/10.1007/BF03345285.
65. Attaoua R, Ait El Mkadem S, Radian S, Fica S, Hanzu F, Albu A, et al. FTO gene associates to metabolic syndrome in women with polycystic ovary syndrome. Biochem Biophys Res Commun. 2008;373:230–4. https://doi.org/10.1016/j.bbrc.2008.06.039.
66. Shroff R, Syrop CH, Davis W, Van Voorhis BJ, Dokras A. Risk of metabolic complications in the new PCOS phenotypes based on the Rotterdam criteria. Fertil Steril. 2007;88:1389–95. https://doi.org/10.1016/j.fertnstert.2007.01.032.
67. Gulcelik NE, Aral Y, Serter R, Koc G. Association of hypoadiponectinemia with metabolic syndrome in patients with polycystic ovary syndrome. J Natl Med Assoc. 2008;100:64–8. https://doi.org/10.1016/S0027-9684(15)31176-7.
68. Ezeh U, Yildiz BO, Azziz R. Referral bias in defining the phenotype and prevalence of obesity in polycystic ovary syndrome. J Clin Endocrinol Metab. 2013;98:E1088–96. https://doi.org/10.1210/jc.2013-1295.
69. Rossi B, Sukalich S, Droz J, Griffin A, Cook S, Blumkin A, et al. Prevalence of metabolic syndrome and related characteristics in obese adolescents with and without polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:4780–6. https://doi.org/10.1210/jc.2008-1198.
70. Coviello AD, Sam S, Legro RS, Dunaif A. High prevalence of metabolic syndrome in first-degree male relatives of women with polycystic ovary syndrome is related to high rates of obesity. J Clin Endocrinol Metab. 2009;94:4361–6. https://doi.org/10.1210/jc.2009-1333.
71. Lizneva D, Kirubakaran R, Mykhalchenko K, Suturina L, Chernukha G, Diamond MP, et al. Phenotypes and body mass in women with polycystic ovary syndrome identified in referral versus unselected populations: systematic review and meta-analysis. Fertil Steril. 2016;106:1510–1520.e2. https://doi.org/10.1016/j.fertnstert.2016.07.1121.
72. Yildiz BO, Knochenhauer ES, Azziz R. Impact of obesity on the risk for polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:162–8. https://doi.org/10.1210/jc.2007-1834.
73. Barber TM, Golding SJ, Alvey C, Wass JAH, Karpe F, Franks S, et al. Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:999–1004. https://doi.org/10.1210/jc.2007-2117.
74. Mannerås-Holm L, Leonhardt H, Kullberg J, Jennische E, Odén A, Holm G, et al. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. J Clin Endocrinol Metab. 2011;96:E304–11. https://doi.org/10.1210/jc.2010-1290.
75. Jin CH, Yuk JS, Choi KM, Yi KW, Kim T, Hur JY, et al. Body fat distribution and its associated factors in Korean women with polycystic ovary syndrome. J Obstet Gynaecol Res. 2015;41:1577–83. https://doi.org/10.1111/jog.12767.
76. Dolfing JG, Stassen CM, van Haard PMM, Wolffenbuttel BHR, Schweitzer DH. Comparison of MRI-assessed body fat content between lean women with polycystic ovary syndrome (PCOS) and matched controls: less visceral fat with PCOS. Hum Reprod. 2011;26:1495–500. https://doi.org/10.1093/humrep/der070.
77. Wild RA, Rizzo M, Clifton S, Carmina E. Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis. Fertil Steril. 2011;95:1073-9.e1–11. https://doi.org/10.1016/j.fertnstert.2010.12.027.
78. Toulis KA, Goulis DG, Mintziori G, Kintiraki E, Eukarpidis E, Mouratoglou SA, et al. Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. Hum Reprod Update. 2011;17:741–60. https://doi.org/10.1093/humupd/dmr025.
79. Enkhmaa B, Anuurad E, Zhang W, Abbuthalha A, Kaur P, Visla J, et al. Lipoprotein(a) and apolipoprotein(a) in polycystic ovary syndrome. Clin Endocrinol (Oxf). 2016;84:229–35. https://doi.org/10.1111/cen.12937.
80. Berneis K, Rizzo M, Hersberger M, Rini GB, Di Fede G, Pepe I, et al. Atherogenic forms of dyslipidaemia in women with polycystic ovary syndrome. Int J Clin Pract. 2009;63:56–62. https://doi.org/10.1111/j.1742-1241.2008.01897.x.
81. Elting MW, Korsen TJM, Bezemer PD, Schoemaker J. Prevalence of diabetes mellitus, hypertension and cardiac complaints in a follow-up study of a Dutch PCOS population. Hum Reprod. 2001;16:556–60.
82. Cõ R, Jirkovska A, La V, Platilova H, Zamrazil V. Cardiovascular risk factors in young Czech females with polycystic ovary syndrome. Hum Reprod. 2003;18:980–4. https://doi.org/10.1093/humrep/deg218.
83. Yu H-F, Chen H-S, Rao D-P, Gong J. Association between polycystic ovary syndrome and the risk of pregnancy complications: a PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore). 2016;95:e4863. https://doi.org/10.1097/MD.0000000000004863.

84. Holte J, Gennarelli G, Berne C, Bergh T, Lithell H. Elevated ambulatory day-time blood pressure in women with polycystic ovary syndrome: a sign of a pre-hypertensive state? Hum Reprod. 1996;11:23–8.
85. Luque-Ramírez M, Alvarez-Blasco F, Mendieta-Azcona C, Botella-Carretero JI, Escobar-Morreale HF. Obesity is the major determinant of the abnormalities in blood pressure found in young women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92:2141–8. https://doi.org/10.1210/jc.2007-0190.
86. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol Metab. 2006;91:492–7. https://doi.org/10.1210/jc.2005-1666.
87. Patel SS, Truong U, King M, Ferland A, Moreau KL, Dorosz J, et al. Obese adolescents with polycystic ovarian syndrome have elevated cardiovascular disease risk markers. Vasc Med. 2017;22(2):85–95. https://doi.org/10.1177/1358863X16682107.
88. Ollila M-ME, West S, Keinänen-Kiukaanniemi S, Jokelainen J, Auvinen J, Puukka K, et al. Overweight and obese but not normal weight women with PCOS are at increased risk of Type 2 diabetes mellitus—a prospective, population-based cohort study. Hum Reprod. 2017;32:423–31. https://doi.org/10.1093/humrep/dew329.
89. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 1999;84:165–9. https://doi.org/10.1210/jcem.84.1.5393.
90. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. J Clin Endocrinol Metab. 2005;90:3236–42. https://doi.org/10.1210/jc.2004-1843.
91. Boudreaux MY, Talbott EO, Kip KE, Brooks MM, Witchel SF. Risk of T2DM and impaired fasting glucose among PCOS subjects: results of an 8-year follow-up. Curr Diab Rep. 2006;6:77–83.
92. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999;22:141–6.
93. Weerakiet S, Srisombut C, Bunnag P, Sangtong S, Chuangsoongnoen N, Rojanasakul A. Prevalence of type 2 diabetes mellitus and impaired glucose tolerance in Asian women with polycystic ovary syndrome. Int J Gynaecol Obstet. 2001;75:177–84.
94. Talbott EO, Zborowski JV, Rager JR, Kip KE, Xu X, Orchard TJ. Polycystic ovarian syndrome (PCOS): a significant contributor to the overall burden of type 2 diabetes in women. J Womens Health (Larchmt). 2007;16:191–7. https://doi.org/10.1089/jwh.2006.0098.
95. Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988–1994 and 2005–2006. Diabetes Care. 2009;32:287–94. https://doi.org/10.2337/dc08-1296.
96. Peppard HR, Marfori J, Iuorno MJ, Nestler JE. Prevalence of polycystic ovary syndrome among premenopausal women with type 2 diabetes. Diabetes Care. 2001;24:1050–2.
97. Conn JJ, Jacobs HS, Conway GS. The prevalence of polycystic ovaries in women with type 2 diabetes mellitus. Clin Endocrinol (Oxf). 2000;52:81–6.
98. Cussons AJ, Stuckey BGA, Watts GF. Cardiovascular disease in the polycystic ovary syndrome: new insights and perspectives. Atherosclerosis. 2006;185:227–39. https://doi.org/10.1016/j.atherosclerosis.2005.10.007.
99. Randeva HS, Tan BK, Weickert MO, Lois K, Nestler JE, Sattar N, et al. Cardiometabolic aspects of the polycystic ovary syndrome. Endocr Rev. 2012;33:812–41. https://doi.org/10.1210/er.2012-1003.
100. Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation. 2000;102:42–7.
101. Haffner SM, Mykkänen L, Festa A, Burke JP, Stern MP. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. Circulation. 2000;101:975–80.
102. Sprung VS, Atkinson G, Cuthbertson DJ, Pugh CJA, Aziz N, Green DJ, et al. Endothelial function measured using flow-mediated dilation in polycystic ovary syndrome: a meta-analysis of the observational studies. Clin Endocrinol (Oxf). 2013;78:438–46. https://doi.org/10.1111/j.1365-2265.2012.04490.x.
103. Meyer ML, Malek AM, Wild RA, Korytkowski MT, Talbott EO. Carotid artery intima-media thickness in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2012;18:112–26. https://doi.org/10.1093/humupd/dmr046.
104. Vryonidou A, Papatheodorou A, Tavridou A, Terzi T, Loi V, Vatalas I-A, et al. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:2740–6. https://doi.org/10.1210/jc.2004-2363.
105. Shroff R, Kerchner A, Maifeld M, Van Beek EJR, Jagasia D, Dokras A. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. J Clin Endocrinol Metab. 2007;92:4609–14. https://doi.org/10.1210/jc.2007-1343.

106. Talbott EO, Zborowski JV, Rager JR, Boudreaux MY, Edmundowicz DA, Guzick DS. Evidence for an association between metabolic cardiovascular syndrome and coronary and aortic calcification among women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2004;89:5454–61. https://doi.org/10.1210/jc.2003-032237.
107. Talbott EO, Zborowski J, Rager J, Stragand JR. Is there an independent effect of polycystic ovary syndrome (PCOS) and menopause on the prevalence of subclinical atherosclerosis in middle aged women? Vasc Health Risk Manag. 2008;4:453–62.
108. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF, Fitzpatrick LA. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88:2562–8. https://doi.org/10.1210/jc.2003-030334.
109. Orio F, Palomba S, Spinelli L, Cascella T, Tauchmanovà L, Zullo F, et al. The cardiovascular risk of young women with polycystic ovary syndrome: an observational, analytical, prospective case-control study. J Clin Endocrinol Metab. 2004;89:3696–701. https://doi.org/10.1210/jc.2003-032049.
110. Wang ET, Ku IA, Shah SJ, Daviglus ML, Schreiner PJ, Konety SH, et al. Polycystic ovary syndrome is associated with higher left ventricular mass index: The CARDIA Women's Study. J Clin Endocrinol Metab. 2012;97:4656–62. https://doi.org/10.1210/jc.2012-1597.
111. Yarali H, Yildirir A, Aybar F, Kabakçi G, Bükülmez O, Akgül E, et al. Diastolic dysfunction and increased serum homocysteine concentrations may contribute to increased cardiovascular risk in patients with polycystic ovary syndrome. Fertil Steril. 2001;76:511–6.
112. Chang AY, Ayers C, Minhajuddin A, Jain T, Nurenberg P, de Lemos JA, et al. Polycystic ovarian syndrome and subclinical atherosclerosis among women of reproductive age in the Dallas heart study. Clin Endocrinol (Oxf). 2011;74:89–96. https://doi.org/10.1111/j.1365-2265.2010.03907.x.
113. de Groot PCM, Dekkers OM, Romijn JA, Dieben SWM, Helmerhorst FM. PCOS, coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis. Hum Reprod Update. 2011;17:495–500. https://doi.org/10.1093/humupd/dmr001.
114. Shaw LJ, Bairey Merz CN, Azziz R, Stanczyk FZ, Sopko G, Braunstein GD, et al. Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health–National Heart, Lung, and Blood Institute sponsored Women's Ischemia Syndrome Evaluation. J Clin Endocrinol Metab. 2008;93:1276–84. https://doi.org/10.1210/jc.2007-0425.
115. Solomon CG, Hu FB, Dunaif A, Rich-Edwards JE, Stampfer MJ, Willett WC, et al. Menstrual cycle irregularity and risk for future cardiovascular disease. J Clin Endocrinol Metab. 2002;87:2013–7. https://doi.org/10.1210/jcem.87.5.8471.
116. Rocha ALL, Faria LC, Guimarães TCM, Moreira GV, Cândido AL, Couto CA, et al. Non – alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta – analysis. J Endocrinol Invest. 2017;40(12):1279–88. https://doi.org/10.1007/s40618-017-0708-9.
117. Jacome-Sosa MM, Parks EJ. Fatty acid sources and their fluxes as they contribute to plasma triglyceride concentrations and fatty liver in humans. Curr Opin Lipidol. 2014;25:213–20. https://doi.org/10.1097/MOL.0000000000000080.
118. Sunny NE, Bril F, Cusi K. Mitochondrial adaptation in nonalcoholic fatty liver disease: novel mechanisms and treatment strategies. Trends Endocrinol Metab. 2017;28:250–60. https://doi.org/10.1016/j.tem.2016.11.006.
119. Belan M, Pelletier C, Baillargeon J-P. Alanine aminotransferase is a marker of lipotoxicity consequences and hyperandrogenemia in women with polycystic ovary syndrome. Metab Syndr Relat Disord. 2017;15(3):145–52. https://doi.org/10.1089/met.2016.0119.
120. Chen M-J, Chiu H-M, Chen C-L, Yang W-S, Yang Y-S, Ho H-N. Hyperandrogenemia is independently associated with elevated alanine aminotransferase activity in young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2010;95:3332–41. https://doi.org/10.1210/jc.2009-2698.
121. Jie C, Chunhua W, Yi Z, Yuying W, Wendi X, Tzuchun L, et al. High free androgen index is associated with increased risk of non-alcoholic fatty liver disease in women with polycystic ovary syndrome, independently of obesity and insulin resistance. Int J Obes. 2017;49:1341–7. https://doi.org/10.1038/ijo.2017.116.
122. Vgontzas AN, Bixler EO, Chrousos GP. Metabolic disturbances in obesity versus sleep apnoea: the importance of visceral obesity and insulin resistance. J Intern Med. 2003;254:32–44.
123. Kahal H, Kyrou I, Tahrani AA, Randeva HS. Obstructive sleep apnoea and polycystic ovary syndrome: a comprehensive review of clinical interactions and underlying pathophysiology. Clin Endocrinol (Oxf). 2017;87:313–9. https://doi.org/10.1111/cen.13392.
124. Fogel RB, Malhotra A, Pillar G, Pittman SD, Dunaif A, White DP. Increased prevalence of obstructive sleep apnea syndrome in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2001;86:1175–80. https://doi.org/10.1210/jcem.86.3.7316.
125. Tasali E, Van Cauter E, Ehrmann DA. Polycystic ovary syndrome and obstructive sleep apnea. Sleep Med Clin. 2008;3:37–46. https://doi.org/10.1016/j.jsmc.2007.11.001.
126. Tasali E, Van Cauter E, Hoffman L, Ehrmann DA. Impact of obstructive sleep apnea on insulin

resistance and glucose tolerance in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:3878–84. https://doi.org/10.1210/jc.2008-0925.
127. Nandalike K, Agarwal C, Strauss T, Coupey SM, Isasi CR, Sin S, et al. Sleep and cardiometabolic function in obese adolescent girls with polycystic ovary syndrome. Sleep Med. 2012;13:1307–12. https://doi.org/10.1016/j.sleep.2012.07.002.
128. Salley KES, Wickham EP, Cheang KI, Essah PA, Karjane NW, Nestler JE. POSITION STATEMENT: glucose intolerance in polycystic ovary syndrome—a position statement of the Androgen Excess Society. J Clin Endocrinol Metab. 2007;92:4546–56. https://doi.org/10.1210/jc.2007-1549.
129. Schroeder BM, American College of Obstetricians and Gynecologists. ACOG releases guidelines on diagnosis and management of polycystic ovary syndrome. Am Fam Physician. 2003;67:1619–20, 1622
130. American Association of Clinical Endocrinologists Polycystic Ovary Syndrome Writing Committee R, Futterweit W, Nestler J, Reaven G, Jellinger P, Handelsman Y, et al. American Association of Clinical Endocrinologists Position Statement on metabolic and cardiovascular consequences of polycystic ovary syndrome. Endocr Pract. 2005;11:126–34. https://doi.org/10.4158/EP.11.2.125.
131. Gagnon C, Baillargeon J-P. Suitability of recommended limits for fasting glucose tests in women with polycystic ovary syndrome. Can Med Assoc J. 2007;176:933–8. https://doi.org/10.1503/cmaj.060607.
132. Fauser BCJM, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod. 2012;27:14–24. https://doi.org/10.1093/humrep/der396.
133. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ, International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110(3):364–79.
134. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346:393–403. https://doi.org/10.1056/NEJMoa012512.
135. Magkos F, Fraterrigo G, Yoshino J, Okunade AL, Patterson BW, Klein S, et al. Clinical and translational report effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity clinical and translational report effects of moderate and subsequent progressive weight loss. Cell Metab. 2016:591–601. https://doi.org/10.1016/j.cmet.2016.02.005.
136. Weiss EP, Albert SG, Reeds DN, Kress KS, McDaniel JL, Klein S, et al. Effects of matched weight loss from calorie restriction, exercise, or both on cardiovascular disease risk factors: a randomized intervention trial. Am J Clin Nutr. 2016;104:576–86. https://doi.org/10.3945/ajcn.116.131391.
137. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. JAMA. 1989;262:2395–401.
138. Martin KA, Chang RJ, Ehrmann DA, Ibanez L, Lobo RA, Rosenfield RL, et al. Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008;93:1105–20. https://doi.org/10.1210/jc.2007-2437.
139. Watanabe RM, Azen CG, Roy S, Perlman JA, Bergman RN. Defects in carbohydrate metabolism in oral contraceptive users without apparent metabolic risk factors. J Clin Endocrinol Metab. 1994;79:1277–83. https://doi.org/10.1210/jcem.79.5.7962320.
140. Cagnacci A, Ferrari S, Tirelli A, Zanin R, Volpe A. Insulin sensitivity and lipid metabolism with oral contraceptives containing chlormadinone acetate or desogestrel: a randomized trial. Contraception. 2009;79:111–6. https://doi.org/10.1016/j.contraception.2008.09.002.
141. Cagnacci A, Ferrari S, Tirelli A, Zanin R, Volpe A. Route of administration of contraceptives containing desogestrel/etonogestrel and insulin sensitivity: a prospective randomized study. Contraception. 2009;80:34–9. https://doi.org/10.1016/j.contraception.2009.01.012.
142. Lidegaard Ø, Løkkegaard E, Jensen A, Skovlund CW, Keiding N. Thrombotic stroke and myocardial infarction with hormonal contraception. N Engl J Med. 2012;366:2257–66. https://doi.org/10.1056/NEJMoa1111840.
143. Baillargeon J-P, McClish DK, Essah PA, Nestler JE. Association between the current use of low-dose oral contraceptives and cardiovascular arterial disease: a meta-analysis. J Clin Endocrinol Metab. 2005;90:3863–70. https://doi.org/10.1210/jc.2004-1958.
144. Al Khalifah RA, Florez ID, Dennis B, Thabane L, Bassilious E. Metformin or oral contraceptives for adolescents with polycystic ovarian syndrome: a meta-analysis. Pediatrics. 2016;137:e20154089. https://doi.org/10.1542/peds.2015-4089.
145. Tang T, Norman RJ, Balen AH, Lord JM. Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. In: Tang T, editor. Cochrane database system review. Chichester, Wiley; 2003. p. CD003053. https://doi.org/10.1002/14651858.CD003053.
146. Nesti L, Natali A. Metformin effects on the heart and the cardiovascular system: a review of experimental and clinical data. Nutr Metab Cardiovasc Dis. 2017;27:657–69. https://doi.org/10.1016/j.numecd.2017.04.009.

147. Costello MF, Shrestha B, Eden J, Johnson N, Moran LJ. Insulin-sensitising drugs versus the combined oral contraceptive pill for hirsutism, acne and risk of diabetes, cardiovascular disease, and endometrial cancer in polycystic ovary syndrome. In: Costello MF, editor. Cochrane database system review. Chichester: Wiley; 2007. p. CD005552. https://doi.org/10.1002/14651858.CD005552.pub2.
148. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352:854–65. https://doi.org/10.1016/S0140-6736(98)07037-8.
149. Essah PA, Apridonidze T, Iuorno MJ, Nestler JE. Effects of short-term and long-term metformin treatment on menstrual cyclicity in women with polycystic ovary syndrome. Fertil Steril. 2006;86:230–2. https://doi.org/10.1016/j.fertnstert.2005.12.036.
150. Harborne L, Fleming R, Lyall H, Sattar N, Norman J. Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88:4116–23. https://doi.org/10.1210/jc.2003-030424.

Part IV

Treatment

17 Is Insulin Resistance a Treatment Target?

Thomas Reinehr

Introduction

As in adulthood, obesity in childhood contributes to an increased prevalence of cardiovascular risk factors, such as hypertension, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, and impaired glucose metabolism [1–5] (see Table 17.1). The clustering of these risk factors is summarized in the definition of metabolic syndrome (MetS), which is associated with atherosclerosis and cardiovascular diseases leading to increased morbidity and mortality [6–12].

Insulin resistance is regarded as the key mechanism in MetS linking obesity to cardiovascular risk factors [6, 13] and type 2 diabetes mellitus (T2D), in both adults and children [14–16]. Confirmatory factor analysis of adult data suggests one pathophysiological mechanism underlying all cardiovascular risk factors summarized in the definition of MetS is insulin resistance [17, 18]. Other diseases linked to insulin resistance are polycystic ovarian syndrome (PCOS) [19] and nonalcoholic fatty liver disease (NAFLD), which are also associated with MetS [20].

Recently, based on this observation, pediatricians have increasingly been treating insulin resistance using lifestyle interventions and drugs, such as metformin [21]. Indeed, some studies in adolescents demonstrated a positive effect of metformin on insulin resistance and associated cardiovascular risk factors [22, 23]. However, other randomized controlled trials have reported no effect [24, 25]. Therefore, before basing treatment recommendations on the concept of insulin resistance itself, some shortcomings of its definitions and its measurement in children and adolescents have to be kept in mind, which will be discussed in the following.

Definition of Insulin Resistance

Insulin resistance is defined as the decreased tissue response to insulin-mediated cellular actions. Therefore, insulin resistance means an impairment of insulin action leading to reduced whole-body glucose uptake in response to physiological insulin levels [14]. This is manifested by decreased insulin-stimulated glucose uptake in skeletal muscle and adipose tissue and impaired suppression of hepatic glucose output through glycogenolysis. Insulin resistance results in compensatory increase in insulin secretion. The resulting hyperinsulinemia overcomes the insulin resistance for a while and keeps blood glucose in the normal range. However, when relative

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Table 17.1 Prevalence of cardiovascular risk factors in 26,008 non-Hispanic white children

	Normal weight (BMI > 10th–<90th percentile)	Overweight (BMI 90th–< 97th percentile)	Obese (BMI 97th–< 99.5th percentile)	Extreme obese (BMI ≥ 99.5th percentile)
Hypertension	5%	7%	12%	26%
HDL cholesterol <0.91 mmol/l	2%	4%	10%	15%
Triglycerides >1.7 mmol/l	8%	10%	14%	16%
Impaired fasting glucose	1%	1%	1%	4%
Impaired glucose tolerance	0%	5%	6%	8%
Type 2 diabetes mellitus	0%	0.1%	0.2%	0.4%
Clustering of cardiovascular risk factors	0%	5%	10%	15%

Adapted from [1]
BMI body mass index, *HDL* high-density lipoprotein

β(beta)-cell insufficiency (i.e., insulin secretion insufficient for the level of hyperglycemia) also sets in, overt diabetes develops. Furthermore, insulin resistance is associated with deterioration in fat metabolism [26, 27].

Underlying Mechanism of Insulin Resistance

The mechanisms of insulin resistance involve defects in insulin signaling, skeletal muscle and adipose triglyceride and fatty acid metabolism, glucose uptake, and glucose metabolism, among others. Adipose tissue cytokines, such as adiponectin and leptin, and other obesity genes may be involved too [28]. In humans, euglycemic hyperinsulinemic clamp studies have shown that insulin resistance is determined primarily by the response of skeletal muscle, with more than 75% of infused glucose taken up by muscle and only 2–3% by adipose tissue [29]. Independent of the relation between total body fat and insulin resistance, increased abdominal visceral adipose tissue in obese youth is associated with lower insulin sensitivity and higher acute insulin response [30].

Clinical Picture of Insulin Resistance

Insulin resistance is frequently associated with the presence of acanthosis nigricans, as well as the occurrence of cardiovascular risk factors summarized in the definition of MetS. Acanthosis nigricans is a condition of the skin presenting as pigmentation along with verrucous hypertrophy [31]. A typical clinical presentation of insulin resistance is mild hypertriglyceridemia combined with low HDL cholesterol levels and increased blood pressure [26, 32]. However, these clinical features cannot define or quantify insulin resistance [14].

Measurement of Insulin Resistance

There is an ongoing discussion about how insulin resistance in childhood is best assessed. Standards for measurements of insulin resistance in children have not been established so far [14]. This is due, in part, to the use of a variety of techniques to measure insulin sensitivity, lack of sufficient cohort sizes to establish normative distributions for insulin sensitivity, and lack of adequate longitudinal studies to relate definitions of insulin resistance to long-term outcomes.

The euglycemic hyperinsulinemic clamp is the "gold standard" for measuring insulin sensitivity [14]. The frequently sampled intravenous glucose tolerance test (FSIVGTT) and steady-state plasma glucose (SSPG) methods are also valid measurements [14]. However, each of these methods is time consuming and costly, requires IV infusions and frequent blood sampling in a research setting, and is burdensome for participants. Therefore, these techniques are useful for research but not for clinical practice.

Clinicians prefer simple tools, such as fasting glucose. However, fasting glucose showed only a weak correlation to continuously measured blood glucose and insulin resistance [33]. Impaired glucose tolerance (IGT) demonstrated a better association with continuously measured blood glucose and insulin resistance [33]. However, the reproducibility of pathological glucose levels in oral glucose tolerance tests is low, therefore limiting its value on an individual level [32, 34, 35]. It has been suggested that hemoglobin A1c (HbA1c) may be a better parameter to describe insulin resistance, since it demonstrates the best correlation to continuous glucose measurements [33]. However, there are no studies in childhood proving the relationship between insulin resistance and HbA1c. Likely, all parameters—such as fasting glucose, glucose in oral glucose tolerance tests, and HbA1c levels—that depend on the measurement of glucose without linking to simultaneous insulin concentrations will fail to describe insulin resistance since there is a narrow range of fasting glucose even among obese children with insulin resistance due to compensatory increased insulin secretion that maintains euglycemia until β(beta)-cell decompensation occurs [21]. Thus, glucose-based measures are more reflective of β(beta)-cell function.

Fasting insulin levels are also not a reliable tool for individual assessment of insulin resistance [14, 36]. The accuracy of fasting insulin as a measure of insulin sensitivity has been compared through correlation analyses with the euglycemic hyperinsulinemic clamp, FSIVGTT, and SSPG and found to be disappointingly poor [37–39]. The value of fasting insulin is limited by great intra- and interindividual variability [36]. Insulin secretion is pulsatile and normal values change physiologically based on pubertal stage [40], making them difficult to interpret in adolescents. Furthermore, the insulin assay itself can be a source of error; testing of aliquots of a common sample assayed in different laboratories has shown disparate results [41]. Sample processing can also introduce error, as insulin levels decrease rapidly if the sample is not frozen [36]. In conclusion, fasting insulin is an unreliable clinical measure of insulin resistance in an individual child and should not be used for decision making in daily practice [14].

The homeostasis model assessment (HOMA)

$$\mathrm{HOMA} = \mathrm{Insulin}\left[\mathrm{mU/l}\right] \times \mathrm{Glucose}\left[\mathrm{mmol/l}\right] / 22.5$$

and the quantitative insulin-sensitivity check index (QUICKI)

$$\mathrm{QUICKI} = 1 / \left(\log\left(\mathrm{Glucose}\left[\mathrm{mg/dl}\right]\right) + \log\left(\mathrm{Insulin}\left[\mu\{\mathrm{mu}\}\,\mathrm{U/ml}\right]\right)\right)$$

are alternative diagnostic tests for insulin resistance that need only a simultaneous fasting determination of glucose and insulin [42, 43]. Both indices use a mathematical formula that adjusts for individual variability in insulin and glucose secretion and clearance. Although the goal of these methods was to improve the accuracy of fasting insulin as a measure of insulin resistance by the addition of fasting glucose, it is now agreed that they yield similar results to fasting insulin alone. For instance, HOMA, the most widely used of the surrogate measures in children, is highly correlated with fasting insulin (r = 0.95) in children [37]. These high correlations can be attributed to the narrow range of fasting glucose, even among insulin-resistant children [21, 37]. In contrast, there is a 53-fold variation in fasting insulin in children with and without insulin resistance [37]. Therefore, on an individual level HOMA and QUICKI have the same problems of reliability as described above for fasting insulin levels.

A further less-intensive method, the measurement of insulin during the oral glucose tolerance test (OGTT), offers the advantage of a smaller number of blood samples. Based on these measurements, indices such as ISI Cederholm

$$\text{ISI Cederholm} = \left(75{,}000 + \text{glucose 0 min}\left[\text{mmol/l}\right] - \text{glucose 120 min}\left[\text{mmol/l}\right]\right) \times 1.15 \times 180 \times 0.19 \times \text{weight}\left[\text{kg}\right]) / (120 \times \text{mean glucose}\left[\text{mmol/l}\right] \times \log\left(\text{mean insulin}\left[\text{pmol/l}\right]\right)$$

are calculated [44]. Good correlations were reported in adult studies comparing OGTT-based indices with the euglycemic hyperinsulinemic clamp [45]. However, the correlation in children is less clear. First reports in a small group of obese children reported only moderate correlations [46]. Finally, this index, as well as other indices such as HOMA, cannot distinguish between peripheral and hepatic insulin resistance [36].

Using the calculation of HOMA and QUICKI as models, a formula using fat metabolism has been suggested to describe insulin resistance [26, 27]. Instead of glucose, free fatty acids (FFAs) were used for the mathematical calculation of insulin resistance. While these calculations correlated well with cardiovascular risk factors [26, 27], the same problems seen for HOMA and QUICKI were also true for these calculations, since the great majority of variability is accounted for by the insulin levels, while the FFA levels differ to a lesser degree between insulin-resistant and nonresistant humans.

In summary, an accurate assessment of insulin resistance requires an invasive and impractical test (e.g., the hyperinsulinemic euglycemic clamp technique). The Insulin Resistance in Children Consensus Conference Group stated that there is no justification for screening children for insulin resistance by HOMA, QUICKI, fasting insulin, or other indices [14]. However, having a uniform internationally accepted definition of insulin resistance that can be measured under clinical conditions would be very helpful for the description of populations in different research studies.

Influence Factors on Insulin Resistance

The two most important biological conditions associated with insulin resistance in childhood besides obesity are ethnicity and puberty [14]. In studies of adult twins, approximately half of the variance in insulin sensitivity and secretion can be attributed to genetic factors [47]. Healthy children with a family history of type 2 diabetes mellitus are more insulin resistant, with an impaired balance between insulin sensitivity and secretion [48]. Using a variety of methods, studies show that non-Hispanic black, Hispanic, Pima Indian, and Asian children are less insulin sensitive than non-Hispanic white children [49, 50]. The insulin resistance in minority ethnic groups is manifested as lower insulin-stimulated glucose uptake, concomitant with hyperinsulinemia, evidence of increased insulin secretion from the β(beta)-cell and decreased insulin clearance [49, 50].

Pubertal stage has been identified as an additional major influence factor on insulin resistance. Puberty onset is characterized by a physiological ~30% reduction of insulin sensitivity that is reversed when puberty is complete [51–54]. Furthermore, impaired glucose tolerance and impaired fasting glucose are also more frequent in pubertal obese adolescents compared to prepubertal children [55] and normalize at the end of puberty [56]. In a longitudinal study in 253 overweight Hispanic youths, insulin resistance increased in both sexes in early puberty with a recovery in late puberty [57]. In non-Hispanic white children, the same changes of insulin resistance during puberty have been reported [58]. Pinhas-Hamiel and colleagues reported an increase of insulin levels during puberty [59]. A rise of insulin resistance has been reported already before puberty when adrenarche starts [60, 61]. Moreover, it has been reported that insulin resistance increases during puberty in obese children more than in normal-weight children [62–64]. Furthermore, it is well-known that glucose metabolism frequently deteriorates during puberty in children suffering from type 1 diabetes mellitus and improves at the end of puberty [65].

The reasons for changes of insulin resistance during puberty are not yet well-understood. The

increase in growth hormone, sex hormone, and insulin-like growth factor-1 (IGF-1) that occurs during puberty is thought to be the cause of this form of insulin resistance [66]. Furthermore, puberty has an effect on the fat oxidation rates during exercise in both overweight and normal-weight girls resulting in increased insulin resistance [67]. A temporal relationship between insulin resistance and the pubertal decrease in physical activity in peripubertal Hispanic and non-Hispanic black females has been reported [68]. Furthermore, concentrations of sex hormones, adipocytokines, and inflammatory cytokines change dramatically during pubertal development, making an influence on insulin resistance probable [69, 70]: Adiponectin concentrations had been negatively correlated to many cardiovascular risk factors and decrease with onset of puberty in males [71]. An association between insulin resistance and leptin has also been reported [72]. Sex hormone binding globulin levels also predict insulin resistance and cardiovascular risk factors during puberty [73]. However, the observed relationships between various adipocytokines and insulin resistance during puberty were weak in longitudinal studies, suggesting additional important influences [69].

Interestingly, puberty is also influenced by insulin resistance. In mouse models, an interaction between insulin and leptin signaling was reported during the peripubertal period in the neurons responsible for pubertal development [74]. Furthermore, a study in obese children reported advanced onset of puberty after metformin therapy—a drug that decreases insulin resistance [75]. Therefore, there seems to be a bidirectional interaction between insulin resistance and puberty.

The compensatory increase in insulin secretion during puberty may be blunted in non-Hispanic and Hispanic youths, thus increasing their risk for T2D around the time of puberty [21, 76–78]. This points toward genetic factors modulating both insulin resistance and insulin secretion [78, 79].

Furthermore, other factors influencing insulin resistance have been identified in children. Hormones secreted by the muscle, such as irisin, are also related to insulin resistance [80]. A chronic inflammatory process is also likely involved in the relationship between obesity and insulin resistance, since inflammation increases insulin resistance through different pathways [81]. A disturbed secretion of adipocytokines and inflammatory markers could be observed especially in mesenteric fat [72, 81]. Therefore, it is not surprising that some studies in children and adolescents reported a stronger correlation between waist circumference and insulin resistance than the correlation between body mass index (BMI) and insulin resistance [82–85].

Normal Values for Insulin Resistance

There are currently no internationally accepted normal values for insulin concentrations in children analyzed by age, sex, pubertal stage, and genetic background available. This lack of normal values makes it difficult even for research measurements using rigorous approaches—such as euglycemic hyperinsulinemic clamp, the frequently sampled IV glucose tolerance test, and steady-state plasma glucose—to define at which exact cut point insulin resistance starts. Normal values for HOMA have been reported [40], but the proposed cutoffs are not adjusted for pubertal stage. Normal values for insulin adapted to pubertal stage have been proposed (for example, definition of insulin resistance by fasting insulin: ≥15 mU/l prepubertal, ≥30 mU/l pubertal, ≥20 mU/l late/postpubertal) [77]. However, studies validating these proposed cutoffs are missing.

Insulin Resistance as an Independent Cardiovascular Risk Factor

Classical cardiovascular risk factors—such as hypertension, dyslipidemia, and impaired glucose tolerance—are related to morbidity and mortality of obesity [15, 86]. Even though they are all based at least in part on insulin resistance,

the use of insulin resistance itself as a treatment goal, rather than management of classical cardiovascular risk factors, is only meaningful if insulin resistance is itself related to morbidity and mortality.

An independent effect of insulin resistance on cardiovascular risk in children has been suggested. Fasting insulin levels in 6- to 9-year-old children predicted their blood pressure at age 9–15 years [87]; and in 5- to 9-year-old Pima Indian children, fasting insulin was associated with the level of weight gain during the subsequent 9 years of childhood [88]. The Bogalusa Heart Study has shown a strong relationship over an 8-year period between persistently high fasting insulin levels and the development of cardiovascular risk factors in children and young adults [89]. In studies of insulin resistance in childhood that used the euglycemic insulin clamp, an important independent association of both body fatness and insulin resistance with increased cardiovascular risk factors was shown, as well as an interaction between body fatness and insulin resistance, so that the presence of both was associated with a level of cardiovascular risk greater than that expected with either fatness or insulin resistance alone [90]. However, none of these studies prove that insulin resistance itself, and not the associated classical cardiovascular risk factors, is related to morbidity and mortality.

Indeed, the role of insulin in the development of cardiovascular morbidity remains controversial. Several lines of evidence suggest insulin may directly promote cardiovascular pathology. Insulin stimulates mitogen-activated protein kinase, mitogenesis, and plasminogen activator inhibitor-1 within vascular smooth muscle cells [91] and stimulates endothelin-1 production, with subsequent vascular smooth muscle growth [92]. Insulin stimulates *ras*-p21 in vascular smooth muscle, which promotes increased effects of other growth factors, such as platelet-derived growth factor [93]. The vascular endothelial cell insulin receptor knockout mouse has lower blood pressure and endothelin-1 levels than its wild-type counterpart [94].

Conversely, other lines of evidence suggest that insulin may be antiatherogenic: Insulin inhibits the inflammatory transcription factor nuclear factor-κ(kappa)B [95] and decreases tumor necrosis factor-α(alpha) [96]. As with other hormone-receptor interactions, the duration and amplitude of insulin effects may play a role, because chronic hyperstimulation by excessive ligand may lead to alternative cellular responses (e.g., cortisol) or tachyphylaxis (e.g., opioids), which would alter hormone action.

Furthermore, not all patients with insulin resistance develop MetS [97]. Therefore, in addition to obesity, other metabolic and pathological factors (inflammatory factors, adipocytokines, cortisol, oxidative stress, vascular factors, heredity, and lifestyle factors) are operative in this process [17].

Studies Treating Insulin Resistance

There are several studies in childhood demonstrating an improvement of insulin resistance by lifestyle intervention, weight loss, and increased physical activity [98–100]. However, all these studies are based on indices, such as HOMA, and not on the gold standard euglycemic hyperinsulinemic clamp or other rigorous measures of insulin sensitivity.

Some studies demonstrated a positive effect of metformin on insulin resistance and the associated cardiovascular risk factors [14, 22, 23]. However, other randomized controlled trails reported no effect [24, 25]. This may be explained by the fact that children in the untreated control group move from mid- to late puberty as the age ranges of these studies suggest. This change of pubertal status is associated with an improvement of cardiovascular risk factors (see above) [101].

Arguments for Using Insulin Resistance as Treatment Goal

There are some important arguments for using insulin resistance as a treatment goal (see Fig. 17.1). First, all cardiovascular risk factors determining morbidity and mortality in obesity are related to insulin resistance; it is clear that insulin-resistant obese children have significantly

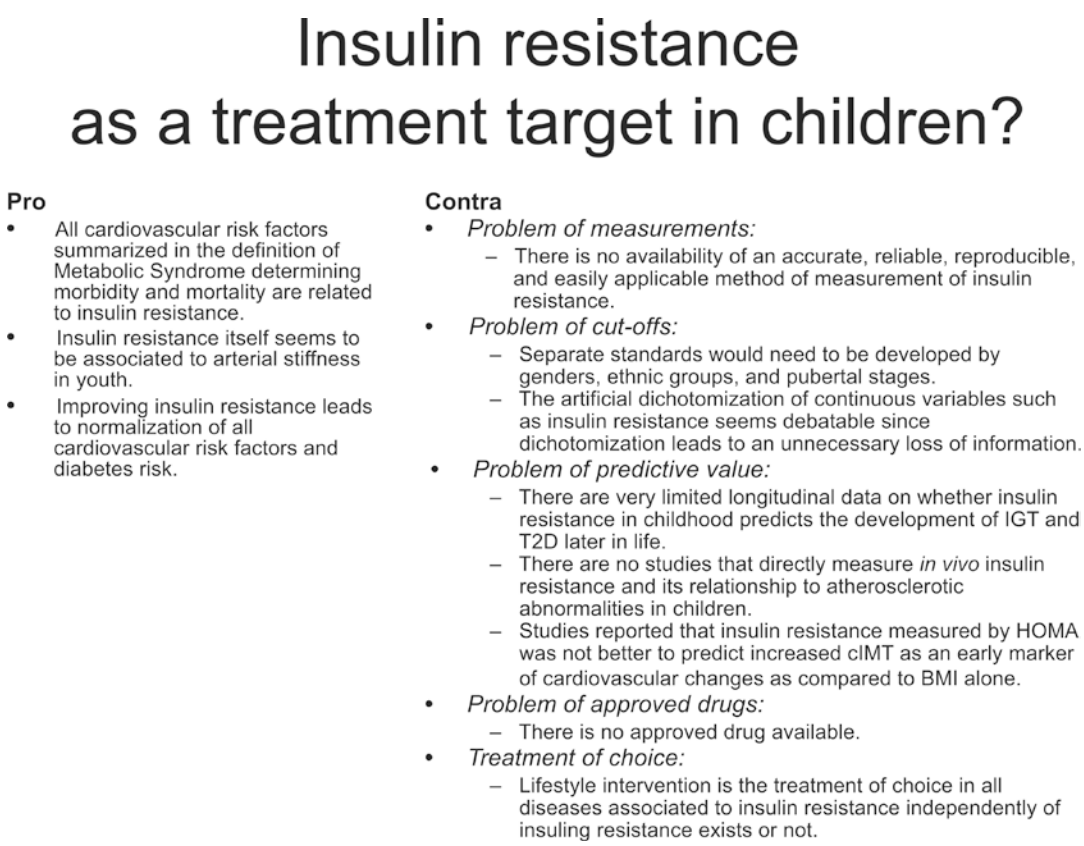

Fig. 17.1 Arguments pro and contra the insulin resistance as treatment target in childhood. Abbreviations: *IGT* impaired glucose tolerance, *T2D* type 2 diabetes, *HOMA* homeostasis model assessment, *cIMT* carotid intima-media thickness, *BMI* body mass index

greater cardiovascular risk profiles, including the metabolic syndrome [17, 90]. Initial observations suggest a relationship between insulin resistance and arterial stiffness in youth [102, 103]. A role for insulin resistance in the early abnormalities of vascular smooth muscle is proposed based on the observation that circulating biomarkers of endothelial dysfunction (intercellular adhesion molecule and E-selectin) are highest, whereas the antiatherogenic adipocytokine adiponectin is lowest among the most insulin-resistant youths [104]. Furthermore, insulin resistance is a risk factor for prediabetes and type 2 diabetes in adults [14].

Improving insulin resistance leads to normalization of all cardiovascular risk factors [98, 105] and diabetes risk. Weight loss and increased physical activity improve insulin resistance [6, 105–116]. These changes in insulin resistance paralleled the changes in cardiovascular risk factors during puberty [63, 101, 117] in both non-Hispanic whites [101] and Afro-Caribbean girls [118].

Arguments Against Using Insulin Resistance as Treatment Goal

Even if the concept of insulin resistance and its association with cardiovascular risk factors is convincing, there are several shortcomings in the definition and measurement of insulin resistance that make it impossible to use insulin resistance as treatment goal in the clinical setting (see Fig. 17.1). Furthermore, there is a lack of evidence in childhood that insulin resistance has a higher cardiovascular risk than the sum of the classical cardiovascular risk factors, such as

hypertension, dyslipidemia, and impaired glucose tolerance. Additionally, the degree of insulin resistance is not stable in adolescents and there are no approved drugs for this indication available for children.

Problem of Measurements

Today there is no availability of an accurate, reliable, reproducible, and easily applicable method of measurement of insulin resistance [14]. It is impractical to use any methods requiring multiple samples because of the complexity, time, and cost of testing. Fasting insulin as an index of insulin resistance or insulin resistance indices such as HOMA or Matsuda may be applicable in epidemiological studies using large populations of children and/or a well-defined cohort, but not on the individual level [14].

Problem of Cutoffs

Even if a uniformly reliable insulin assay became available, separate standards would need to be developed by sex, ethnic group, and pubertal stage [14, 101]. Without such specific cutoffs, treatment goals based on insulin resistance cannot be determined. However, the use of cutoff points for insulin resistance in the absence of longitudinal outcome studies represents a major concern, since this implies that values above the specified thresholds are associated with excess risk—although the rationale for the different cutoff points has never been delineated in children and adolescents [12]. Moreover, the artificial dichotomization of continuous variables, such as insulin resistance, seems debatable since dichotomization leads to an unnecessary loss of information [119]. In fact, insulin resistance is not even linear, which opens up the issue of how risk might be weighted more appropriately.

Indeed, the use of rigid cutoff points, such as in the definition of MetS for cardiovascular risk factors and insulin resistance, reduces its prognostic value in both adults and children. Mente and colleagues reported an underestimation of myocardial infarction in adults using the dichotomous variable MetS instead of the continuous variables, blood pressure or lipids [120]. Fadini et al., as well as Baldassare and colleagues, reported no increased risk in MetS compared to the sum of its individual components based on carotid intima-media thickness (cIMT) measurements. CIMT is a noninvasive, reliable, and predictive marker for early atherosclerotic changes [121, 122]. We have recently reported that the sum of the individual components of the different MetS definitions was superior to predict presence of increased cIMT in obese adolescents compared to the all-or-nothing variable: occurrence of MetS [123]. Furthermore, adding the MetS indicator to the individual components added no additional information to prediction of increased cIMT [123, 124].

Problem of Predictive Value

There are very limited longitudinal data on whether insulin resistance in childhood predicts the development of impaired glucose tolerance (IGT) and type 2 diabetes mellitus later in life [14]. A recent longitudinal study has shown that obese adolescents progressing to IGT manifest primary defects in β(beta)-cell function that are aggravated by a progressive increase in insulin resistance [125]. However, another study reported a low predictive value of impaired glucose tolerance for later type 2 diabetes in the next 3–5 years in adolescents. Furthermore, the high reversion rate (66% to 75%) to normal glucose tolerance in youth contrasts to a conversion rate of 30% from impaired glucose tolerance to type 2 diabetes in 5 years in adults [126, 127]. These findings may be attributed, at least in part, to the fact that many adolescents in the longitudinal studies move from mid- to late or postpuberty [35, 56]. Moreover, there also seems to be a genetic contribution, since in Sweden impaired fasting glucose is more frequent than in mid-European countries and conversion to type 2 diabetes more frequent [128, 129].

There are no studies that directly measure in vivo insulin resistance and its relationship to atheroscle-

rotic abnormalities in children. Furthermore, studies reported that insulin resistance measured by HOMA was not a better predictor of increased cIMT than BMI alone [130]. Accordingly, some studies reported that cardiovascular risk factors correlated stronger to degree of overweight than insulin resistance [1, 131]. Obese children and adults without cardiovascular risk factors have been classified as metabolic healthy obese [82, 132, 133]. A total of 6–40% of obese adults [134, 135] and 6–36% of obese children [82–85] are metabolically healthy. However, longitudinal studies have demonstrated that even obese children without cardiovascular risk factors can switch to an unhealthy metabolic state without change in their weight status [1, 101]. Also in adults, the status of metabolic healthy—characterized by the absence of insulin resistance and cardiovascular risk factors—is not a steady state [136], questioning the whole concept of metabolic healthy since there is no evidence for decreased mortality [83–85].

While autopsy studies have shown that the extent of early atherosclerosis of the aorta and coronary arteries is directly associated with levels of lipids, blood pressure, and obesity in childhood and adolescence, this evidence is lacking for insulin resistance [137, 138]. In conclusion, up to now no outcome study in childhood has proven an increased mortality or morbidity due to insulin resistance [12, 139].

Problem of Approved Drugs

Even though metformin improves insulin resistance in several studies in children [14, 22, 23], no consistent metabolic effect or change in cardiovascular risk factors has been demonstrated and it has to be stressed that metformin has not been approved for the treatment of children with insulin resistance.

Summary

The reported prevalences of hypertension, dyslipidemia, and disturbed glucose metabolism in obese children and adolescents underline the necessity for screening, since most of these disorders are asymptomatic but related to later cardiovascular disease. Indications for treatment, including antihypertensive and lipid- or glucose-lowering drugs and bariatric surgery, should be based on weighing of the cardiovascular risk factors themselves, keeping in mind the pubertal stage, rather than on one single variable, such as insulin resistance. Since puberty and genetics influence insulin resistance, it is questionable to use definitions of insulin resistance in adolescents not accounting for pubertal stage and ethnic background. Furthermore, we do not currently have feasible and reliable measurements of insulin resistance that can be used in clinical practice; surrogate measures, such as fasting insulin, are poor estimates of insulin sensitivity [14]. There is also a lack of validated cutoffs to define insulin resistance in children. Because of these limitations, there is no justification for treating insulin resistance in children, though insulin resistance is associated with cardiovascular risk factor, MetS, prediabetes, type 2 diabetes, as well as polycystic ovarian syndrome and fatty liver disease [14]. Therefore, the mere presence of these abnormalities should call for intervention to treat the associated obesity by lifestyle intervention, surgery, or medications to reduce weight or improve insulin sensitivity without a need to measure insulin resistance [14].

Future research should aim at the following:

1. How to best measure insulin sensitivity in children and adolescents
2. Standardization of insulin measurements
3. Identification of strong surrogate biomarkers of insulin resistance
4. The potential role of both lifestyle intervention and medications in the prevention and treatment of insulin resistance

Having a uniform internationally accepted definition of insulin resistance for children and adolescents adjusted by sex, ethnic background, and pubertal stage would be very helpful for the description of populations in different studies. What is probably needed is not a dichotomous

definition of insulin resistance but a more complex weighted scoring system that takes into account the magnitude of all of the risk factors, their interaction, and other important patient-specific characteristics [17].

References

1. I'Allemand D, Wiegand S, Reinehr T, Muller J, Wabitsch M, Widhalm K, et al. Cardiovascular risk in 26,008 European overweight children as established by a multicenter database. Obesity (Silver Spring). 2008;16(7):1672–9.
2. Csabi G, Torok K, Jeges S, Molnar D. Presence of metabolic cardiovascular syndrome in obese children. Eur J Pediatr. 2000;159(1–2):91–4.
3. Chu NF, Rimm EB, Wang DJ, Liou HS, Shieh SM. Clustering of cardiovascular disease risk factors among obese schoolchildren: the Taipei Children Heart Study. Am J Clin Nutr. 1998;67(6):1141–6.
4. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. Pediatrics. 1999;103(6 Pt 1):1175–82.
5. Han JC, Lawlor DA, Kimm SY. Childhood obesity. Lancet. 2010;375(9727):1737–48.
6. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415–28.
7. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24(4):683–9.
8. Reaven GM. Insulin resistance and compensatory hyperinsulinemia: role in hypertension, dyslipidemia, and coronary heart disease. Am Heart J. 1991;121(4 Pt 2):1283–8.
9. Grundy SM. Obesity, metabolic syndrome, and coronary atherosclerosis. Circulation. 2002;105(23):2696–8.
10. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. N Engl J Med. 2010;362(6):485–93.
11. Twig G, Yaniv G, Levine H, Leiba A, Goldberger N, Derazne E, et al. Body-mass index in 2.3 million adolescents and cardiovascular death in adulthood. N Engl J Med. 2016;374(25):2430–40.
12. Reinehr T, de Sousa G, Toschke AM, Andler W. Comparison of metabolic syndrome prevalence using eight different definitions: a critical approach. Arch Dis Child. 2007;92(12):1067–72.
13. Vukovic R, Milenkovic T, Mitrovic K, Todorovic S, Plavsic L, Vukovic A, et al. Preserved insulin sensitivity predicts metabolically healthy obese phenotype in children and adolescents. Eur J Pediatr. 2015;174(12):1649–55.
14. Levy-Marchal C, Arslanian S, Cutfield W, Sinaiko A, Druet C, Marcovecchio ML, et al. Insulin resistance in children: consensus, perspective, and future directions. J Clin Endocrinol Metab. 2010;95(12):5189–98.
15. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev. 1995;75(3):473–86.
16. Ten S, Maclaren N. Insulin resistance syndrome in children. J Clin Endocrinol Metab. 2004;89(6):2526–39.
17. Steinberger J, Daniels SR, Eckel RH, Hayman L, Lustig RH, McCrindle B, et al. Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2009;119(4):628–47.
18. Pladevall M, Singal B, Williams LK, Brotons C, Guyer H, Sadurni J, et al. A single factor underlies the metabolic syndrome: a confirmatory factor analysis. Diabetes Care. 2006;29(1):113–22.
19. Lass N, Kleber M, Winkel K, Wunsch R, Reinehr T. Effect of lifestyle intervention on features of polycystic ovarian syndrome, metabolic syndrome, and intima-media thickness in obese adolescent girls. J Clin Endocrinol Metab. 2011;96(11):3533–40.
20. Fishbein MH, Miner M, Mogren C, Chalekson J. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to severity of steatosis. J Pediatr Gastroenterol Nutr. 2003;36(1):54–61.
21. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004;350(23):2362–74.
22. Kendall D, Vail A, Amin R, Barrett T, Dimitri P, Ivison F, et al. Metformin in obese children and adolescents: the MOCA trial. J Clin Endocrinol Metab. 2013;98(1):322–9.
23. Freemark M, Bursey D. The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. Pediatrics. 2001;107(4):E55.
24. Rynders C, Weltman A, Delgiorno C, Balagopal P, Damaso L, Killen K, et al. Lifestyle intervention improves fitness independent of metformin in obese adolescents. Med Sci Sports Exerc. 2012;44(5):786–92.
25. Wiegand S, l'Allemand D, Hubel H, Krude H, Burmann M, Martus P, et al. Metformin and placebo therapy both improve weight management and fasting insulin in obese insulin-resistant adolescents: a prospective, placebo-controlled, randomized study. Eur J Endocrinol. 2010;163(4):585–92.
26. Reinehr T, Kiess W, Andler W. Insulin sensitivity indices of glucose and free fatty acid metabolism in

obese children and adolescents in relation to serum lipids. Metabolism. 2005;54(3):397–402.
27. Hershkop K, Besor O, Santoro N, Pierpont B, Caprio S, Weiss R. Adipose insulin resistance in obese adolescents across the spectrum of glucose tolerance. J Clin Endocrinol Metab. 2016;101(6):2423–31.
28. Bastian W. Genes with linkage or association with type 2 diabetes mellitus. J Pediatr Endocrinol Metab. 2002;15(Suppl 1):471–84.
29. DeFronzo RA, Ferrannini E. The pathogenesis of non-insulin-dependent diabetes: an update. Medicine (Baltimore). 1982;61(3):125–40.
30. Bacha F, Saad R, Gungor N, Janosky J, Arslanian SA. Obesity, regional fat distribution, and syndrome X in obese black versus white adolescents: race differential in diabetogenic and atherogenic risk factors. J Clin Endocrinol Metab. 2003;88(6):2534–40.
31. Burke JP, Hale DE, Hazuda HP, Stern MP. A quantitative scale of acanthosis nigricans. Diabetes Care. 1999;22(10):1655–9.
32. Reinehr T. Metabolic syndrome in children and adolescents: a critical approach considering the interaction between pubertal stage and insulin resistance. Curr Diab Rep. 2016;16(1):8.
33. Chan CL, Pyle L, Newnes L, Nadeau KJ, Zeitler PS, Kelsey MM. Continuous glucose monitoring and its relationship to hemoglobin A1c and oral glucose tolerance testing in obese and prediabetic youth. J Clin Endocrinol Metab. 2015;100(3):902–10.
34. Libman IM, Barinas-Mitchell E, Bartucci A, Robertson R, Arslanian S. Reproducibility of the oral glucose tolerance test in overweight children. J Clin Endocrinol Metab. 2008;93(11):4231–7.
35. Kleber M, Desousa G, Papcke S, Wabitsch M, Reinehr T. Impaired glucose tolerance in obese white children and adolescents: three to five year follow-up in untreated patients. Exp Clin Endocrinol Diabetes. 2011;119(3):172–6.
36. Wallace TM, Matthews DR. The assessment of insulin resistance in man. Diabet Med. 2002 Jul;19(7):527–34.
37. Schwartz B, Jacobs DR Jr, Moran A, Steinberger J, Hong CP, Sinaiko AR. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. Diabetes Care. 2008;31(4):783–8.
38. Gungor N, Saad R, Janosky J, Arslanian S. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr. 2004;144(1):47–55.
39. Brandou F, Brun JF, Mercier J. Limited accuracy of surrogates of insulin resistance during puberty in obese and lean children at risk for altered glucoregulation. J Clin Endocrinol Metab. 2005;90(2):761–7.
40. Allard P, Delvin EE, Paradis G, Hanley JA, O'Loughlin J, Lavallee C, et al. Distribution of fasting plasma insulin, free fatty acids, and glucose concentrations and of homeostasis model assessment of insulin resistance in a representative sample of Quebec children and adolescents. Clin Chem. 2003;49(4):644–9.
41. Marcovina S, Bowsher RR, Miller WG, Staten M, Myers G, Caudill SP, et al. Standardization of insulin immunoassays: report of the American Diabetes Association Workgroup. Clin Chem. 2007;53(4):711–6.
42. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
43. Hrebicek J, Janout V, Malincikova J, Horakova D, Cizek L. Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention. J Clin Endocrinol Metab. 2002;87(1):144–7.
44. Penesova A, Radikova Z. Comparison of insulin sensitivity indices calculated from standard 3-sampled and frequently sampled oral glucose tolerance test. Endocr Regul. 2004;38(4):167–71.
45. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, Van HT, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295–301.
46. Yeckel CW, Weiss R, Dziura J, Taksali SE, Dufour S, Burgert TS, et al. Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. J Clin Endocrinol Metab. 2004;89(3):1096–101.
47. Poulsen P, Levin K, Petersen I, Christensen K, Beck-Nielsen H, Vaag A. Heritability of insulin secretion, peripheral and hepatic insulin action, and intracellular glucose partitioning in young and old Danish twins. Diabetes. 2005;54(1):275–83.
48. Arslanian SA, Bacha F, Saad R, Gungor N. Family history of type 2 diabetes is associated with decreased insulin sensitivity and an impaired balance between insulin sensitivity and insulin secretion in white youth. Diabetes Care. 2005;28(1):115–9.
49. Goran MI, Bergman RN, Cruz ML, Watanabe R. Insulin resistance and associated compensatory responses in african-american and Hispanic children. Diabetes Care. 2002;25(12):2184–90.
50. Whincup PH, Gilg JA, Papacosta O, Seymour C, Miller GJ, Alberti KG, et al. Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children. BMJ. 2002;324(7338):635.
51. Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes. 2001;50(11):2444–50.
52. Yin J, Li M, Xu L, Wang Y, Cheng H, Zhao X, et al. Insulin resistance determined by Homeostasis Model Assessment (HOMA) and associations with metabolic syndrome among Chinese children and teenagers. Diabetol Metab Syndr. 2013;5(1):71.
53. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. Pediatr Res. 2006;60(6):759–63.

54. Goran MI, Shaibi GQ, Weigensberg MJ, Davis JN, Cruz ML. Deterioration of insulin sensitivity and beta-cell function in overweight Hispanic children during pubertal transition: a longitudinal assessment. Int J Pediatr Obes. 2006;1(3):139–45.
55. Reinehr T, Wabitsch M, Kleber M, de Sousa G, Denzer C, Toschke AM. Parental diabetes, pubertal stage, and extreme obesity are the main risk factors for prediabetes in children and adolescents: a simple risk score to identify children at risk for prediabetes. Pediatr Diabetes. 2009;10(6):395–400.
56. Kleber M, Lass N, Papcke S, Wabitsch M, Reinehr T. One-year follow-up of untreated obese white children and adolescents with impaired glucose tolerance: high conversion rate to normal glucose tolerance. Diabet Med. 2010;27(5):516–21.
57. Kelly LA, Lane CJ, Weigensberg MJ, Toledo-Corral CM, Goran MI. Pubertal changes of insulin sensitivity, acute insulin response, and beta-cell function in overweight Latino youth. J Pediatr. 2011;158(3):442–6.
58. Brufani C, Tozzi A, Fintini D, Ciampalini P, Grossi A, Fiori R, et al. Sexual dimorphism of body composition and insulin sensitivity across pubertal development in obese Caucasian subjects. Eur J Endocrinol. 2009;160(5):769–75.
59. Pinhas-Hamiel O, Lerner-Geva L, Copperman NM, Jacobson MS. Lipid and insulin levels in obese children: changes with age and puberty. Obesity (Silver Spring). 2007;15(11):2825–31.
60. Raab J, Haupt F, Kordonouri O, Scholz M, Wosch A, Ried C, et al. Continuous rise of insulin resistance before and after the onset of puberty in children at increased risk for type 1 diabetes – a cross-sectional analysis. Diabetes Metab Res Rev. 2013;29(8):631–5.
61. Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). Diabetes Care. 2012 Mar;35(3):536–41.
62. Pilia S, Casini MR, Foschini ML, Minerba L, Musiu MC, Marras V, et al. The effect of puberty on insulin resistance in obese children. J Endocrinol Invest. 2009;32(5):401–5.
63. Xu L, Li M, Yin J, Cheng H, Yu M, Zhao X, et al. Change of body composition and adipokines and their relationship with insulin resistance across pubertal development in obese and non-obese Chinese children: the BCAMS Study. Int J Endocrinol. 2012;2012:389108.
64. Pilia S, Casini MR, Foschini ML, Minerba L, Musiu MC, Marras V, et al. The effect of puberty on insulin resistance in obese children. J Endocrinol Invest. 2009 May;32(5):401–5.
65. Hainan E. Diabetes and puberty. Am J Nurs. 2014 Oct;114(10):12.
66. Moran A, Jacobs DR Jr, Steinberger J, Cohen P, Hong CP, Prineas R, et al. Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. J Clin Endocrinol Metab. 2002 Oct;87(10):4817–20.
67. Chu L, Riddell MC, Schneiderman JE, McCrindle BW, Hamilton JK. The effect of puberty on fat oxidation rates during exercise in overweight and normal-weight girls. J Appl Physiol. 2014;116(1):76–82.
68. Spruijt-Metz D, Belcher BR, Hsu YW, McClain AD, Chou CP, Nguyen-Rodriguez S, et al. Temporal relationship between insulin sensitivity and the pubertal decline in physical activity in peripubertal Hispanic and African American females. Diabetes Care. 2013 Nov;36(11):3739–45.
69. Kayser BD, Toledo-Corral CM, Alderete TL, Weigensberg MJ, Goran MI. Temporal relationships between adipocytokines and diabetes risk in Hispanic adolescents with obesity. Obesity (Silver Spring). 2015;23(7):1479–85.
70. Agirbasli M, Agaoglu NB, Orak N, Caglioz H, Ocek T, Poci N, et al. Sex hormones and metabolic syndrome in children and adolescents. Metabolism. 2009;58(9):1256–62.
71. Jurimae J, Gruodyte R, Saar M, Cicchella A, Stefanelli C, Passariello C, et al. Plasma visfatin and adiponectin concentrations in physically active adolescent girls: relationships with insulin sensitivity and body composition variables. J Pediatr Endocrinol Metab. 2011;24(7–8):419–25.
72. Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin--the classical, resistin--the controversial, adiponectin--the promising, and more to come. Best Pract Res Clin Endocrinol Metab. 2005;19(4):525–46.
73. Sorensen K, Aksglaede L, Munch-Andersen T, Aachmann-Andersen NJ, Petersen JH, Hilsted L, et al. Sex hormone-binding globulin levels predict insulin sensitivity, disposition index, and cardiovascular risk during puberty. Diabetes Care. 2009;32(5):909–14.
74. Qiu X, Dao H, Wang M, Heston A, Garcia KM, Sangal A, et al. Insulin and leptin signaling interact in the mouse Kiss1 neuron during the peripubertal period. PLoS One. 2015;10(5):e0121974.
75. Astiz S, Gonzalez-Bulnes A, Astiz I, Barbero A, Perez-Solana ML, Garcia-Real I. Advanced onset of puberty after metformin therapy in swine with thrifty genotype. Exp Physiol. 2014;99(9):1241–52.
76. Adam TC, Hasson RE, Lane CJ, Davis JN, Weigensberg MJ, Spruijt-Metz D, et al. Fasting indicators of insulin sensitivity: effects of ethnicity and pubertal status. Diabetes Care. 2011;34(4):994–9.
77. Viner RM, Segal TY, Lichtarowicz-Krynska E, Hindmarsh P. Prevalence of the insulin resistance syndrome in obesity. Arch Dis Child. 2005 Jan;90(1):10–4.
78. Dowling AR, Nedorezov LB, Qiu X, Marino JS, Hill JW. Genetic factors modulate the impact of pubertal androgen excess on insulin sensitivity and fertility. PLoS One. 2013;8(11):e79849.

79. Reinehr T, Toschke AM. Onset of puberty and cardiovascular risk factors in untreated obese children and adolescents: a 1-year follow-up study. Arch Pediatr Adolesc Med. 2009;163(8):709–15.
80. Reinehr T, Elfers C, Lass N, Roth CL. Irisin and its relation to insulin resistance and puberty in obese children: a longitudinal analysis. J Clin Endocrinol Metab. 2015;100(5):2123–30.
81. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006;17(1):4–12.
82. Prince RL, Kuk JL, Ambler KA, Dhaliwal J, Ball GD. Predictors of metabolically healthy obesity in children. Diabetes Care. 2014;37(5):1462–8.
83. Bokor S, Frelut ML, Vania A, Hadjiathanasiou CG, Anastasakou M, Malecka-Tendera E, et al. Prevalence of metabolic syndrome in European obese children. Int J Pediatr Obes. 2008;3(Suppl 2):3–8.
84. Cruz ML, Weigensberg MJ, Huang TT, Ball G, Shaibi GQ, Goran MI. The metabolic syndrome in overweight Hispanic youth and the role of insulin sensitivity. J Clin Endocrinol Metab. 2004;89(1):108–13.
85. Shaibi GQ, Goran MI. Examining metabolic syndrome definitions in overweight Hispanic youth: a focus on insulin resistance. J Pediatr. 2008;152(2):171–6.
86. Ruige JB, Assendelft WJ, Dekker JM, Kostense PJ, Heine RJ, Bouter LM. Insulin and risk of cardiovascular disease: a meta-analysis. Circulation. 1998;97(10):996–1001.
87. Taittonen L, Uhari M, Nuutinen M, Turtinen J, Pokka T, Akerblom HK. Insulin and blood pressure among healthy children. Cardiovascular risk in young Finns. Am J Hypertens. 1996;9(3):194–9.
88. Odeleye OE, de Courten M, Pettitt DJ, Ravussin E. Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children. Diabetes. 1997;46(8):1341–5.
89. Bao W, Srinivasan SR, Berenson GS. Persistent elevation of plasma insulin levels is associated with increased cardiovascular risk in children and young adults. The Bogalusa Heart Study. Circulation. 1996;93(1):54–9.
90. Sinaiko AR, Steinberger J, Moran A, Prineas RJ, Vessby B, Basu S, et al. Relation of body mass index and insulin resistance to cardiovascular risk factors, inflammatory factors, and oxidative stress during adolescence. Circulation. 2005;111(15):1985–91.
91. Begum N, Song Y, Rienzie J, Ragolia L. Vascular smooth muscle cell growth and insulin regulation of mitogen-activated protein kinase in hypertension. Am J Physiol. 1998;275(1 Pt 1):C42–9.
92. Nagai M, Kamide K, Rakugi H, Takiuchi S, Imai M, Kida I, et al. Role of endothelin-1 induced by insulin in the regulation of vascular cell growth. Am J Hypertens. 2003;16(3):223–8.
93. Goalstone ML, Natarajan R, Standley PR, Walsh MF, Leitner JW, Carel K, et al. Insulin potentiates platelet-derived growth factor action in vascular smooth muscle cells. Endocrinology. 1998;139(10):4067–72.
94. Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, et al. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. J Clin Invest. 2003;111(9):1373–80.
95. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, et al. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab. 2001;86(7):3257–65.
96. Fraker DL, Merino MJ, Norton JA. Reversal of the toxic effects of cachectin by concurrent insulin administration. Am J Physiol. 1989;256(6 Pt 1):E725–31.
97. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med. 2003;163(4):427–36.
98. Reinehr T, Lass N, Toschke C, Rothermel J, Lanzinger S, Holl RW. Which amount of BMI-SDS reduction is necessary to improve cardiovascular risk factors in overweight children? J Clin Endocrinol Metab. 2016;101(8):3171–9.
99. Savoye M, Nowicka P, Shaw M, Yu S, Dziura J, Chavent G, et al. Long-term results of an obesity program in an ethnically diverse pediatric population. Pediatrics. 2011;127(3):402–10.
100. Oude LH, Baur L, Jansen H, Shrewsbury VA, O'Malley C, Stolk RP, et al. Interventions for treating obesity in children. Cochrane Database Syst Rev. 2009;1:CD001872.
101. Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. J Clin Endocrinol Metab. 2015;100(1):301–8.
102. Gungor N, Thompson T, Sutton-Tyrrell K, Janosky J, Arslanian S. Early signs of cardiovascular disease in youth with obesity and type 2 diabetes. Diabetes Care. 2005;28(5):1219–21.
103. Reinehr T, Wunsch R. Intima media thickness-related risk factors in childhood obesity. Int J Pediatr Obes. 2011;6(Suppl 1):46–52.
104. Lee S, Gungor N, Bacha F, Arslanian S. Insulin resistance: link to the components of the metabolic syndrome and biomarkers of endothelial dysfunction in youth. Diabetes Care. 2007;30(8):2091–7.
105. Reinehr T, Kiess W, Kapellen T, Andler W. Insulin sensitivity among obese children and adolescents, according to degree of weight loss. Pediatrics. 2004;114(6):1569–73.
106. Reinehr T, de Sousa G, Andler W. Longitudinal analyses among overweight, insulin resistance, and cardiovascular risk factors in children. Obes Res. 2005;13(10):1824–33.

107. Reinehr T, de Sousa G, Toschke AM, Andler W. Long-term follow-up of cardiovascular disease risk factors in children after an obesity intervention. Am J Clin Nutr. 2006;84(3):490–6.
108. Sung RY, Yu CW, Chang SK, Mo SW, Woo KS, Lam CW. Effects of dietary intervention and strength training on blood lipid level in obese children. Arch Dis Child. 2002;86(6):407–10.
109. Reinehr T, Andler W. Changes in the atherogenic risk factor profile according to degree of weight loss. Arch Dis Child. 2004;89(5):419–22.
110. Coppen AM, Risser JA, Vash PD. Metabolic syndrome resolution in children and adolescents after 10 weeks of weight loss. J Cardiometab Syndr. 2008;3(4):205–10.
111. Reinehr T, Kleber M, Toschke AM. Lifestyle intervention in obese children is associated with a decrease of the metabolic syndrome prevalence. Atherosclerosis. 2009;207(1):174–80.
112. Savoye M, Shaw M, Dziura J, Tamborlane WV, Rose P, Guandalini C, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. JAMA. 2007;297(24):2697–704.
113. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, et al. Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med. 2002;347(19):1483–92.
114. Uusitupa M, Laakso M, Sarlund H, Majander H, Takala J, Penttila I. Long term effects of a very low calorie diet on metabolic control and cardiovascular risk factors in the treatment of obese non-insulin-dependent diabetics. Int J Obes (Lond). 1989;13(Suppl 2):163–4.
115. Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. Int J Obes Relat Metab Disord. 1999;23(5):528–36.
116. Inoue DS, De Mello MT, Foschini D, Lira FS, De Piano GA, Da Silveira Campos RM, et al. Linear and undulating periodized strength plus aerobic training promote similar benefits and lead to improvement of insulin resistance on obese adolescents. J Diabetes Complications. 2015;29(2):258–64.
117. Tobisch B, Blatniczky L, Barkai L. Cardiometabolic risk factors and insulin resistance in obese children and adolescents: relation to puberty. Pediatr Obes. 2015;10(1):37–44.
118. Boyne MS, Thame M, Osmond C, Fraser RA, Gabay L, Taylor-Bryan C, et al. The effect of earlier puberty on cardiometabolic risk factors in Afro-Caribbean children. J Pediatr Endocrinol Metab. 2014;27(5–6):453–60.
119. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med. 2006;25(1):127–41.
120. Mente A, Yusuf S, Islam S, McQueen MJ, Tanomsup S, Onen CL, et al. Metabolic syndrome and risk of acute myocardial infarction a case-control study of 26,903 subjects from 52 countries. J Am Coll Cardiol. 2010;55(21):2390–8.
121. Baldassarre D, Werba JP, Castelnuovo S, Frigerio B, Amato M, Ravani A, et al. The metabolic syndrome predicts carotid intima-media thickness no better than the sum of individual risk factors in a lipid clinic population. Atherosclerosis. 2010;210(1):214–9.
122. Fadini GP, Coracina A, Inchiostro S, Tiengo A, Avogaro A, de Kreutzenberg SV. A stepwise approach to assess the impact of clustering cardiometabolic risk factors on carotid intima-media thickness: the metabolic syndrome no-more-than-additive. Eur J Cardiovasc Prev Rehabil. 2008;15(2):190–6.
123. Reinehr T, Wunsch R, Putter C, Scherag A. Relationship between carotid intima-media thickness and metabolic syndrome in adolescents. J Pediatr. 2013;163(2):327–32.
124. Reinehr T, Wunsch R, de Sousa G, Toschke AM. Relationship between metabolic syndrome definitions for children and adolescents and intima-media thickness. Atherosclerosis. 2008;199(1):193–200.
125. Cali AM, Man CD, Cobelli C, Dziura J, Seyal A, Shaw M, et al. Primary defects in beta-cell function further exacerbated by worsening of insulin resistance mark the development of impaired glucose tolerance in obese adolescents. Diabetes Care. 2009;32(3):456–61.
126. Rasmussen SS, Glumer C, Sandbaek A, Lauritzen T, Borch-Johnsen K. Progression from impaired fasting glucose and impaired glucose tolerance to diabetes in a high-risk screening programme in general practice: the ADDITION Study, Denmark. Diabetologia. 2007;50(2):293–7.
127. Rasmussen SS, Glumer C, Sandbaek A, Lauritzen T, Borch-Johnsen K. Determinants of progression from impaired fasting glucose and impaired glucose tolerance to diabetes in a high-risk screened population: 3 year follow-up in the ADDITION study, Denmark. Diabetologia. 2008;51(2):249–57.
128. Hagman E, Reinehr T, Kowalski J, Ekbom A, Marcus C, Holl RW. Impaired fasting glucose prevalence in two nationwide cohorts of obese children and adolescents. Int J Obes (Lond). 2014;38(1):40–5.
129. Hagman E, Danielsson P, Brandt L, Ekbom A, Marcus C. Association between impaired fasting glycaemia in pediatric obesity and type 2 diabetes in young adulthood. Nutr Diabetes. 2016;6(8):e227.
130. Magnussen CG, Koskinen J, Chen W, Thomson R, Schmidt MD, Srinivasan SR, et al. Pediatric metabolic syndrome predicts adulthood metabolic syndrome, subclinical atherosclerosis, and type 2 diabetes mellitus but is no better than body mass index alone: the Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. Circulation. 2010 Oct 19;122(16):1604–11.
131. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. N Engl J Med. 2015;373(14):1307–17.

132. Bluher M. Are there still healthy obese patients? Curr Opin Endocrinol Diabetes Obes. 2012;19(5):341–6.
133. Bluher M. The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. Curr Opin Lipidol. 2010;21(1):38–43.
134. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes. 1998;47(10):1643–9.
135. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med. 2008;168(15):1617–24.
136. Mongraw-Chaffin M, Foster MC, Kalyani RR, Vaidya D, Burke GL, Woodward M, et al. Obesity severity and duration are associated with incident metabolic syndrome: evidence against metabolically healthy obesity from the Multi-Ethnic Study of Atherosclerosis. J Clin Endocrinol Metab. 2016;101(11):4117–24.
137. McGill HC Jr, McMahan CA, Zieske AW, Tracy RE, Malcom GT, Herderick EE, et al. Association of Coronary Heart Disease Risk Factors with microscopic qualities of coronary atherosclerosis in youth. Circulation. 2000;102(4):374–9.
138. McGill HC Jr, McMahan CA, Herderick EE, Tracy RE, Malcom GT, Zieske AW, et al. Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery. PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. Arterioscler Thromb Vasc Biol. 2000;20(3):836–45.
139. Brambilla P, Lissau I, Flodmark CE, Moreno LA, Widhalm K, Wabitsch M, et al. Metabolic risk-factor clustering estimation in children: to draw a line across pediatric metabolic syndrome. Int J Obes (Lond). 2007;31(4):591–600.

18

Exercise in Metabolic Syndrome and Diabetes: A Central Role for Insulin Sensitivity

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Introduction

Exercise is considered the cornerstone of treatment for metabolic syndrome and type 2 diabetes mellitus (T2D), yet exercise is demonstrably more difficult and capacity impaired in T2D. Insulin resistance (IR) at many levels appears to be a key factor in diabetes-related exercise deficits. Conversely, the benefits of exercise in metabolic syndrome and T2D are likely related, in large part, to improvements in insulin sensitivity. This chapter provides an overview of the current understanding of the bidirectional relationship between exercise and IR and of the benefits of exercise in metabolic syndrome, prediabetes, and diabetes.

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Exercise and Mortality

The current literature, including meta-analyses covering more than 2.6 million person-years of study, provides indisputable support for the association of physical activity and physical fitness with lower cardiovascular disease (CVD) risk (reviewed in [1, 2]). 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 [3]. 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. 18.1). Overall, the benefits of fitness were greater than the benefits of physical activity, with the fittest population achieving a two-thirds reduction in relative risk of CVD compared to the least fit population. A 2009 meta-analysis of 33 studies included more than 100,000 participants (men and women and multiple races and ethnicities) and nearly 7000 deaths [4]. This meta-analysis found a highly significant inverse correlation of cardiorespiratory fitness (CRF) with all-cause and cardiac mortality, where each one metabolic equivalent (MET) improvement in maximum exercise capacity conferred a 13–15% decrease in mortality. It has also been demonstrated that improving CRF later in life (from unfit to fit) confers significant mortality benefits, thus strengthening the arguments for a cause-and-effect relationship [5–9].

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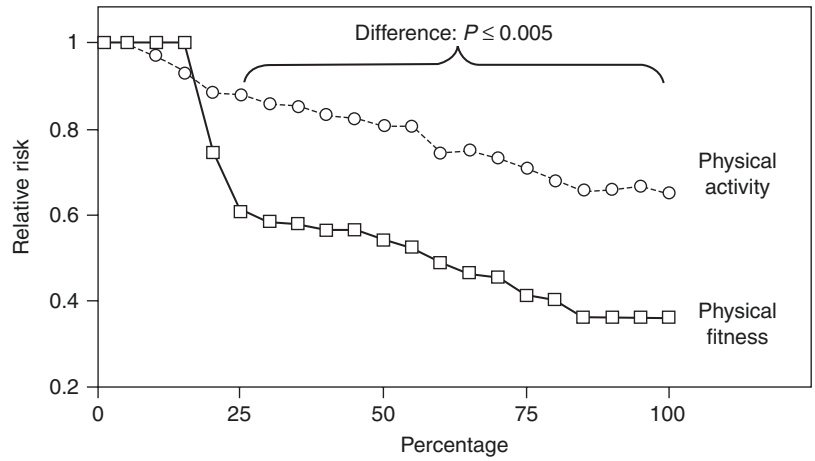

Fig. 18.1 Estimated dose–response curve for the relative risk of CVD by fitness and physical activity. (Reprinted with permission from Williams [3])

Conversely, sedentary behavior has been endorsed by the US Centers for Disease Control (CDC) as a leading cause of premature mortality [10]. As little as 2 hours per day of TV viewing was found to confer 20%, 15%, and 13% increased risks of T2D, cardiovascular disease, and all-cause mortality, respectively [11]. A burden of disease analysis found that physical inactivity accounted for 6% of all cardiovascular disease, 7% of T2D, and 9% of all premature mortality in 2008 [12]. The increased mortality associated with sitting time and TV-viewing time can be eliminated or attenuated by the addition of high levels of daily moderate intensity exercise to the largely sedentary day [13].

Exercise and Mortality in Insulin-Resistant States

Other studies have demonstrated that the relationship between physical activity or CRF and CVD or overall mortality also exists in populations with comorbid conditions, including T2D, obesity, prediabetes, and metabolic syndrome [3, 14–18] independent of race, sex, and methodology. For example, a substudy of the Aerobics Center Longitudinal Study that focused on women with prediabetes or undiagnosed diabetes (n = 3044) demonstrated an inverse correlation between CRF and all-cause mortality, with the greatest benefit occurring when exercise capacity exceeded 7 METs (very roughly corresponding to a VO_2max of 22–23 ml O_2/kg/min) [19]. The same observation held true for a cohort of 1263 diabetic men [14] and the mortality benefit of CRF was observed even in obese subjects. In other studies in people with diabetes, lower habitual physical activity was associated with increased mortality [17, 20].

It is well accepted that CVD risk and mortality are increased in individuals with diabetes and/or the metabolic syndrome relative to those without these conditions and, as described above, that physical activity decreases CVD risk and mortality in both insulin-sensitive and insulin-resistant populations. 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 (reviewed in [21]). These studies suggest that improvement in insulin sensitivity may be a major contributor to the cardiovascular 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 lifestyle [22]. This is consistent with the findings of Bonora et al. in a 15-year prospective study of 839 new cases of symptomatic CVD [23]. In this study, homeostatic model assessment of insulin resistance (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, body mass index (BMI), triglyceride (TG) level, high-sensitivity C-reactive protein (hsCRP), adiponectin, fibrinogen, vascular cell adhesion molecule 1 (VCAM-1), and oxidized low-density lipoprotein (LDL). Thus, it seems likely that IR itself (or the underlying cause thereof) increases CVD risk and that exercise attenuates this increased risk by improving IR.

This central role for IR in mediating negative health effects of poor fitness and, conversely, insulin sensitization in mediating the beneficial effects of exercise is challenged, however, by other literature. Some studies suggest that physical activity may provide full protection from the excess CVD risk in insulin-resistant states [15, 24]. 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 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. 18.2) [24], such that the most active 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 lower cardiovascular fitness in these groups [16]. However, neither of these studies clearly demonstrated that improving CRF in individuals with IR improved CVD risk and mortality independent of improvements in insulin sensitivity. Thus, 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 (reviewed in [2])—but this effect could be a result of improved insulin sensitivity concomitant with CRF improvement. Still, two other recent lines of evidence may further challenge the hypothesis that IR is a direct contributor to increased CVD and mortality risk. Adiponectin, a presumed beneficial anti-inflammatory adipokine and marker of insulin sensitivity, has been shown in many studies to be positively correlated with cardiometabolic risk and mortality (reviewed in [25]). In addition, Kim et al. used *National Health and Nutrition*

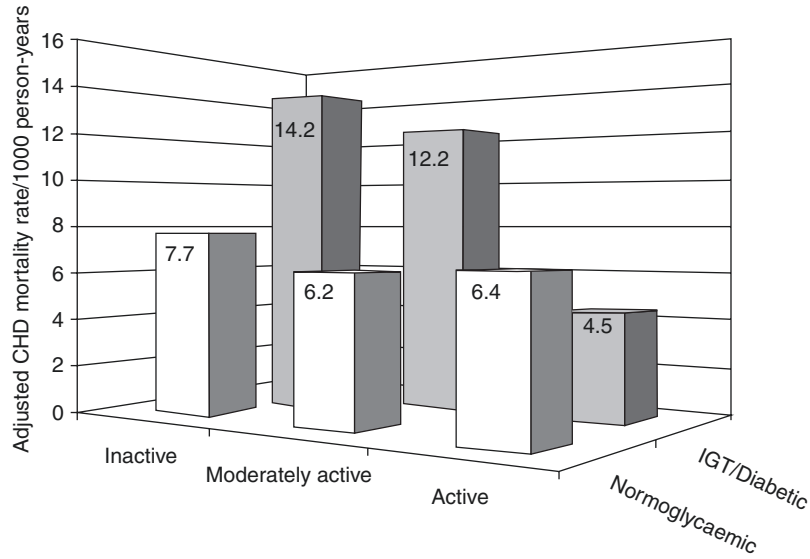

Fig. 18.2 Age-adjusted CVD mortality rates by leisure time activity in normoglycemic men (6056) vs. IGT/diabetes men (352) in the Whitehall Study. (Adapted by Gill and Malakova from data from the Whitehall Study [24]. Reprinted with permission from Gill and Malkova [2]). $P = 0.006$ for trend in normoglycemic men, $P = 0.003$ for trend in men with IGT/diabetes

Examination Survey (NHANES) data to demonstrate that HOMA-IR (an admittedly imperfect measure of IR) was associated with higher mortality in the lean population but, paradoxically, with lower mortality in the obese segment of the population [26].

The evidence linking exercise, physical fitness, and sedentary behavior to mortality is admittedly epidemiological, observational, cohort data and not the preferred randomized controlled trial data. 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 (discussed later). Whether improved insulin sensitivity is an independent contributor to lower mortality with improved CRF remains an open question.

Specific Benefits of Exercise in Insulin-Resistant States

Prevention of Diabetes

There is strong evidence for a role of exercise in the prevention of diabetes. Early epidemiological and sociological evidence demonstrated a strong inverse correlation between habitual physical activity and 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 [27]. This epidemiological relationship between physical inactivity and diabetes risk has been observed across diverse populations, including male and female college alumni, registered nurses, and British men (reviewed in [28]). These observations were followed by a set of large prospective studies: the Finnish Diabetes Prevention Study [29], Da Qing Study [30], and the Diabetes Prevention Program [31]. In all of these studies, a diet and exercise intervention prevented transition from impaired glucose tolerance to diabetes in 50–60% of individuals. Importantly, 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. The beneficial effect of achieving the exercise goal on prevention of diabetes in the Diabetes Prevention Project was also evident even among those participants who did not lose weight [32]. The reasons for exercise being so successful in preventing diabetes are not completely known but likely include multiple factors (discussed further below) including: improved insulin sensitivity through decreased visceral adiposity even in the absence of weight loss, enhanced fatty acid utilization, increased mitochondrial function and/or content, improved vascular and microvascular function, and modulation of inflammation and oxidative stress [2, 21].

Improved Glycemic Control in Diabetes

Physical activity/exercise is recognized as a cornerstone of the treatment of patients with T2D. More than 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 [33]. Since that observation, numerous studies and reviews have confirmed the beneficial effects of various forms of exercise or decreased or interrupted sedentary time on glycemic control for patients with T2D [34–44] and those at risk for diabetes [29–31, 43, 45]. However, some studies have suggested that long-duration, high-intensity, and/or combined exercise programs may be required to achieve significant improvements in chronic glycemic control, as measured by HbA1c [41, 46]. These salutary effects on blood sugar involve both acute (bout) and long-term (training) effects discussed further below, including improvements in insulin sensitivity, inflammatory state, oxidant load, and mitochondrial function, as well as improvements in multiple aspects of vascular function and changes in body composition.

Effects of Exercise on Glucose Metabolism

Glucose metabolism in response to exercise has been extensively studied, as it poses an important clinical challenge. Multiple studies have now demonstrated improved insulin sensitivity or lowered insulin levels with an exercise intervention [40, 41, 45, 47, 48]. 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 up to 72 hours 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 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 [49]. The overall benefits of regular exercise on IR 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 metabolism, including in persons with IR with or without T2D, although there is some variability in the impact of acute exercise on immediate glucose control due to variable activation of the sympathetic nervous system. The impact and duration of effects of an exercise bout on glucose uptake are dependent upon intensity, duration, and type (aerobic/resistance) of exercise, as well as on postexercise diet, possibly depending on the rate at which glycogen stores are repleted (reviewed in [41, 50, 51]). In general, much of the metabolic benefit of exercise on insulin action may be due to the most recent bout(s) of exercise [52–54]. Studies in rodents and humans have shown that exercise-induced insulin-independent glucose uptake occurs only during and shortly after exercise, but improved insulin-stimulated glucose uptake (ISGU) persists for 24–48 hours after an acute bout [54]. Therefore, repeated exercise, probably daily or at least every other day, is needed for long-term, bout effect benefits on glucose metabolism. The increase in ISGU occurs at the level of both liver and muscle tissue [55], including in individuals with T2D [56] where it results in improved glucose tolerance [34]. However, studies have shown that while ISGU improves with an exercise bout in individuals with IR, it remains less robust than postexercise ISGU in healthy controls [54].

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 contraction-related signaling events: (1) release of calcium from the sarcoplasmic reticulum leading to activation of calcium/calmodulin-dependent protein kinase (CaMK) II and (2) activation of AMP-activated protein kinase (AMPK) due to the increase in the AMP:ATP ratio that occurs with continued muscle contraction (reviewed in [51]). Use of inhibitors to block either of these pathways in rodent skeletal muscle blocks acute contraction-mediated increases in glucose transport.

Later in a bout of exercise, this insulin-independent enhanced glucose utilization appears to be supplemented or replaced by an increase in skeletal muscle ISGU. Both skeletal muscle adaptive responses to metabolic demand and vascular adaptation to exercise are necessary for this more prolonged, insulin-dependent increase in glucose uptake with exercise. 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 (reviewed in [57]). However, others have found that this postexercise insulin sensitivity does not involve increases in insulin signaling per se or require new protein synthesis. In contrast, they find that insulin sensitization does occur, as evidenced by a left shift of the insulin dose response curve, with concomitant lowering of serum insulin concentrations,

but this is in the absence of any increase in direct measures of insulin signaling (reviewed in [51, 58]). Instead increased skeletal muscle ISGU coincides with persistent increased 160-kDa Akt substrate (AS160 or TBC1D4) phosphorylation and glycogen synthase activity and not with Akt activity [54, 59, 60].

Other signaling mechanisms triggered by exercise also appear to contribute to insulin sensitization. For instance, interleukin 6 (IL-6), usually produced by adipocytes, is also produced at high levels by exercising skeletal muscle resulting in a ~100-fold increase in serum IL-6 during an exercise bout (reviewed in [61]). IL-6 has been shown to stimulate glucagon-like peptide-1 (GLP-1) secretion from intestinal L cells and the pancreas, leading to improved insulin responsiveness [62, 63]. IL-6, together with the increased AMP:ATP ratio, also results in exercise-induced increases in AMPK activity, further contributing to improved insulin sensitivity [64]. 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 [65]. 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 [66]. 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, the IL-6-deficient mice are obese and have several features of metabolic syndrome, including impaired glucose tolerance. Clearly the IL-6 bout response plays a crucial role in both acute metabolic responses and long-term adaptations to exercise.

Most recently, inositol hexakisphosphate kinase-1 (IP6K1) also has been implicated. This kinase generates an inositol pyrophosphate (IP7) that binds to and prevents activation of Akt. A recent study demonstrated downregulation of IP6K1 with muscle contraction that could result in enhanced Akt phosphorylation independent of increased PI3 kinase activation [67].

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 (reviewed in [21, 50]). 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, at least up to 72 hours after the last bout of exercise [68, 69]. However, it remains unclear whether insulin sensitization persists beyond a prolonged bout effect after cessation of exercise in the absence of weight loss or weight redistribution [50, 70]. These studies also compared exercise regimens consisting of moderate versus 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 (reviewed in [21]). 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, VO_2max was not an independent predictor of insulin sensitivity or fasting insulin concentration after adjusting for measures of total or visceral adiposity (total body fat and waist circumference). Conversely, after adjustment for fitness (VO_2max), fatness remained an independent predictor of both measures of insulin responsiveness [71]. 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, while waist circumference explained approximately 20% of the variance [72]. These results again suggest a much more significant effect from fatness than from fitness and suggest that insulin sensitivity is more tightly associated with low visceral adiposity than with high 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 versus the highest fitness quintile of 4.6 and 1.8, respectively, before and after adjustment for abdominal fat (visceral and subcutaneous). Thus, when considering the overall metabolic risk, rather than IR alone, adiposity and fitness both appear to play important roles [73].

Non-glucocentric Cardiometabolic Health Benefits of Exercise

Beyond the effects on muscle glucose uptake, multiple possible contributors to and mediators of the beneficial effects of physical activity on overall cardiometabolic health have been proposed. These include, but are not limited to, effects on inflammation, oxidative stress, the vasculature, mitochondrial function, and the gut microbiome that may provide cardiometabolic benefit through improved insulin sensitivity, as well as through other independent mechanisms, and are discussed briefly as follows. A detailed discussion of the vast and conflicting data on these mediators and likely bidirectional cause-and-effect relationships between them is beyond the scope of this chapter. Reviews of these topics are referenced where possible.

Vascular Effects of Exercise

In addition to direct effects on muscle glucose uptake, muscle vascular responses to exercise are likely to play a role in the apparent insulin sensitization that occurs with exercise. Sjoberg et al. explored postexercise insulin-stimulated muscle perfusion and glucose uptake in an exercised leg compared to the contralateral non-exercised leg 4 hours after an acute single leg exercise bout. They found that L-NMMA-mediated nitric oxide synthase inhibition blocked the insulin-stimulated increase in microvascular perfusion and abrogated the increased glucose uptake in the exercised leg [59, 60]. This suggests that an insulin-dependent vascular response to exercise provides enhanced insulin, substrate, and oxygen delivery to the skeletal muscle and is an essential contributor to the increased ISGU occurring after an acute exercise bout. Two aspects of endothelial function are likely to be involved: changes in total muscle blood flow and, perhaps more importantly, changes in blood flow distribution resulting from the endothelium-mediated increased delivery of glucose (as well as oxygen, insulin, and other substrates) specifically to muscle fibers that are most metabolically active. Thus, it is likely that endothelial dysfunction in diabetes contributes to IR by impairing appropriate blood flow and/or distribution [74, 75]. Conversely, the often-demonstrated improvements in endothelial responsiveness with exercise (reviewed in [59, 76–81]) likely contribute to improved glucose homeostasis in response to regular physical activity. Lee et al. have found that low- to moderate-intensity exercise is at least as beneficial as high-intensity or combined endurance and resistance exercise for endothelial function, as measured by flow-mediated dilation, consistent with other reports of significant metabolic and mortality benefits even for a change from sedentary to low levels of activity [77]. These vascular defects in IR will be discussed more fully in the section on T2D: *Role of Insulin Resistance in Exercise Defects*.

Two other aspects of vascular structure and communication with the muscle, capillary density, and the interstitial extracellular matrix (ECM) also play a role in IR and the response to exercise (reviewed in [82, 83]). Muscle capillary density is known to be decreased in IR, impairing insulin and substrate delivery to the muscle. Capillary density is improved with exercise, thus correcting the defect in delivery (reviewed in

[84]). However, insulin and substrates that reach the active muscle must then also exit the vasculature and enter the interstitial space. Evidence in animals and humans suggests that inflammation-mediated changes in the ECM with increased collagen synthesis and defective integrin signaling also affect insulin sensitivity. The mechanism is not understood but may involve direct effects on insulin signaling, interference with insulin entry into the interstitial space, or simply through capillary rarefication [82, 83].

Inflammation and Oxidant Stress

Inflammation is one of the universal mechanisms contributing to the initiation and progression of atherosclerosis [85] and the development of T2D [86–88]. It is also thought to play a role in the development of IR in these conditions [89]. Thus, the anti-inflammatory effects of exercise may contribute to insulin sensitization. However, the inflammatory consequences of exercise remain unclear. In general, short-term moderate-intensity exercise interventions have a modest positive impact on a subset of circulating cytokines, such as IL-1 and IL-18, CRP, and tumor necrosis factor-alpha (TNFα[alpha]); presumed anti-inflammatory markers such as adiponectin and IL-6; and inflammation-related cell adhesion molecules such as VCAM, intercellular adhesion molecule (ICAM), and the selectins [90–94], 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 people with diabetes [91]. 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 [90]. A recent study comparing two exercise modalities in individuals at risk for T2D found improved IR and increased IL-1 receptor antagonist (IL-1 RA) concentrations, independent of the exercise modality (high versus moderate intensity). However, CRP and IL-6 were not affected by either exercise intervention [95].

Overall, the most consistent findings from a large body of literature on exercise and inflammation support anti-inflammatory effects of exercise in the form of lower TNFα(alpha), increased IL-1 receptor antagonist (IL-1RA), and transiently increased anti-inflammatory interleukins IL-6 and IL-10 [88]. One of the most consistent findings is a pronounced, but transient, bout effect on IL-6 that is now widely considered to have significant beneficial anti-inflammatory effects in addition to the metabolic effects described previously (reviewed in [88]). Infusion of recombinant IL-6 has been shown to inhibit production of TNFα in response to endotoxin. Other evidence supports a role for IL-6 in suppression of TNFα production and in the synthesis or release of other anti-inflammatory molecules, such as IL-1RA and soluble TNFα receptor (reviewed in [94]).

In contrast, the effect of exercise on the commonly used clinical marker of inflammation, hsCRP, remains controversial. A recent review of the evidence concluded that there is insufficient evidence for CRP lowering with exercise [96]. One of the largest studies to address this issue is the HERITAGE Family Study of 652 healthy sedentary adults [97]. 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, the effect of exercise on inflammation may be dependent upon the baseline status of the population, as well as on the nature, intensity, and regularity of the exercise intervention and on the timing of measurement of specific markers relative to the last bout of exercise.

Another proposed mechanism for variability in the inflammatory response to exercise invokes oxidant stress. The increased metabolic rate in exercising muscle results in increased generation of oxidants that can cause tissue damage. With training, levels of antioxidant enzyme systems appear to increase and oxidant damage decreases (reviewed in [98–101]). The net result is, therefore, a balance of two opposing forces: inflammation and oxidant stress from unaccustomed activity and exercise bouts, with compensatory and beneficial anti-inflammatory, antioxidant, and tissue repair responses with training (reviewed in [99–101]). Several studies have demonstrated that antioxidants actually impair the training response to exercise, pos-

sible by attenuating the oxidant burst with exercise and, subsequently, the adaptive responses to the oxidant burst [102, 103]. Thus, this apparently harmful oxidant burst from exercise may actually be an essential contributor to the benefits of exercise.

Mitochondrial Function and Fatty Acid Oxidation

Sedentary behavior, obesity, IR, and T2D are all associated with mitochondrial dysfunction, decreased mitochondrial content, and altered mitochondrial dynamics in what is likely a bidirectional cause-and-effect relationship (reviewed in [104–109]). While the mechanism is not understood, some studies have implicated inflammation and meta-fibrosis induced by mitochondrial oxidant stress from overnutrition in the etiology of these mitochondrial defects (reviewed in [110]). A deficiency in skeletal muscle oxidative capacity, especially for fatty acid oxidation, is now thought to be a major factor in IR and T2D. Thus, another mechanism by which exercise may improve insulin sensitivity is through beneficial effects on mitochondrial function and muscle oxidative capacity.

In fact, multiple studies have now demonstrated increases in fat oxidation and mitochondrial content with various exercise interventions, with or without calorie restriction or weight loss. Goodpaster et al. studied the effects of training combined with caloric reduction on insulin sensitivity and fat oxidation and found that fasting fat oxidation increased by nearly 20% after the intervention and was a stronger predictor of insulin sensitivity than weight loss or fitness [111]. A similar intervention was found to induce about a 50% increase in skeletal muscle mitochondrial electron transport chain activity [112, 113]. More recently, this group performed a study comparing the effects of 16-week interventions with either caloric restriction-induced weight loss or exercise without weight loss. Both interventions improved insulin sensitivity to similar degrees, but only the exercise intervention increased mitochondrial content and electron transport chain and fatty acid oxidation enzyme activities [114]. An older study looked at effects of a physical activity intervention in a sedentary overweight-to-obese population without caloric restriction and measured fat oxidation and insulin sensitivity both after two to four bouts of exercise (<1 week of training) and after 6 weeks of training. Significant and maximal increases in insulin sensitivity and fat oxidation were already present after two to four bouts of exercise in the absence of changes in weight or fat distribution [115], suggesting that effects on mitochondrial fatty acid oxidation may also occur as a bout effect of exercise. The idea that improved fat oxidation and mitochondrial function result from a bout of exercise is supported by other literature. For instance, Holloway et al. demonstrated that during 120 minutes of aerobic exercise, whole body fat oxidation, mitochondrial palmitate oxidation, and CPT1 activity increased progressively throughout the exercise bout [116]. In another study, a single bout of exercise was able to prevent the IR induced by lipid/heparin infusion. Compared to a sedentary control day, the exercise bout increased enzymes of triglyceride synthesis, decreased muscle diacylglycerol levels, and increased whole body fat oxidation throughout the lipid infusion [117]. Like the bout effect of exercise on glucose disposal, this effect is mediated at least in part by the activation of AMP kinase by muscle contraction [118, 119]. 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. These and many other studies demonstrate that exercise, both endurance and resistance, increase mitochondrial biogenesis and improve oxidative metabolism, fatty acid oxidation, and insulin sensitivity (reviewed in [104, 105, 108, 112, 113, 116, 120–124]).

Weight Loss/Maintenance

The worsening epidemic of obesity is clearly an underlying factor in the increasing prevalence and earlier onset of metabolic syndrome and T2D. 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. Multiple reviews and meta-analyses of weight loss studies that included an isolated exercise intervention have consistently found that exercise training without dietary change results in minimal absolute weight loss (reviewed in [125–130]).

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 [131]. Similarly, in the National Weight Control Registry comparison of a group of subjects who have maintained a substantial weight loss for greater 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 [132]. 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 [133]. 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 resulting from their exercise regimen.

Lipids, Blood Pressure, and Cardiorespiratory Fitness

The role of exercise interventions in altering lipid levels remains unclear (reviewed in [134–140]). In general, studies with longer interventions (>6 months) of higher intensity and longer bout duration in metabolically unhealthy populations have been most likely to show increases in high-density lipoprotein cholesterol (HDLC) levels and reductions in cholesterol and triglyceride (TG) levels. Two meta-analyses found conflicting results with regard to the HDLC-raising effects of exercise. A meta-analysis of studies of 2–12 months of exercise in subjects with T2D found a significant decrease in TG levels, but no significant change in HDLC or low-density lipoprotein cholesterol (LDLC) levels [141]. In contrast, a meta-analysis of randomized controlled trials of exercise intervention with lipid endpoints [142] 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 2 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.

High blood pressure (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 [143]. 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 comorbidities. Several meta-analyses have assessed longitudinal intervention studies with different forms of exercise to determine the impact of exercise training on BP [144–146]. Studies have included both hypertensive and normotensive subjects and some have compared aerobic versus resistance versus isometric exercise. Overall these analyses demonstrated a small (average ~3 mm Hg), but clinically and statistically significant, decline in both systolic and diastolic average BP at rest, with a greater reduction in older hypertensive subjects.

Discussion of the effects of exercise on these and other parameters is complicated by the nonresponder phenomenon. In the HERITAGE study, individual responses to exercise were noted [147, 148]. For instance, although mean insulin sensitivity improved, individually 58% of subjects improved, while the remaining 42%

remained stable or became more insulin resistant. Similar results were found for HDLC-raising and BP-lowering effects. In addition, the response in one parameter did not predict response in other parameters. A combined analysis of several large exercise trials further supported the issue of non-response to exercise, again including a lack of effect on lipid levels, BP, and insulin sensitivity [149]. This combined analysis of ~700 individuals who underwent a variety of exercise interventions found that only about half of the individuals had a positive response to exercise for these measures, while about 10% had a statistically significant worsening of each of these parameters. Interestingly, the adverse responses were independent of type and intensity or exercise and occurred despite improvement in VO_2 max, the commonly used measure of CRF in these studies. Most adverse responders had a negative response in only one parameter (31% of all participants), with <1% having a negative response to three or more parameters. Conversely, the HART-D study demonstrated that significant metabolic benefits were seen with exercise even in participants who were nonresponders with respect to CRF [150]. Thus, responses to exercise are likely quite individual [151], but it is clear that virtually everyone has a beneficial response to exercise in some, if not all, known cardiometabolic measures.

Gut Microbiome

Effects of the gut microbiome on insulin sensitivity are increasingly well established and the interaction between exercise, IR, and the microbiome is just now being elucidated. Several lines of evidence briefly reviewed here suggest that the gut microbiome affects insulin sensitivity. For instance, Sepideh et al. treated individuals with mild prediabetes and fatty liver for 2 months with a multispecies probiotic in a randomized double-blind placebo-controlled trial of 25 participants in each group. Fasting insulin, HOMA-IR, and several inflammatory markers improved significantly in the probiotic group [152]. A high-fat diet has been shown to decrease overall diversity and the bacteroidetes-to-firmicutes ratio in the distal rodent intestine—changes associated with propensity for weight gain and with inflammation and IR in animals and man (reviewed in [153]).

Additional evidence suggests that exercise alters the gut microbiome in a direction that may be associated with improved insulin sensitivity. Preclinical studies have now demonstrated that exercise, in various forms, is able to counter the ill effects of a high-fat diet on the distal intestinal microbiome independent of weight changes [154–157]. For instance, Denou et al. studied male mice on chow versus high-fat diet (HFD) with and without high-intensity interval exercise training in the HFD group. HFD decreased genetic diversity and the bacteroidetes-to-firmicutes ratio in the gut microbiome. Exercise training did not mitigate weight gain on the HFD, but the trained mice had significant reversal of the HFD-induced changes in the gut microbiome and were significantly more insulin-sensitive [156]. Studies in humans are very limited, but also suggest that exercise effects on the gut microbiome may contribute to the insulin-sensitizing effects of exercise. Three studies have found a correlation between physical fitness and gut microbiome composition. Yang et al. demonstrated correlations of physical fitness with several components of the gut microbiome in premenopausal women [158]. However, this relationship was lost when the model was adjusted for percent body fat, suggesting that fatness, not fitness, was the driving factor. Estaki et al. studied young healthy adults of both sexes and also found a correlation between fitness and gut microbiome diversity and healthy (i.e., butyrate producing) composition [159]. Finally, Clarke et al. compared professional athletes during a training period (rugby team members) to nonathlete controls. The athletes demonstrated increased gut microbiome diversity compared to controls, especially overweight and obese controls, but diet differences between the groups also appeared to play a role [160].

Overall, there is strong evidence for a relationship between exercise and/or fitness and the insulin sensitivity-impacting health and diversity of the gut microbiome in animals, with much weaker, though suggestive, evidence in people.

An ongoing study in men with obesity-related sleep disorders may shed more light on this topic [161].

Other Novel Mediators

MicroRNA

Exercise has been shown to alter microRNA (miRNA) levels in the blood, heart, and skeletal muscle, as well as other tissues (reviewed in [162]). In particular, evidence supports a protective role for exercise-induced miRNA changes in the diabetic heart [163]. There is wide speculation and diverse evidence for metabolic consequences of these miRNA changes. This is likely to be a field of intense future study with potential impact on our understanding of the relationships among exercise, insulin sensitivity, and the benefits of exercise. However, the field is too new to allow for a concise review or clear conclusions from this evidence at this time.

Natriuretic Peptides

A growing body of evidence suggests that natriuretic peptides (NPs) have roles beyond cardiovascular function. These peptides, secreted by myocardium and endothelial cells, appear to have widespread effects not only on cardiovascular function, but also on every tissue known to be involved in metabolic homeostasis. Recent studies have shown an inverse correlation between NPs and obesity, while exercise has been shown to increase circulating NPs. Thus NPs may also play a key role in the metabolic benefits of exercise and/or exercise deficits in IR (reviewed in [164]).

Myokines

As discussed previously, production of IL-6 by active muscle may play a large role in the beneficial effects of exercise. Other studies have implicated a large array of other myokines produced by contracting skeletal muscle as well (reviewed in [165]). These include other interleukins, irisin, fibroblast growth factors, neurotrophic factors, and more.

Summary of Exercise Benefits

In general, evidence suggests that the benefits of physical activity on the overall metabolic state result from a combination of single and cumulative bout effects on insulin sensitivity, largely in the skeletal muscle and the vasculature, as well as training effects, likely through body composition and/or fitness, on insulin sensitivity and other metabolic syndrome criteria. Many different mechanisms contribute to these cardiometabolic changes and the interplay between them is not fully understood and continues to be the subject of active research.

The Reality of Exercise in Diabetes

Unfortunately, despite the extensive data indicating the importance of physical activity and exercise in diabetes, 60–80% of adults with T2D do not exercise sufficiently and adherence to exercise programs is low in these patients [166–169]. Sedentary people are more likely to develop diabetes, so established lifestyle habits are likely to contribute to this statistic. Importantly, however, another clear physiological contributor to this relationship is the underappreciated impairment in functional exercise capacity and exercise tolerance in people with diabetes. In age, sex, activity, and BMI-matched individuals (youth and adults) with uncomplicated diabetes (type 1 and type 2), there is a 20–30% decrease in CRF [170–173]. This exercise impairment also occurs in other insulin-resistant, diabetes risk states, such as polycystic ovarian syndrome (PCOS) and metabolic syndrome [174, 175]. Thus, a bidirectional adverse relationship between sedentary lifestyle and IR may also occur in these prediabetic conditions. In this section we will review the data demonstrating exercise impairment and intolerance in diabetes, the reported contributors to this relationship, the interaction between exercise and insulin sensitivity, and newer reports demonstrating microvascular contributors to the relationship between exercise, insulin action, muscle fatigue, and CFR.

Effects of Type 2 Diabetes on Exercise Performance

Maximal Exercise Capacity

Multiple studies have now demonstrated that people with apparently uncomplicated diabetes (adults and youth, type 1 and type 2) have reduced CRF compared with people without diabetes who are matched for age, weight, and/or physical activity, as evidenced by a lower VO_2max during incremental exercise (e.g., Table 18.1) [37, 38, 172, 176–181]. The overall difference in VO_2max between people with and without diabetes is approximately 20%. The mechanisms for this impairment have not been completely elucidated. However, based upon available data, IR, cardiac, and peripheral factors limiting systemic oxygen delivery and defects in tissue oxygen extraction may all play a role (see below).

Submaximal Exercise Tolerance and Oxygen Uptake Kinetics (VO_2 Kinetics)

There are also exercise abnormalities during submaximal exercise in T2D. During the early stages of an incremental exercise test, oxygen consumption (VO_2) increases with each increase in work rate. In individuals without diabetes, there is a predictable increase in VO_2 to meet the metabolic demand for a given increase in workload (~10.1 ml/min/watt) [182]. The VO_2-to-workload 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 [183]. Similar to maximal O_2 consumption, the rate of increase in VO_2 per increase with increasing workload is altered in people with T2D compared with healthy controls [172]. Submaximal constant-load exercise studies have demonstrated defects in the ability to increase oxygen consumption with increasing demand in T2D. 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, cardiac and vascular adaptations of oxygen delivery, and pulmonary gas exchange. The primary component of VO_2 kinetics is described by a time constant (tau2) reflecting the time to reach ~63% of the increase in VO_2. The VO_2 kinetic response is slowed in women with T2D compared to nondiabetic women of similar body mass index and physical activity levels in the absence of any clinical evidence of CVD [172] (Table 18.2). Women with T2D had not only a lower VO_2max, but also

Table 18.1 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

Modified with permission from Regensteiner et al. [172, 179]

RER respiratory exchange ratio

$*P < 0.05$ for difference between T2D and controls. $**P < 0.05$ for difference between lean and other two groups. Data are mean ± SD

Table 18.2 Submaximal exercise kinetics

	LC	OC	DM
VO_2 kinetics			
20 W Tau (sec)	21.4 ± 8.9	18.4 ± 9.9	42.6 ± 23.8∗
30 W Tau (sec)	28.8 ± 5.3	27.8 ± 8.9	36.8 ± 6.2∗
80 W Tau (sec)	42.8 ± 7.5	41.2 ± 8.2	55.7 ± 20.6
Heart rate kinetics			
20 W Tau (sec)	8.5 ± 4.6	10.6 ± 8.2	23.8 ± 16.2∗
30 W Tau (sec)	23.9 ± 13.8	14.2 ± 8.0	40.7 ± 11.9∗
80 W Tau (sec)	41.2 ± 14.8	43.3 ± 11.3	72.3 ± 21.5∗

Modified with permission from Regensteiner et al. [172]

LC lean controls, *OC* overweight controls, *DM* T2 diabetes, *W* watts, *Tau* the monoexponential time constant of VO_2

$*P < 0.05$ difference between T2D and both control groups. Data are mean ± SD

reduced VO_2 at all submaximal workloads tested (Fig. 18.3) and slower VO_2 kinetic and heart rate kinetic responses. This was true compared to both lean and obese controls (Table 18.2), suggesting that T2D, rather than obesity per se, is responsible for the observed exercise impairments. Of note, this defect in the kinetics of oxygen increase at submaximal workloads may be of particular relevance to the decreased activity in patients with T2D, as it has an impact on the rate of perceived exertion (RPE) at any exercise level. This may discourage physical activity as the effort required seems higher for individuals with T2D, even at very low workloads [181, 184].

VO_2 kinetics may be limited by defects in oxygen delivery or by the inertia of oxidative metabolism in the working tissue [185–188]. Thus, the time constant of phase 2 VO_2 kinetics is sensitive to alterations in oxygen exchange at the lungs, cardiac output, endothelial function, microvascular distribution, oxygen diffusion, and rates of tissue oxygen consumption and is prolonged in patient groups with abnormal pulmonary, CV, or metabolic responses to exercise. Potential mechanisms for this abnormal response in T2D include a decrease in oxygen delivery due to impaired endothelial or cardiac function, defects in blood flow distribution at the tissue level, and/or an abnormality of muscle oxidative metabolism.

Role of Insulin Resistance in Exercise Defects

Many reports demonstrate a positive correlation between insulin sensitivity and VO_2max [189–191]. In adults and adolescents with uncomplicated T2D, IR was a significant independent predictor of VO_2peak and slowed VO_2 kinetics [180]. IR has also been reported to be inversely correlated with VO_2max in several disease states in addition to T2D, including type 1 diabetes, heart failure, and chronic renal failure [192–194]. This decrease in CRF is independent of hyperglycemia or complications of diabetes or of the systemic illness associated with heart and renal failure, as evidenced by the findings of exercise defects in adolescents with uncomplicated early T2D [180], in nondiabetic women with polycystic ovarian syndrome (PCOS) [174], and in the metabolic syndrome [175]. The deficit 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. Furthermore, a direct correlation between insulin sensitivity and physical fitness level has been demonstrated in normotensive men with a family history of hypertension [195] and in healthy nonobese individuals without personal or family history of diabetes [196].

The cause-and-effect relationship between IR and impaired exercise performance has

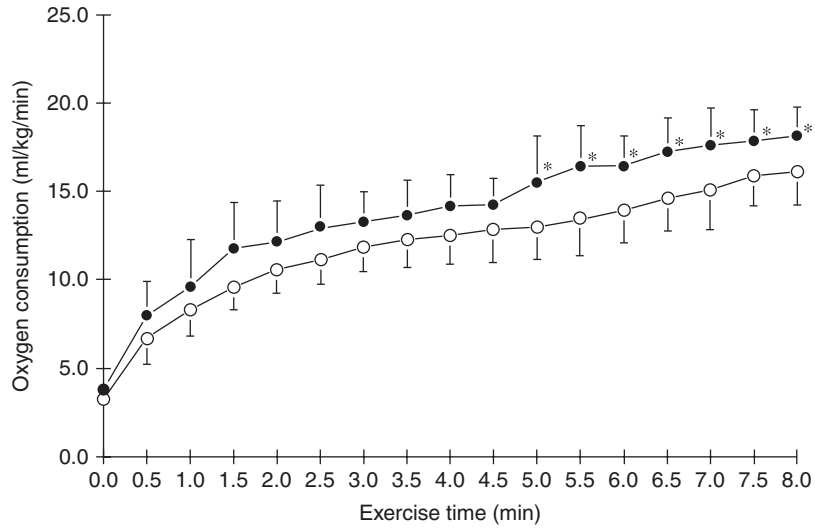

Fig. 18.3 This figure illustrates that oxygen consumption at all submaximal workloads for which there is complete data is reduced in persons with type 2 DM (open circles) compared to nondiabetic controls (closed circles) of similar age and activity levels during graded exercise testing [173]. (Reprinted with permission from Regensteiner et al. [173])

been further addressed through the use of a pharmacological intervention to improve insulin sensitivity. In a study of 20 women with early, uncomplicated T2D randomized to rosiglitazone or placebo, rosiglitazone treatment resulted in a significant 7% improvement in VO_2 max [189]. This improvement correlated with both increased insulin sensitivity and improved endothelial function. Furthermore, in one small study, 6–8-hour lipid infusion to acutely induce IR in healthy individuals prior to exercise testing decreased max workload, slowed kinetics, and increased RPE [196]. Conversely, the addition of exenatide in a well-controlled population with T2D improved glycemia and vascular and cardiac stiffness, but not insulin sensitivity (by HOMA-IR), endothelial function, VO_2peak, or VO_2 kinetics [197]. The implication of all these results is that there is an exercise impairment associated with T2D that is not solely a result of deconditioning from an associated sedentary lifestyle or of hyperglycemia and that appears to be directly related to IR. Thus, the aforementioned 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 that, in turn, make exercise feel more difficult and interfere with exercise adherence.

Potential Mechanisms for Insulin Resistance-Associated Exercise Impairment

There are several metabolic and nonmetabolic sequelae of IR that may contribute to muscle fatigue and to the decreased capacity for exercise in IR (reviewed in [198]). In this section, we will review the relationship between insulin action, cardiac and peripheral endothelial function, and muscle metabolism and mitochondrial function in both cardiac and skeletal muscle. We will further discuss the impact of changes in heart and skeletal muscle perfusion (blood flow and distribution, capillary density, and recruitment) on exercise performance, thus considering how each defect may contribute to exercise impairments in diabetes.

Hyperglycemia

To date no associations have been found between markers of glucoregulation (hemoglobin A1C or fasting serum glucose concentration) and exercise performance [173, 177, 178, 180, 197, 199, 200]. Thus, changes in glycemic control, per se, do not appear to be the primary mediators of exercise defects in T2D.

Vascular Dysfunction

This broad term encompasses several forms of vascular dysfunction that are well established to be present in IR conditions (reviewed in [201]). These include endothelial dysfunction, arterial stiffness, and microvascular defects. The potential relationship of each of these parameters to IR and to exercise defects is discussed as follows.

Arterial Stiffness

Arterial stiffness is widely recognized in IR states [202]. Correlation with decreased VO_2 peak has been reported in some [203, 204] studies. However, pharmacological improvement in arterial stiffness does not appear to translate to improved exercise capacity [197, 205]. In contrast to endothelial dysfunction (a specific defect in endothelial cells), arterial stiffness is multifactorial and results from a combination of endothelial dysfunction, chronic changes in the vessel wall cellularity and structure, vascular smooth muscle dysfunction, and tonic vasoconstriction. Structural stiffening of the vessel wall (increased collagen and elastin degradation) is associated with chronic hypertension and hyperglycemia and correlates with IR, but the causal relationship has not been established. In addition, IR states are associated with increased sympathetic nervous system (SNS) activity [206–208] and renin angiotensin system (RAS) activation [201]. Each of the latter cause vasoconstriction and hence contribute to a state of increased vascular tone and structural arterial stiffness. In the exenatide study referred to earlier, exenatide improved arterial stiffness but did not affect IR and the intervention did not significantly improve exercise

performance [197]. These data suggest that arterial stiffness may not be a primary mediator of IR-associated exercise defects.

Endothelial Dysfunction

It is well established that peripheral endothelial function and vascular reactivity in response to pharmacological vasodilators and cuff ischemia at rest [209, 210], as well as in response to exercise, are abnormal in adults with T2D compared to nondiabetic controls [211, 212]. Furthermore, insulin's physiologic ability to enhance endothelium-dependent vasodilation is markedly impaired in people with diabetes compared to lean control subjects. It has been proposed, and studies support, that IR at the level of the endothelial cell is invariably associated with endothelial dysfunction [213]. For instance, obese subjects with and without T2D have endothelium-dependent vasodilation that is reduced by 40–50% compared with lean control subjects [214]. Jahn et al. demonstrated that IR-related endothelial dysfunction, at least in response to insulin, exists at all levels of the arterial tree (conduit, resistance, and microvascular) in metabolic syndrome [215]. Direct effects of IR on endothelial nitric oxide synthase activation and indirect effects through associated RAS and/or SNS activation and adipokine production by perivascular adipocyte tissue have all been proposed and supported as mediators of IR-associated endothelial dysfunction [201, 216–218]. Thus, the exercise abnormalities observed in T2D could reflect a deficient endothelial dilator and recruitment response to metabolic demand in the heart, as well as in peripheral skeletal muscle. In this scenario, exercise capacity would be limited by peripheral and/or cardiac blood flow, impairing not only demand-associated increases in oxygen delivery, but also in glucose and insulin transport to skeletal and cardiac muscle.

The evidence exploring an interaction between endothelium-dependent endothelial nitric oxide (NO) generation and exercise performance in T2D is surprisingly mixed. Specifically, direct support for a role for endothelial dysfunction in T2D exercise defects comes from the work 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 that correlated with the expected reduction in vasodilation and decreased perfusion of large muscle groups [219]. However, in contrast to the slowing of VO_2 kinetics seen in subjects with T2D, L-NAME induced an acceleration of the rate at which oxygen consumption increased with exercise [219, 220]. This could be explained by the observation that NO appears to competitively interfere with the mitochondrial electron transport chain [221–223], impairing muscle oxidative phosphorylation and slowing muscle VO_2 kinetics. Thus, acute 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. Furthermore, mouse studies have demonstrated an adaptation to chronic L-NAME treatment with restoration of VO_2max over time [224]. If this holds in humans, the chronic endothelial dysfunction of T2D could be overcome by compensatory changes. Together with the fact that VO_2 kinetics are slowed in diabetes, these results imply that changes in exercise parameters in T2D cannot be fully explained by changes in NO synthesis or, presumably, by endothelial dysfunction alone.

Microvascular Dysfunction

The role of the microvascular blood flow distribution in muscle fatigue and CRF has been postulated for decades and is an area of intensive study. Microvascular blood flow and responses to increased demand are more difficult to study quantitatively and a number of techniques have been combined in both clinical and preclinical studies to address the microvascular contribution. These techniques include contrast-enhanced ultrasound (CES), near-infrared spectroscopy (NIRS), and magnetic resonance imaging/magnetic resonance spectroscopy (MRI/MRS) and positron emission tomography (PET) methods in humans and intravital microscopy in rodent muscle and human surrogate muscle (tongue vasculature). A number of groups have defined clear defects in microvascular function in IR and T2D [225–229]. Other studies have clearly demonstrated microvascular recruit-

ment by insulin—for instance, during euglycemic–hyperinsulinemic clamps—that is independent of overall limb blood flow and correlated directly with insulin sensitivity [230] (reviewed in [231]). This microvascular recruitment is thought to be due to insulin-mediated endothelial NO production in the small resistance vessels in the muscle. For example, the skeletal muscle improvement in glucose clearance postexercise bout depends in part upon NO-dependent improvement in skeletal muscle blood flow and in part on increased skeletal muscle insulin responsiveness [59]. In support of this, another study also found that cutaneous microvascular flow in response to acetylcholine iontophoresis was decreased in insulin-resistant women without T2D [232], again supporting a role for IR, rather than hyperglycemia, in this defect. This finding, combined with reports that insulin-mediated capillary recruitment is decreased in people with T2D, places microvascular dysfunction squarely as a mechanistic contributor to and/or result of IR that may also contribute to exercise defects in T2D.

We and others have examined the relationship between abnormal microvascular function in T2D and the CRF impairment. To date, the data are inferential yet compelling. In the heart, we found that postexercise cardiac perfusion was impaired in people with uncomplicated T2D [233]. This perfusion defect correlated with little or no reduction in cardiac index [233, 234]. Despite stable or decreased cardiac function, people with T2D demonstrated lower arteriovenous oxygen extraction and less net muscle blood flow, with a resulting overall decrease in muscle oxygen consumption. Importantly, VO_2max correlated with the skeletal muscle arteriovenous oxygen difference, but not with cardiac output [170, 233]. Since even a modest reduction in cardiac output should increase reliance on oxygen extraction, this suggests that local defects in oxygen transport into and/or oxidative capacity of the exercising skeletal muscle (discussed later) exist in T2D and may contribute to the exercise defects seen in this population. The microvascular perfusion defects suggested by these studies appear to fall into three possible general categories: (1) gross defects in capillary density and structure, (2) decreased capillary recruitment with increased demand (likely reflecting small vessel endothelial function), and (3) dysfunctional distribution of blood flow in response to demand (reviewed in [231, 235]).

The contributors to muscle microvascular perfusion include structural and functional components outlined previously. One of the earliest observed microvascular defects in T2D was reduced skeletal muscle capillary density [236]. In addition altered basement membrane structure has been demonstrated in T2D and is thought to correlate with hyperglycemia and other microvascular complications of T2D [237]. These structural changes could directly contribute to alterations in microvascular hemodynamics that impair O_2 and substrate exchange from capillary to myocyte, as suggested by work in diabetic rodent models [238–240]. The relationship between oxygen diffusion (potentially decreased in T2D) and exercise performance in T2D has not been extensively explored [241, 242].

In order to address capillary recruitment during exercise, we evaluated the impairment seen in VO_2 kinetics in T2D in conjunction with measures of skeletal muscle oxygenation with NIRS in 11 T2D and 11 healthy, sedentary subjects [187]. 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 the T2D subjects that is consistent with a transient imbalance of muscle oxygen delivery relative to muscle VO_2 in T2D [188, 243]. These data suggest a subnormal or delayed microvascular blood flow increase in the skeletal muscle of T2D subjects in response to exercise.

Decreased capillary recruitment and density do not fully explain the failure to increase arterial oxygen extraction in compensation for demand-insufficient oxygen delivery during exercise in IR and T2D. First, muscle contraction itself also triggers microvascular recruitment and studies

by Barrett et al. have suggested that exercise-induced recruitment is independent of insulin and occurs even in the setting of IR without microvascular complications [228, 229]. Thus, increased recruitment of microvascular flow should occur during exercise in uncomplicated IR. Second, if the problem during exercise is solely impaired microvascular blood flow, oxygen extraction from the available blood should increase in response to the demand. While the absence of this compensatory increase in oxygen extraction may result from defects in skeletal muscle oxygen extraction or metabolism (described later), studies and flow modeling by our group and others have suggested that impaired distribution of blood flow may also contribute and is possibly the main contributor. In this model, blood flow within the muscle is not appropriately locally distributed to the muscle fibers with the highest oxygen demand, due either to lack of appropriate perfusion heterogeneity or an excess of inappropriate heterogeneity [74, 75, 188, 235]. The result is excess blood flow and low oxygen extraction in nonworking muscle and insufficient blood flow with maximum possible oxygen extraction in the working muscle. Mathematical modeling suggests that this would result in an overall reduction, or at least failure to compensate for increased demand, in overall oxygen extraction.

Overall there is strong evidence that IR-induced defects in oxygen delivery to working muscle (skeletal and cardiac), including at the level of the microvasculature, contribute to the exercise defects associated with IR and T2D.

Myocardial Dysfunction

It is likely that cardiac factors also contribute to the exercise abnormalities of T2D 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 and in adolescents with T2D (e.g., [244–253]). This condition has been termed "diabetic cardiomyopathy" and generally refers to a finding of subclinically impaired left ventricular (LV) function at rest [244, 247, 249, 250, 254] and/or during exercise [234, 246, 248, 252, 253, 255] in the absence of major coronary disease or hypertension. The aforementioned studies have demonstrated a predominant component of diastolic dysfunction in diabetic cardiomyopathy. Similar subclinical, largely diastolic, dysfunction has also been demonstrated in metabolic syndrome [175].

Clinically, it has been shown that cardiac diastolic dysfunction correlates closely with impairments in CV exercise capacity in heart failure [256], in diabetes [234, 248], and in normal subjects [257]. In our studies of exercise dysfunction in T2D, we have observed that pulmonary capillary wedge pressure rises more steeply and to a greater level with exercise in T2D than in controls [258] and that this cardiac abnormality, which likely represents diastolic dysfunction, correlates with the observed decrease in exercise capacity. In addition, the finding that heart rate kinetics are slowed in diabetes further suggests a cardiac or "central" oxygen delivery component to the exercise impairment [172]. Although resting cardiac output is typically preserved in T2D, the impaired diastolic filling results in a smaller stroke volume and cardiac output is preserved at the expense of a higher heart rate. As a result, heart rate reserve for exercise is decreased in addition to the impaired filling, likely contributing to the decrease in VO_2 max in T2D [234, 255]. In contrast, however, Gurdal et al. found that the decreased exercise capacity in 43 study participants with T2D did not correlate with their mild degree of diastolic dysfunction [259].

Multiple mechanisms, including the IR-associated increase in SNS and RAS activation, have been proposed to play a role in cardiac dysfunction in T2D [234]. We have also observed that the disproportionately increased pulmonary capillary wedge pressure, which correlates with the decrease in exercise capacity in uncomplicated T2D, also correlates with reduced myocardial perfusion [233], suggesting that impaired coronary artery endothelial function and/or the microvascular dysfunction, described previously, leading to cardiac dysfunction may be another mechanism for exercise impairment in T2D.

Metabolic Dysfunction

Cardiac Substrate Utilization in Insulin Resistance

Changes in substrate utilization and metabolic inflexibility are also potential contributors to exercise defects in IR. Simply stated, insulin promotes carbohydrate utilization. In the absence of sufficient insulin signaling in IR, 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 that may limit the ability to mobilize these alternative fuel sources. Compared to skeletal muscle, cardiac muscle preferentially utilizes fatty acids for energy production. However, a further shift in energy production to use of fat over glucose and an inability to shift to glucose under increased energy demand may also contribute to exercise defects in diabetes. This model is supported by studies examining cardiac fuel utilization in IR rodents, in which a fixed, excess reliance on inefficient fat oxidation in the diabetic myocardium relative to nondiabetic controls was demonstrated [260, 261]. 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 (reviewed in [122, 262]). 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 [263–266]. 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 cardiac substrate oxidation.

Skeletal Muscle Mitochondrial Function in Diabetes

As discussed previously, multiple mechanisms are likely to be at play in altering skeletal muscle metabolism during exercise. Defects in skeletal muscle mitochondrial function and, consequently, impaired ability to maintain and restore ATP levels may contribute to exercise defects in T2D. The changes in microvascular function described previously would not only limit oxygen delivery to working muscle, but also substrate and insulin delivery. Hence the shift to the less energy-efficient fatty acid utilization for cardiac muscle may also occur in skeletal muscle, even in the absence of other muscle defects. In contrast to cardiac muscle, however, skeletal muscle can take up glucose in an insulin-independent fashion during muscle contraction as described. Thus, in the setting of exercise, skeletal muscle fibers that are properly perfused may have an excess of substrate (glucose and fatty acids) available, while underperfused muscle fibers lack adequate substrate. The observation of metabolic inflexibility in IR and T2D has established that these conditions impair the ability to adjust to available substrate on the whole-body level [105]. Overall, metabolic inflexibility and microvascular perfusion and distribution defects together present a scenario of metabolic disarray with variably inappropriate substrate utilization in working skeletal muscle. This metabolic disarray is likely to contribute to increased muscle fatigue and greater perceived exertion seen in T2D and thus to decreased peak exercise capacity. Furthermore, we and others have demonstrated decreased mitochondrial function *in vivo* in the skeletal muscle in T2D using MR spectroscopy [267, 268]. However, we have also found that the in vivo defect is largely corrected with supplemental oxygen [269] suggesting that the defect may be largely in oxygen delivery or extraction, rather than an inherent mitochondrial defect. Overall, it appears that both the ability to deliver oxygen appropriately to working skeletal muscle and the ability of the muscle to utilize oxygen during exercise are compromised in insulin-resistant states and contribute to the exercise defects seen in IR and T2D.

Precursors of Insulin Resistance

Alternatively, or in addition to sequelae of IR, it is possible that precursors to IR, such as inflammation, dysfunctional adipose tissue, and altered

sleep patterns, also contribute to exercise defects seen in IR. However, direct evidence that these mechanisms contribute to exercise defects is lacking and further study is needed.

Effects of Exercise Training on Exercise Performance in Type 2 Diabetes

Exercise training can substantially improve exercise performance of individuals with T2D [37, 179, 200, 270]. Improvements in VO_2max in men and women with diabetes ranging from 8 to 30% have been documented [179, 270, 271]. In addition, a decreased heart rate per submaximal workload has been reported after exercise training [200], suggesting improved exercise efficiency, again similar to results in nondiabetic persons. Oxygen uptake kinetics and heart rate kinetics became faster after 4 months of exercise training in persons with T2D, although not in nondiabetic controls, suggesting normalization of the rate of circulatory adjustment to the beginning of exercise, a process that is impaired in sedentary diabetic subjects [179]. The relationship between this training effect and improved insulin sensitivity with exercise has not been explored.

Summary

The relationship between 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, evidence suggests that IR may itself cause defects in exercise capacity. These defects, in turn, may make exercise more difficult and uncomfortable and, thus, 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. Importantly, studies suggest that there may not be one right answer. Results from the HERITAGE study and from the combined analysis of several large studies demonstrate that responses to an exercise program are heterogeneous [147–149, 272]. Details on timing of these measures relative to the last bout of exercise and measures of compliance with exercise may account for some of the variation. The HERITAGE study authors argue that genetic variation accounts for nearly 50% of the heterogeneity in responses to exercise. Thus, patient characteristics that we do not yet understand may determine the best exercise regimen [151].

This chapter provides a strong metabolic argument for increasing physical activity and cardiorespiratory fitness. The question remains of how to enable individuals at risk to successfully engage in physical activity/exercise. 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, supporting a daily exercise recommendation. Furthermore, these bout effects likely persist longer after more intense (i.e., more glycogen-depleting) exercise. Thus, daily or near daily exercise, of at least moderate intensity and duration, provides the most benefit for insulin sensitivity and metabolic health. 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? This dilemma faces both people with IR and their clinical providers. Optimally, exercise recommendations should stress the fact that, while more rigorous and daily is better, any increase in 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 these general recommendations, 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. If one strategy is not effective, health-care providers and wellness coaches need to work with the individual to consider a different way to introduce physical activity. Much as with obesity or smoking cessation, a persistent effort may be needed to enable successful behavior change. Finally, it is critical to acknowledge that 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.

References

1. Harber MP, Kaminsky LA, Arena R, Blair SN, Franklin BA, Myers J, et al. Impact of cardiorespiratory fitness on all-cause and disease-specific mortality: advances since 2009. Prog Cardiovasc Dis. 2017;60(1):11–20.
2. Gill JM, Malkova D. Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. Clin Sci (Lond). 2006;110(4):409–25.
3. Williams PT. Physical fitness and activity as separate heart disease risk factors: a meta-analysis. Med Sci Sports Exerc. 2001;33(5):754–61.
4. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA. 2009;301(19):2024–35.
5. Blair SN, Kohl HW 3rd, Barlow CE, Paffenbarger RS Jr, Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. JAMA. 1995;273(14):1093–8.
6. Gielen S, Laughlin MH, O'Conner C, Duncker DJ. Exercise training in patients with heart disease: review of beneficial effects and clinical recommendations. Prog Cardiovasc Dis. 2015;57(4):347–55.
7. Kokkinos P, Myers J, Faselis C, Panagiotakos DB, Doumas M, Pittaras A, et al. Exercise capacity and mortality in older men: a 20-year follow-up study. Circulation. 2010;122(8):790–7.
8. Laukkanen JA, Zaccardi F, Khan H, Kurl S, Jae SY, Rauramaa R. Long-term change in cardiorespiratory fitness and all-cause mortality: a population-based follow-up study. Mayo Clin Proc. 2016;91(9):1183–8.
9. Shah RV, Murthy VL, Colangelo LA, Reis J, Venkatesh BA, Sharma R, et al. Association of fitness in young adulthood with survival and cardiovascular risk: the Coronary Artery Risk Development in Young Adults (CARDIA) study. JAMA Intern Med. 2016;176(1):87–95.
10. Bauer UE, Briss PA, Goodman RA, Bowman BA. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. Lancet. 2014;384(9937):45–52.
11. Grontved A, Hu FB. Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis. JAMA. 2011;305(23):2448–55.
12. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2012;380(9838):219–29.
13. Ekelund U, Steene-Johannessen J, Brown WJ, Fagerland MW, Owen N, Powell KE, et al. Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. Lancet. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Comment]. 2016;388(10051):1302–10.
14. Wei M, Gibbons LW, Kampert JB, Nichaman MZ, Blair SN. Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. Ann Intern Med. 2000;132(8):605–11.
15. Wei M, Kampert JB, Barlow CE, Nichaman MZ, Gibbons LW, Paffenbarger RS Jr, et al. Relationship

between low cardiorespiratory fitness and mortality in normal-weight, overweight, and obese men. JAMA. 1999;282(16):1547–53.
16. Katzmarzyk PT, Church TS, Janssen I, Ross R, Blair SN. Metabolic syndrome, obesity, and mortality: impact of cardiorespiratory fitness. Diabetes Care. 2005;28(2):391–7.
17. Church TS, Cheng YJ, Earnest CP, Barlow CE, Gibbons LW, Priest EL, et al. Exercise capacity and body composition as predictors of mortality among men with diabetes. Diabetes Care. 2004;27(1):83–8.
18. McAuley PA, Myers JN, Abella JP, Tan SY, Froelicher VF. Exercise capacity and body mass as predictors of mortality among male veterans with type 2 diabetes. Diabetes Care. 2007;30(6):1539–43.
19. Lyerly GW, Sui X, Lavie CJ, Church TS, Hand GA, Blair SN. The association between cardiorespiratory fitness and risk of all-cause mortality among women with impaired fasting glucose or undiagnosed diabetes mellitus. Mayo Clin Proc. 2009;84(9):780–6.
20. Gregg EW, Gerzoff RB, Caspersen CJ, Williamson DF, Narayan KM. Relationship of walking to mortality among US adults with diabetes. Arch Intern Med. 2003;163(12):1440–7.
21. Gill JM. Physical activity, cardiorespiratory fitness and insulin resistance: a short update. Curr Opin Lipidol. 2007;18(1):47–52.
22. Byberg L, Zethelius B, McKeigue PM, Lithell HO. Changes in physical activity are associated with changes in metabolic cardiovascular risk factors. Diabetologia. 2001;44(12):2134–9.
23. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Meigs JB, et al. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in caucasian subjects from the general population: the Bruneck study. Diabetes Care. 2007;30(2):318–24.
24. Batty GD, Shipley MJ, Marmot M, Smith GD. Physical activity and cause-specific mortality in men with Type 2 diabetes/impaired glucose tolerance: evidence from the Whitehall study. Diabet Med. 2002;19(7):580–8.
25. Menzaghi C, Trischitta V. The adiponectin paradox for all-cause and cardiovascular mortality. Diabetes. 2018;67(1):12–22.
26. Kim KS, Lee YM, Lee IK, Kim DJ, Jacobs DR Jr, Lee DH. Paradoxical associations of insulin resistance with total and cardiovascular mortality in humans. J Gerontol A Biol Sci Med Sci. 2015;70(7):847–53.
27. Ravussin E, Valencia ME, Esparza J, Bennett PH, Schulz LO. Effects of a traditional lifestyle on obesity in Pima Indians. Diabetes Care. 1994;17(9):1067–74.
28. Kelley DE, Goodpaster BH. Effects of exercise on glucose homeostasis in Type 2 diabetes mellitus. Med Sci Sports Exerc. 2001;33(6 Suppl):S495–501; discussion S28–9.
29. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344(18):1343–50.
30. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care. 1997;20(4):537–44.
31. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.
32. Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L, et al. Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes Care. 2006;29(9):2102–7.
33. Allen F, Stillman E, Fitz R. Total dietary regulation in the treatment of diabetes. Exercise. New York: Rockefeller Institute of Medical Research; 1919. p. 486–99.
34. Rogers MA, Yamamoto C, King DS, Hagberg JM, Ehsani AA, Holloszy JO. Improvement in glucose tolerance after 1 wk of exercise in patients with mild NIDDM. Diabetes Care. 1988;11(8):613–8.
35. Wei M, Schwertner HA, Blair SN. The association between physical activity, physical fitness, and type 2 diabetes mellitus. Compr Ther. 2000;26(3):176–82.
36. Ruderman N, Apelian AZ, Schneider SH. Exercise in therapy and prevention of type II diabetes. Implications for blacks. Diabetes Care. 1990;13(11):1163–8.
37. Schneider SH, Khachadurian AK, Amorosa LF, Clemow L, Ruderman NB. Ten-year experience with an exercise-based outpatient life-style modification program in the treatment of diabetes mellitus. Diabetes Care. 1992;15(11):1800–10.
38. Schneider SH, Elouzi EB. The role of exercise in type II diabetes mellitus. Prev Cardiol. 2000;3(2):77–82.
39. Eves ND, Plotnikoff RC. Resistance training and type 2 diabetes: Considerations for implementation at the population level. Diabetes Care. 2006;29(8):1933–41.
40. Duvivier BM, Schaper NC, Hesselink MK, van Kan L, Stienen N, Winkens B, et al. Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. Diabetologia. 2017;60(3):490–8.
41. Grace A, Chan E, Giallauria F, Graham PL, Smart NA. Clinical outcomes and glycaemic responses to different aerobic exercise training intensities in type II diabetes: a systematic review and meta-analysis. Cardiovasc Diabetol. 2017;16(1):37.
42. Lee J, Kim D, Kim C. Resistance training for glycemic control, muscular strength, and lean body mass in old type 2 diabetic patients: a meta-analysis. Diabetes Ther. 2017;8(3):459–73.
43. Dempsey PC, Larsen RN, Winkler EAH, Owen N, Kingwell BA, Dunstan DW. Prolonged uninterrupted sitting elevates postprandial hyperglycaemia

proportional to degree of insulin resistance. Diabetes Obes Metab. 2018;20(6):1526–30.
44. Castaneda C, Layne JE, Munoz-Orians L, Gordon PL, Walsmith J, Foldvari M, et al. A randomized controlled trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes. Diabetes Care. 2002;25(12):2335–41.
45. Zhang X, Imperatore G, Thomas W, Cheng YJ, Lobelo F, Norris K, et al. Effect of lifestyle interventions on glucose regulation among adults without impaired glucose tolerance or diabetes: A systematic review and meta-analysis. Diabetes Res Clin Pract. 2017;123:149–64.
46. Church TS, Blair SN, Cocreham S, Johannsen N, Johnson W, Kramer K, et al. Effects of aerobic and resistance training on hemoglobin A1c levels in patients with type 2 diabetes: a randomized controlled trial. JAMA. 2010;304(20):2253–62.
47. Kang SJ, Kim JH, Gang Z, Yook YS, Yoon JR, Ha GC, et al. Effects of 12-week circuit exercise program on obesity index, appetite regulating hormones, and insulin resistance in middle-aged obese females. J Phys Ther Sci. 2018;30(1):169–73.
48. Marson EC, Delevatti RS, Prado AK, Netto N, Kruel LF. Effects of aerobic, resistance, and combined exercise training on insulin resistance markers in overweight or obese children and adolescents: a systematic review and meta-analysis. Prev Med. 2016;93:211–8.
49. Storlien L, Oakes ND, Kelley DE. Metabolic flexibility. Proc Nutr Soc. 2004;63(2):363–8.
50. Keshel TE, Coker RH. Exercise training and insulin resistance: a current review. J Obes Weight Loss Ther. 2015;5(Suppl 5).
51. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. J Appl Physiol. 2005;99(1):338–43.
52. Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes. 1987;36(4):434–9.
53. Ruderman NB, Ganda OP, Johansen K. The effect of physical training on glucose tolerance and plasma lipids in maturity-onset diabetes. Diabetes. 1979;28(Suppl 1):89–92.
54. Cartee GD. Mechanisms for greater insulin-stimulated glucose uptake in normal and insulin-resistant skeletal muscle after acute exercise. Am J Physiol Endocrinol Metab. 2015;309(12):E949–59.
55. Galassetti P, Coker RH, Lacy DB, Cherrington AD, Wasserman DH. Prior exercise increases net hepatic glucose uptake during a glucose load. Am J Physiol. 1999;276(6 Pt 1):E1022–9.
56. O'Gorman DJ, Karlsson HK, McQuaid S, Yousif O, Rahman Y, Gasparro D, et al. Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. Diabetologia. 2006;49(12):2983–92.
57. Zierath JR. Invited review: exercise training-induced changes in insulin signaling in skeletal muscle. J Appl Physiol. 2002;93(2):773–81.
58. Wojtaszewski JF, Jorgensen SB, Frosig C, MacDonald C, Birk JB, Richter EA. Insulin signalling: effects of prior exercise. Acta Physiol Scand. 2003;178(4):321–8.
59. Sjoberg KA, Frosig C, Kjobsted R, Sylow L, Kleinert M, Betik AC, et al. Exercise increases human skeletal muscle insulin sensitivity via coordinated increases in microvascular perfusion and molecular signaling. Diabetes. 2017;66(6):1501–10.
60. Sylow L, Kleinert M, Richter EA, Jensen TE. Exercise-stimulated glucose uptake – regulation and implications for glycaemic control. Nat Rev Endocrinol. 2017;13(3):133–48.
61. Morettini M, Palumbo MC, Sacchetti M, Castiglione F, Mazza C. A system model of the effects of exercise on plasma Interleukin-6 dynamics in healthy individuals: role of skeletal muscle and adipose tissue. PLoS One. 2017;12(7):e0181224.
62. Allen TL, Whitham M, Febbraio MA. IL-6 muscles in on the gut and pancreas to enhance insulin secretion. Cell Metab. 2012;15(1):8–9.
63. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med. 2011;17(11):1481–9.
64. Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, et al. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes. 2006;55(10):2688–97.
65. Faldt J, Wernstedt I, Fitzgerald SM, Wallenius K, Bergstrom G, Jansson JO. Reduced exercise endurance in interleukin-6-deficient mice. Endocrinology. 2004;145(6):2680–6.
66. Ruderman NB, Keller C, Richard AM, Saha AK, Luo Z, Xiang X, et al. Interleukin-6 regulation of AMP-activated protein kinase: potential role in the systemic response to exercise and prevention of the metabolic syndrome. Diabetes. 2006;55(Suppl 2):S48–54.
67. Naufahu J, Elliott B, Markiv A, Dunning-Foreman P, McGrady M, Howard D, et al. High intensity exercise decreases IP6K1 muscle content & improves insulin sensitivity (SI2∗) in glucose intolerant individuals. J Clin Endocrinol Metab. 2018;103(4):1479–90.
68. Coker RH, Hays NP, Williams RH, Brown AD, Freeling SA, Kortebein PM, et al. Exercise-induced changes in insulin action and glycogen metabolism in elderly adults. Med Sci Sports Exerc. 2006;38(3):433–8.
69. DiPietro L, Dziura J, Yeckel CW, Neufer PD. Exercise and improved insulin sensitivity in older women: evidence of the enduring benefits of higher intensity training. J Appl Physiol. 2006;100(1):142–9.
70. Way KL, Hackett DA, Baker MK, Johnson NA. The effect of regular exercise on insulin sensitivity in type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes Metab J. 2016;40(4):253–71.

71. Christou DD, Gentile CL, DeSouza CA, Seals DR, Gates PE. Fatness is a better predictor of cardiovascular disease risk factor profile than aerobic fitness in healthy men. Circulation. 2005;111(15):1904–14.
72. Racette SB, Evans EM, Weiss EP, Hagberg JM, Holloszy JO. Abdominal adiposity is a stronger predictor of insulin resistance than fitness among 50-95 year olds. Diabetes Care. 2006;29(3):673–8.
73. Lee S, Kuk JL, Katzmarzyk PT, Blair SN, Church TS, Ross R. Cardiorespiratory fitness attenuates metabolic risk independent of abdominal subcutaneous and visceral fat in men. Diabetes Care. 2005;28(4):895–901.
74. Frisbee JC, Goodwill AG, Frisbee SJ, Butcher JT, Wu F, Chantler PD. Microvascular perfusion heterogeneity contributes to peripheral vascular disease in metabolic syndrome. J Physiol. 2016;594(8):2233–43.
75. McClatchey PM, Frisbee JC, Reusch JEB. A conceptual framework for predicting and addressing the consequences of disease-related microvascular dysfunction. Microcirculation. 2017;24(6).
76. Ashor AW, Lara J, Siervo M, Celis-Morales C, Oggioni C, Jakovljevic DG, et al. Exercise modalities and endothelial function: a systematic review and dose-response meta-analysis of randomized controlled trials. Sports Med. 2015;45(2):279–96.
77. Lee JH, Lee R, Hwang MH, Hamilton MT, Park Y. The effects of exercise on vascular endothelial function in type 2 diabetes: a systematic review and meta-analysis. Diabetol Metab Syndr. 2018;10:15.
78. Montero D, Walther G, Benamo E, Perez-Martin A, Vinet A. Effects of exercise training on arterial function in type 2 diabetes mellitus: a systematic review and meta-analysis. Sports Med. 2013;43(11):1191–9.
79. Ramos JS, Dalleck LC, Tjonna AE, Beetham KS, Coombes JS. The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. Sports Med. 2015;45(5):679–92.
80. Kolka C. Treating diabetes with exercise – focus on the microvasculature. J Diabetes Metab. 2013;4:308.
81. Olver TD, Laughlin MH. Endurance, interval sprint, and resistance exercise training: impact on microvascular dysfunction in type 2 diabetes. Am J Physiol Heart Circ Physiol. 2016;310(3):H337–50.
82. Williams AS, Kang L, Wasserman DH. The extracellular matrix and insulin resistance. Trends Endocrinol Metab. 2015;26(7):357–66.
83. Williams IM, Valenzuela FA, Kahl SD, Ramkrishna D, Mezo AR, Young JD, et al. Insulin exits skeletal muscle capillaries by fluid-phase transport. J Clin Invest. 2018;128(2):699–714.
84. Jansson PA. Endothelial dysfunction in insulin resistance and type 2 diabetes. J Intern Med. 2007;262(2):173–83.
85. Libby P. Vascular biology of atherosclerosis: overview and state of the art. Am J Cardiol. 2003;91(3A):3A–6A.
86. Festa A, D'Agostino R Jr, Rich SS, Jenny NS, Tracy RP, Haffner SM. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Circulation. 2003;107(19):2422–7.
87. Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. Circulation. 2006;113(14):1753–9.
88. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest. 2017;47(8):600–11.
89. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006;116(7):1793–801.
90. Olson TP, Dengel DR, Leon AS, Schmitz KH. Changes in inflammatory biomarkers following one-year of moderate resistance training in overweight women. Int J Obes (Lond). 2007;31(6):996–1003.
91. Zoppini G, Targher G, Zamboni C, Venturi C, Cacciatori V, Moghetti P, et al. Effects of moderate-intensity exercise training on plasma biomarkers of inflammation and endothelial dysfunction in older patients with type 2 diabetes. Nutr Metab Cardiovasc Dis. 2006;16(8):543–9.
92. Leick L, Lindegaard B, Stensvold D, Plomgaard P, Saltin B, Pilegaard H. Adipose tissue interleukin-18 mRNA and plasma interleukin-18: effect of obesity and exercise. Obesity (Silver Spring). 2007;15(2):356–63.
93. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. Int J Sports Med. 2000;21(1):21–4.
94. Petersen AM, Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. J Physiol Pharmacol. 2006;57(Suppl 10):43–51.
95. Martins FM, de Paula SA, Nunes PRP, Michelin MA, Murta EFC, Resende E, et al. High-intensity body weight training is comparable to combined training in changes in muscle mass, physical performance, inflammatory markers and metabolic health in postmenopausal women at high risk for type 2 diabetes mellitus: a randomized controlled clinical trial. Exp Gerontol. 2018;107:108–15.
96. Melo LC, Dativo-Medeiros J, Menezes-Silva CE, Barbosa FT, de Sousa-Rodrigues CF, Rabelo LA. Physical exercise on inflammatory markers in type 2 diabetes patients: a systematic review of randomized controlled trials. Oxid Med Cell Longev. 2017;2017:8523728.
97. Lakka TA, Lakka HM, Rankinen T, Leon AS, Rao DC, Skinner JS, et al. Effect of exercise training on plasma levels of C-reactive protein in healthy adults: the HERITAGE Family Study. Eur Heart J. 2005;26(19):2018–25.
98. Powers SK, Ji LL, Leeuwenburgh C. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med Sci Sports Exerc. 1999;31(7):987–97.

99. Avloniti A, Chatzinikolaou A, Deli CK, Vlachopoulos D, Gracia-Marco L, Leontsini D, et al. Exercise-induced oxidative stress responses in the pediatric population. Antioxidants (Basel). 2017;6(1).
100. Huang CJ, McAllister MJ, Slusher AL, Webb HE, Mock JT, Acevedo EO. Obesity-related oxidative stress: the impact of physical activity and diet manipulation. Sports Med Open. 2015;1(1):32.
101. Pesta D, Roden M. The janus head of oxidative stress in metabolic diseases and during physical exercise. Curr Diab Rep. 2017;17(6):41.
102. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A. 2009;106(21):8665–70.
103. Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD. Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. Med Sci Sports Exerc. 2011;43(6):1017–24.
104. Dube JJ, Coen PM, DiStefano G, Chacon AC, Helbling NL, Desimone ME, et al. Effects of acute lipid overload on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance. Am J Physiol Endocrinol Metab. 2014;307(12):E1117–24.
105. Goodpaster BH, Sparks LM. Metabolic flexibility in health and disease. Cell Metab. 2017;25(5):1027–36.
106. Hesselink MK, Schrauwen-Hinderling V, Schrauwen P. Skeletal muscle mitochondria as a target to prevent or treat type 2 diabetes mellitus. Nat Rev Endocrinol. 2016;12(11):633–45.
107. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science. 2005;307(5708):384–7.
108. Pesta DH, Goncalves RLS, Madiraju AK, Strasser B, Sparks LM. Resistance training to improve type 2 diabetes: working toward a prescription for the future. Nutr Metab (Lond). 2017;14:24.
109. Theurey P, Rieusset J. Mitochondria-associated membranes response to nutrient availability and role in metabolic diseases. Trends Endocrinol Metab. 2017;28(1):32–45.
110. Lark DS, Wasserman DH. Meta-fibrosis links positive energy balance and mitochondrial metabolism to insulin resistance. F1000Res. 2017;6:1758.
111. Goodpaster BH, Katsiaras A, Kelley DE. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabetes. 2003;52(9):2191–7.
112. Menshikova EV, Ritov VB, Ferrell RE, Azuma K, Goodpaster BH, Kelley DE. Characteristics of skeletal muscle mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity. J Appl Physiol. 2007;103(1):21–7.
113. Menshikova EV, Ritov VB, Toledo FG, Ferrell RE, Goodpaster BH, Kelley DE. Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity. Am J Physiol Endocrinol Metab. 2005;288(4):E818–25.
114. Menshikova EV, Ritov VB, Dube JJ, Amati F, Stefanovic-Racic M, Toledo FGS, et al. Calorie restriction-induced weight loss and exercise have differential effects on skeletal muscle mitochondria despite similar effects on insulin sensitivity. J Gerontol A Biol Sci Med Sci. 2017;73(1):81–7.
115. Gan SK, Kriketos AD, Ellis BA, Thompson CH, Kraegen EW, Chisholm DJ. Changes in aerobic capacity and visceral fat but not myocyte lipid levels predict increased insulin action after exercise in overweight and obese men. Diabetes Care. 2003;26(6):1706–13.
116. Holloway GP, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, et al. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. J Physiol. 2006;571(Pt 1):201–10.
117. Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest. 2007;117(6):1690–8.
118. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res. 2007;100(3):328–41.
119. Hardie DG. AMP-activated protein kinase: a key system mediating metabolic responses to exercise. Med Sci Sports Exerc. 2004;36(1):28–34.
120. Fealy CE, Mulya A, Lai N, Kirwan JP. Exercise training decreases activation of the mitochondrial fission protein dynamin-related protein-1 in insulin-resistant human skeletal muscle. J Appl Physiol (1985). 2014;117(3):239–45.
121. Menshikova EV, Ritov VB, Fairfull L, Ferrell RE, Kelley DE, Goodpaster BH. Effects of exercise on mitochondrial content and function in aging human skeletal muscle. J Gerontol A Biol Sci Med Sci. 2006;61(6):534–40.
122. Hafstad AD, Boardman N, Aasum E. How exercise may amend metabolic disturbances in diabetic cardiomyopathy. Antioxid Redox Signal. 2015;22(17):1587–605.
123. Hansen D, De Strijcker D, Calders P. Impact of endurance exercise training in the fasted state on muscle biochemistry and metabolism in healthy subjects: can these effects be of particular clinical benefit to type 2 diabetes mellitus and insulin-resistant patients? Sports Med. 2017;47(3):415–28.
124. Zampieri S, Mammucari C, Romanello V, Barberi L, Pietrangelo L, Fusella A, et al. Physical exercise in aging human skeletal muscle increases mitochondrial calcium uniporter expression levels and affects mitochondria dynamics. Physiol Rep. 2016;4(24).
125. Batsis JA, Gill LE, Masutani RK, Adachi-Mejia AM, Blunt HB, Bagley PJ, et al. Weight loss interventions in older adults with obesity: a systematic review of randomized controlled trials since 2005. J Am Geriatr Soc. 2017;65(2):257–68.
126. Cheng CC, Hsu CY, Liu JF. Effects of dietary and exercise intervention on weight loss and body composition in obese postmenopausal women: a systematic review and meta-analysis. Menopause. 2018;25(7):772–82.

127. Johansson K, Neovius M, Hemmingsson E. Effects of anti-obesity drugs, diet, and exercise on weight-loss maintenance after a very-low-calorie diet or low-calorie diet: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr. 2014;99(1):14–23.
128. Nascimento SL, Pudwell J, Surita FG, Adamo KB, Smith GN. The effect of physical exercise strategies on weight loss in postpartum women: a systematic review and meta-analysis. Int J Obes (Lond). 2014;38(5):626–35.
129. Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The role of exercise and physical activity in weight loss and maintenance. Prog Cardiovasc Dis. 2014;56(4):441–7.
130. Thorogood A, Mottillo S, Shimony A, Filion KB, Joseph L, Genest J, et al. Isolated aerobic exercise and weight loss: a systematic review and meta-analysis of randomized controlled trials. Am J Med. 2011;124(8):747–55.
131. Rodearmel SJ, Wyatt HR, Barry MJ, Dong F, Pan D, Israel RG, et al. A family-based approach to preventing excessive weight gain. Obesity (Silver Spring). 2006;14(8):1392–401.
132. Phelan S, Wyatt HR, Hill JO, Wing RR. Are the eating and exercise habits of successful weight losers changing? Obesity (Silver Spring). 2006;14(4):710–6.
133. Pavlou KN, Krey S, Steffee WP. Exercise as an adjunct to weight loss and maintenance in moderately obese subjects. Am J Clin Nutr. 1989;49(5 Suppl):1115–23.
134. Ring-Dimitriou S, von Duvillard SP, Paulweber B, Stadlmann M, Lemura LM, Peak K, et al. Nine months aerobic fitness induced changes on blood lipids and lipoproteins in untrained subjects versus controls. Eur J Appl Physiol. 2007;99(3):291–9.
135. Escola-Gil JC, Julve J, Griffin BA, Freeman D, Blanco-Vaca F. HDL and lifestyle interventions. Handb Exp Pharmacol. 2015;224:569–92.
136. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Summary of American Heart Association Diet and Lifestyle Recommendations revision 2006. Arterioscler Thromb Vasc Biol. 2006;26(10):2186–91.
137. Riccardi G, Vaccaro O, Costabile G, Rivellese AA. How well can we control dyslipidemias through lifestyle modifications? Curr Cardiol Rep. 2016;18(7):66.
138. Wesnigk J, Bruyndonckx L, Hoymans VY, De Guchtenaere A, Fischer T, Schuler G, et al. Impact of lifestyle intervention on HDL-induced eNOS activation and cholesterol efflux capacity in obese adolescent. Cardiol Res Pract. 2016;2016:2820432.
139. Zhang X, Devlin HM, Smith B, Imperatore G, Thomas W, Lobelo F, et al. Effect of lifestyle interventions on cardiovascular risk factors among adults without impaired glucose tolerance or diabetes: a systematic review and meta-analysis. PLoS One. 2017;12(5):e0176436.
140. Fikenzer K, Fikenzer S, Laufs U, Werner C. Effects of endurance training on serum lipids. Vascul Pharmacol. 2018;101:9–20.
141. Thomas DE, Elliott EJ, Naughton GA. Exercise for type 2 diabetes mellitus. Cochrane Database Syst Rev. 2006;3:CD002968.
142. Kodama S, Tanaka S, Saito K, Shu M, Sone Y, Onitake F, et al. Effect of aerobic exercise training on serum levels of high-density lipoprotein cholesterol: a meta-analysis. Arch Intern Med. 2007;167(10):999–1008.
143. Jennings G, Nelson L, Nestel P, Esler M, Korner P, Burton D, et al. The effects of changes in physical activity on major cardiovascular risk factors, hemodynamics, sympathetic function, and glucose utilization in man: a controlled study of four levels of activity. Circulation. 1986;73(1):30–40.
144. Fagard RH, Cornelissen VA. Effect of exercise on blood pressure control in hypertensive patients. Eur J Cardiovasc Prev Rehabil. 2007;14(1):12–7.
145. Inder JD, Carlson DJ, Dieberg G, McFarlane JR, Hess NC, Smart NA. Isometric exercise training for blood pressure management: a systematic review and meta-analysis to optimize benefit. Hypertens Res. 2016;39(2):88–94.
146. Pal S, Radavelli-Bagatini S, Ho S. Potential benefits of exercise on blood pressure and vascular function. J Am Soc Hypertens. 2013;7(6):494–506.
147. Bouchard C, Rankinen T. Individual differences in response to regular physical activity. Med Sci Sports Exerc. 2001;33(6 Suppl):S446–51; discussion S52–3.
148. Boule NG, Weisnagel SJ, Lakka TA, Tremblay A, Bergman RN, Rankinen T, et al. Effects of exercise training on glucose homeostasis: the HERITAGE Family Study. Diabetes Care. 2005;28(1):108–14.
149. Bouchard C, Blair SN, Church TS, Earnest CP, Hagberg JM, Hakkinen K, et al. Adverse metabolic response to regular exercise: is it a rare or common occurrence? PLoS One. 2012;7(5):e37887.
150. Pandey A, Swift DL, McGuire DK, Ayers CR, Neeland IJ, Blair SN, et al. Metabolic effects of exercise training among fitness-nonresponsive patients with type 2 diabetes: the HART-D study. Diabetes Care. 2015;38(8):1494–501.
151. Sparks LM. Exercise training response heterogeneity: physiological and molecular insights. Diabetologia. 2017;60(12):2329–36.
152. Sepideh A, Karim P, Hossein A, Leila R, Hamdollah M, Mohammad EG, et al. Effects of multistrain probiotic supplementation on glycemic and inflammatory indices in patients with nonalcoholic fatty liver disease: a double-blind randomized clinical trial. J Am Coll Nutr. 2016;35(6):500–5.
153. Baothman OA, Zamzami MA, Taher I, Abubaker J, Abu-Farha M. The role of gut microbiota in the development of obesity and diabetes. Lipids Health Dis. 2016;15:108.
154. Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S, Dougherty J, et al. Exercise prevents

weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PLoS One. 2014;9(3):e92193.
155. Matsumoto M, Inoue R, Tsukahara T, Ushida K, Chiji H, Matsubara N, et al. Voluntary running exercise alters microbiota composition and increases n-butyrate concentration in the rat cecum. Biosci Biotechnol Biochem. 2008;72(2):572–6.
156. Denou E, Marcinko K, Surette MG, Steinberg GR, Schertzer JD. High-intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity. Am J Physiol Endocrinol Metab. 2016;310(11):E982–93.
157. Welly RJ, Liu TW, Zidon TM, Rowles JL 3rd, Park YM, Smith TN, et al. Comparison of diet versus exercise on metabolic function and gut microbiota in obese rats. Med Sci Sports Exerc. 2016;48(9):1688–98.
158. Yang Y, Shi Y, Wiklund P, Tan X, Wu N, Zhang X, et al. The association between cardiorespiratory fitness and gut microbiota composition in premenopausal women. Nutrients. 2017;25:9(8).
159. Estaki M, Pither J, Baumeister P, Little JP, Gill SK, Ghosh S, et al. Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. Microbiome. 2016;4(1):42.
160. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, et al. Exercise and associated dietary extremes impact on gut microbial diversity. Gut. 2014;63(12):1913–20.
161. Tan X, Saarinen A, Mikkola TM, Tenhunen J, Martinmaki S, Rahikainen A, et al. Effects of exercise and diet interventions on obesity-related sleep disorders in men: study protocol for a randomized controlled trial. Trials. 2013;14:235.
162. Masi LN, Serdan TD, Levada-Pires AC, Hatanaka E, Silveira LD, Cury-Boaventura MF, et al. Regulation of gene expression by exercise-related micrornas. Cell Physiol Biochem. 2016;39(6):2381–97.
163. Lew JK, Pearson JT, Schwenke DO, Katare R. Exercise mediated protection of diabetic heart through modulation of microRNA mediated molecular pathways. Cardiovasc Diabetol. 2017;16(1):10.
164. Verboven K, Hansen D, Jocken JWE, Blaak EE. Natriuretic peptides in the control of lipid metabolism and insulin sensitivity. Obes Rev. 2017;18(11):1243–59.
165. Carson BP. The potential role of contraction-induced myokines in the regulation of metabolic function for the prevention and treatment of type 2 diabetes. Front Endocrinol (Lausanne). 2017;8:97.
166. Morrato EH, Hill JO, Wyatt HR, Ghushchyan V, Sullivan PW. Physical activity in u.s. Adults with diabetes and at risk for developing diabetes, 2003. Diabetes Care. 2007;30(2):203–9.
167. Krug LM, Haire-Joshu D, Heady SA. Exercise habits and exercise relapse in persons with non-insulin-dependent diabetes mellitus. Diabetes Educ. 1991;17(3):185–8.
168. Zhao G, Ford ES, Li C, Mokdad AH. Compliance with physical activity recommendations in US adults with diabetes. Diabet Med. 2008;25(2):221–7.
169. Luoma KA, Leavitt IM, Marrs JC, Nederveld AL, Regensteiner JG, Dunn AL, et al. How can clinical practices pragmatically increase physical activity for patients with type 2 diabetes? A systematic review. Transl Behav Med. 2017;7(4):751–72.
170. Baldi JC, Aoina JL, Oxenham HC, Bagg W, Doughty RN. Reduced exercise arteriovenous O2 difference in Type 2 diabetes. J Appl Physiol. 2003;94(3):1033–8.
171. Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation. 2003;107(24):3040–6.
172. Regensteiner JG, Bauer TA, Reusch JE, Brandenburg SL, Sippel JM, Vogelsong AM, et al. Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. J Appl Physiol. 1998;85(1):310–7.
173. Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR. Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. Med Sci Sports Exerc. 1995;27(6):875–81.
174. Orio F Jr, Giallauria F, Palomba S, Cascella T, Manguso F, Vuolo L, et al. Cardiopulmonary impairment in young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91(8):2967–71.
175. Wong CY, O'Moore-Sullivan T, Fang ZY, Haluska B, Leano R, Marwick TH. Myocardial and vascular dysfunction and exercise capacity in the metabolic syndrome. Am J Cardiol. 2005;96(12):1686–91.
176. Kemmer FW, Tacken M, Berger M. Mechanism of exercise-induced hypoglycemia during sulfonylurea treatment. Diabetes. 1987;36(10):1178–82.
177. Kjaer M, Hollenbeck CB, Frey-Hewitt B, Galbo H, Haskell W, Reaven GM. Glucoregulation and hormonal responses to maximal exercise in non-insulin-dependent diabetes. J Appl Physiol. 1990;68(5):2067–74.
178. Schneider SH, Khachadurian AK, Amorosa LF, Gavras H, Fineberg SE, Ruderman NB. Abnormal glucoregulation during exercise in type II (non-insulin-dependent) diabetes. Metabolism. 1987;36(12):1161–6.
179. Brandenburg SL, Reusch JE, Bauer TA, Jeffers BW, Hiatt WR, Regensteiner JG. Effects of exercise training on oxygen uptake kinetic responses in women with type 2 diabetes. Diabetes Care. 1999;22(10):1640–6.
180. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. J Clin Endocrinol Metab. 2009;94(10):3687–95.
181. Huebschmann AG, Kohrt WM, Herlache L, Wolfe P, Daugherty S, Reusch JE, et al. Type 2 diabetes exaggerates exercise effort and impairs exercise performance in older women. BMJ Open Diabetes Res Care. 2015;3(1):e000124.

182. Wasserman K, Hansen JE, Sue DY, Whipp BJ, Casaburi R. Principles of exercise testing and interpretation. 2nd ed. Philadelphia: Lea & Febiger; 1994.
183. Hansen JE, Sue DY, Oren A, Wasserman K. Relation of oxygen uptake to work rate in normal men and men with circulatory disorders. Am J Cardiol. 1987;59(6):669–74.
184. Huebschmann AG, Reis EN, Emsermann C, Dickinson LM, Reusch JE, Bauer TA, et al. Women with type 2 diabetes perceive harder effort during exercise than nondiabetic women. Appl Physiol Nutr Metab. 2009;34(5):851–7.
185. Jones AM, Poole DC. Oxygen uptake dynamics: from muscle to mouth--an introduction to the symposium. Med Sci Sports Exerc. 2005;37(9):1542–50.
186. Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD. Muscle O2 uptake kinetics in humans: implications for metabolic control. J Appl Physiol. 1996;80(3):988–98.
187. Bauer TA, Reusch JE, Levi M, Regensteiner JG. Skeletal muscle deoxygenation after the onset of moderate exercise suggests slowed microvascular blood flow kinetics in type 2 diabetes. Diabetes Care. 2007;30(11):2880–5.
188. Mason McClatchey P, Bauer TA, Regensteiner JG, Schauer IE, Huebschmann AG, Reusch JEB. Dissociation of local and global skeletal muscle oxygen transport metrics in type 2 diabetes. J Diabetes Complications. 2017;31(8):1311–7.
189. Regensteiner JG, Bauer TA, Reusch JE. Rosiglitazone improves exercise capacity in individuals with type 2 diabetes. Diabetes Care. 2005;28(12):2877–83.
190. Reusch JE, Regensteiner JG, Watson PA. Novel actions of thiazolidinediones on vascular function and exercise capacity. Am J Med. 2003;115(Suppl 8A):69S–74S.
191. Seibaek M, Vestergaard H, Burchardt H, Sloth C, Torp-Pedersen C, Nielsen SL, et al. Insulin resistance and maximal oxygen uptake. Clin Cardiol. 2003;26(11):515–20.
192. Eidemak I, Feldt-Rasmussen B, Kanstrup IL, Nielsen SL, Schmitz O, Strandgaard S. Insulin resistance and hyperinsulinaemia in mild to moderate progressive chronic renal failure and its association with aerobic work capacity. Diabetologia. 1995;38(5):565–72.
193. Swan JW, Anker SD, Walton C, Godsland IF, Clark AL, Leyva F, et al. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. J Am Coll Cardiol. 1997;30(2):527–32.
194. Nadeau KJ, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, Hull A, et al. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. J Clin Endocrinol Metab. 2010;95(2):513–21.
195. Endre T, Mattiasson I, Hulthen UL, Lindgarde F, Berglund G. Insulin resistance is coupled to low physical fitness in normotensive men with a family history of hypertension. J Hypertens. 1994;12(1):81–8.
196. Schauer I, editor. Effect of acute induction of insulin resistance on exercise parameters. Philadelphia: American Diabetes Association; 2012.
197. Scalzo RL, Moreau KL, Ozemek C, Herlache L, McMillin S, Gilligan S, et al. Exenatide improves diastolic function and attenuates arterial stiffness but does not alter exercise capacity in individuals with type 2 diabetes. J Diabetes Complications. 2017;31(2):449–55.
198. Wahl MP, Scalzo RL, Regensteiner JG, Reusch JEB. Mechanisms of aerobic exercise impairment in diabetes: a narrative review. Front Endocrinol (Lausanne). 2018;9:181.
199. Modan M, Meytes D, Rozeman P, Yosef SB, Sehayek E, Yosef NB, et al. Significance of high HbA1 levels in normal glucose tolerance. Diabetes Care. 1988;11(5):422–8.
200. Schneider SH, Amorosa LF, Khachadurian AK, Ruderman NB. Studies on the mechanism of improved glucose control during regular exercise in type 2 (non-insulin-dependent) diabetes. Diabetologia. 1984;26(5):355–60.
201. Manrique C, Lastra G, Sowers JR. New insights into insulin action and resistance in the vasculature. Ann N Y Acad Sci. 2014;1311:138–50.
202. Mikola H, Pahkala K, Niinikoski H, Ronnemaa T, Viikari JSA, Jula A, et al. Cardiometabolic determinants of carotid and aortic distensibility from childhood to early adulthood. Hypertension. 2017;70(2):452–60.
203. Jung JY, Min KW, Ahn HJ, Kwon HR, Lee JH, Park KS, et al. Arterial stiffness by aerobic exercise is related with aerobic capacity, physical activity energy expenditure and total fat but not with insulin sensitivity in obese female patients with type 2 diabetes. Diabetes Metab J. 2014;38(6):439–48.
204. Tazawa Y, Mori N, Ogawa Y, Ito O, Kohzuki M. Arterial stiffness measured with the cuff oscillometric method is predictive of exercise capacity in patients with cardiac diseases. Tohoku J Exp Med. 2016;239(2):127–34.
205. Attina TM, Drummond ID, Malatino LS, Maxwell SR, Webb DJ. Phosphodiesterase type 5 inhibition improves arterial stiffness after exercise but not exercise capacity in hypertensive men. Am J Hypertens. 2013;26(3):342–50.
206. Lambert GW, Straznicky NE, Lambert EA, Dixon JB, Schlaich MP. Sympathetic nervous activation in obesity and the metabolic syndrome--causes, consequences and therapeutic implications. Pharmacol Ther. 2010;126(2):159–72.
207. Mancia G, Bousquet P, Elghozi JL, Esler M, Grassi G, Julius S, et al. The sympathetic nervous system and the metabolic syndrome. J Hypertens. 2007;25(5):909–20.
208. Thorp AA, Schlaich MP. Relevance of sympathetic nervous system activation in obesity and metabolic syndrome. J Diabetes Res. 2015;2015:341583.
209. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodi-

lation in patients with non-insulin-dependent diabetes mellitus. J Am Coll Cardiol. 1996;27(3):567–74.
210. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, et al. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia. 1992;35(8):771–6.
211. Kingwell BA, Formosa M, Muhlmann M, Bradley SJ, McConell GK. Type 2 diabetic individuals have impaired leg blood flow responses to exercise: role of endothelium-dependent vasodilation. Diabetes Care. 2003;26(3):899–904.
212. Regensteiner JG, Popylisen S, Bauer TA, Lindenfeld J, Gill E, Smith S, et al. Oral L-arginine and vitamins E and C improve endothelial function in women with type 2 diabetes. Vasc Med. 2003;8(3):169–75.
213. Yki-Jarvinen H. Insulin resistance and endothelial dysfunction. Best Pract Res Clin Endocrinol Metab. 2003;17(3):411–30.
214. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest. 1996;97(11):2601–10.
215. Jahn LA, Hartline L, Rao N, Logan B, Kim JJ, Aylor K, et al. Insulin enhances endothelial function throughout the arterial tree in healthy but not metabolic syndrome subjects. J Clin Endocrinol Metab. 2016;101(3):1198–206.
216. McArdle MJ, deGoma EM, Cohen DL, Townsend RR, Wilensky RL, Giri J. Beyond blood pressure: percutaneous renal denervation for the management of sympathetic hyperactivity and associated disease states. J Am Heart Assoc. 2015;4(3):e001415.
217. Boa BC, Yudkin JS, van Hinsbergh VW, Bouskela E, Eringa EC. Exercise effects on perivascular adipose tissue: endocrine and paracrine determinants of vascular function. Br J Pharmacol. 2017;174(20):3466–81.
218. Gu P, Xu A. Interplay between adipose tissue and blood vessels in obesity and vascular dysfunction. Rev Endocr Metab Disord. 2013;14(1):49–58.
219. Jones AM, Wilkerson DP, Campbell IT. Nitric oxide synthase inhibition with L-NAME reduces maximal oxygen uptake but not gas exchange threshold during incremental cycle exercise in man. J Physiol. 2004;560(Pt 1):329–38.
220. Jones AM, Wilkerson DP, Koppo K, Wilmshurst S, Campbell IT. Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary. VO2 kinetics in the transition to moderate-intensity exercise in man. J Physiol. 2003;552(Pt 1):265–72.
221. Xie YW, Shen W, Zhao G, Xu X, Wolin MS, Hintze TH. Role of endothelium-derived nitric oxide in the modulation of canine myocardial mitochondrial respiration in vitro. Implications for the development of heart failure. Circ Res. 1996;79(3):381–7.
222. Shen W, Hintze TH, Wolin MS. Nitric oxide. An important signaling mechanism between vascular endothelium and parenchymal cells in the regulation of oxygen consumption. Circulation. 1995;92(12):3505–12.
223. Shen W, Zhang X, Zhao G, Wolin MS, Sessa W, Hintze TH. Nitric oxide production and NO synthase gene expression contribute to vascular regulation during exercise. Med Sci Sports Exerc. 1995;27(8):1125–34.
224. Wojewoda M, Przyborowski K, Sitek B, Zakrzewska A, Mateuszuk L, Zoladz JA, et al. Effects of chronic nitric oxide synthase inhibition on V'O2max and exercise capacity in mice. Naunyn Schmiedebergs Arch Pharmacol. 2017;390(3):235–44.
225. Liu J, Jahn LA, Fowler DE, Barrett EJ, Cao W, Liu Z. Free fatty acids induce insulin resistance in both cardiac and skeletal muscle microvasculature in humans. J Clin Endocrinol Metab. 2011;96(2):438–46.
226. Liu Z, Liu J, Jahn LA, Fowler DE, Barrett EJ. Infusing lipid raises plasma free fatty acids and induces insulin resistance in muscle microvasculature. J Clin Endocrinol Metab. 2009;94(9):3543–9.
227. Eggleston EM, Jahn LA, Barrett EJ. Early microvascular recruitment modulates subsequent insulin-mediated skeletal muscle glucose metabolism during lipid infusion. Diabetes Care. 2013;36(1):104–10.
228. Inyard AC, Chong DG, Klibanov AL, Barrett EJ. Muscle contraction, but not insulin, increases microvascular blood volume in the presence of free fatty acid-induced insulin resistance. Diabetes. 2009;58(11):2457–63.
229. Womack L, Peters D, Barrett EJ, Kaul S, Price W, Lindner JR. Abnormal skeletal muscle capillary recruitment during exercise in patients with type 2 diabetes mellitus and microvascular complications. J Am Coll Cardiol. 2009;53(23):2175–83.
230. Wang N, Chai W, Zhao L, Tao L, Cao W, Liu Z. Losartan increases muscle insulin delivery and rescues insulin's metabolic action during lipid infusion via microvascular recruitment. Am J Physiol Endocrinol Metab. 2013;304(5):E538–45.
231. Kusters YH, Barrett EJ. Muscle microvasculature's structural and functional specializations facilitate muscle metabolism. Am J Physiol Endocrinol Metab. 2016;310(6):E379–87.
232. Pienaar PR, Micklesfield LK, Levitt NS, Gooding K, Shore AC, Goedecke JH, et al. Insulin resistance is associated with lower acetylcholine-induced microvascular reactivity in nondiabetic women. Metab Syndr Relat Disord. 2014;12(3):178–84.
233. Regensteiner JG, Bauer TA, Reusch JE, Quaife RA, Chen MY, Smith SC, et al. Cardiac dysfunction during exercise in uncomplicated type 2 diabetes. Med Sci Sports Exerc. 2009;41(5):977–84.
234. Baldi JC, Wilson GA, Wilson LC, Wilkins GT, Lamberts RR. The type 2 diabetic heart: its role in exercise intolerance and the challenge to find effective exercise interventions. Sports Med. 2016;46(11):1605–17.

235. Mason McClatchey P, Wu F, Olfert IM, Ellis CG, Goldman D, Reusch JE, et al. Impaired tissue oxygenation in metabolic syndrome requires increased microvascular perfusion heterogeneity. J Cardiovasc Transl Res. 2017;10(1):69–81.
236. He J, Watkins S, Kelley DE. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. Diabetes. 2001;50(4):817–23.
237. Williamson JR, Kilo C. Capillary basement membranes in diabetes. Diabetes. 1983;32(Suppl 2):96–100.
238. Behnke BJ, Kindig CA, McDonough P, Poole DC, Sexton WL. Dynamics of microvascular oxygen pressure during rest-contraction transition in skeletal muscle of diabetic rats. Am J Physiol Heart Circ Physiol. 2002;283(3):H926–32.
239. Kindig CA, Sexton WL, Fedde MR, Poole DC. Skeletal muscle microcirculatory structure and hemodynamics in diabetes. Respir Physiol. 1998;111(2):163–75.
240. Padilla DJ, McDonough P, Behnke BJ, Kano Y, Hageman KS, Musch TI, et al. Effects of Type II diabetes on capillary hemodynamics in skeletal muscle. Am J Physiol Heart Circ Physiol. 2006;291(5):H2439–44.
241. MacRury SM, Small M, MacCuish AC, Lowe GD. Association of hypertension with blood viscosity in diabetes. Diabet Med. 1988;5(9):830–4.
242. McMillan DE. Exercise and diabetic microangiopathy. Diabetes. 1979;28(Suppl 1):103–6.
243. Reusch JE, Bridenstine M, Regensteiner JG. Type 2 diabetes mellitus and exercise impairment. Rev Endocr Metab Disord. 2013;14(1):77–86.
244. Baldi JC, Aoina JL, Whalley GA, Carrick-Ranson G, Walsh HA, O'Shaughnessy H, et al. The effect of type 2 diabetes on diastolic function. Med Sci Sports Exerc. 2006;38(8):1384–8.
245. Bouchard A, Sanz N, Botvinick EH, Phillips N, Heilbron D, Byrd BF 3rd, et al. Noninvasive assessment of cardiomyopathy in normotensive diabetic patients between 20 and 50 years old. Am J Med. 1989;87(2):160–6.
246. Mustonen JN, Uusitupa MI, Tahvanainen K, Talwar S, Laakso M, Lansimies E, et al. Impaired left ventricular systolic function during exercise in middle-aged insulin-dependent and noninsulin-dependent diabetic subjects without clinically evident cardiovascular disease. Am J Cardiol. 1988;62(17):1273–9.
247. Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG. Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care. 2001;24(1):5–10.
248. Poirier P, Garneau C, Bogaty P, Nadeau A, Marois L, Brochu C, et al. Impact of left ventricular diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 diabetes mellitus. Am J Cardiol. 2000;85(4):473–7.
249. Regan TJ, Lyons MM, Ahmed SS, Levinson GE, Oldewurtel HA, Ahmad MR, et al. Evidence for cardiomyopathy in familial diabetes mellitus. J Clin Invest. 1977;60(4):884–99.
250. Shimizu M, Sugihara N, Kita Y, Shimizu K, Shibayama S, Takeda R. Increase in left ventricular chamber stiffness in patients with non-insulin dependent diabetes mellitus. Jpn Circ J. 1991;55(7):657–64.
251. Robillon JF, Sadoul JL, Jullien D, Morand P, Freychet P. Abnormalities suggestive of cardiomyopathy in patients with type 2 diabetes of relatively short duration. Diabete Metab. 1994;20(5):473–80.
252. Bacha F, Gidding SS, Pyle L, Levitt Katz L, Kriska A, Nadeau KJ, et al. Relationship of cardiac structure and function to cardiorespiratory fitness and lean body mass in adolescents and young adults with type 2 diabetes. J Pediatr. 2016;177:159–66.e1.
253. Bjornstad P, Truong U, Dorosz JL, Cree-Green M, Baumgartner A, Coe G, et al. Cardiopulmonary dysfunction and adiponectin in adolescents with type 2 diabetes. J Am Heart Assoc. 2016;5(3):e002804.
254. Uusitupa M, Mustonen J, Laakso M, Vainio P, Lansimies E, Talwar S, et al. Impairment of diastolic function in middle-aged type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic patients free of cardiovascular disease. Diabetologia. 1988;31(11):783–91.
255. Miller TM, Gilligan S, Herlache LL, Regensteiner JG. Sex differences in cardiovascular disease risk and exercise in type 2 diabetes. J Invest Med. 2012;60(4):664–70.
256. Borlaug BA, Melenovsky V, Russell SD, Kessler K, Pacak K, Becker LC, et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. Circulation. 2006;114(20):2138–47.
257. Vanoverschelde JJ, Essamri B, Vanbutsele R, d'Hondt A, Cosyns JR, Detry JR, et al. Contribution of left ventricular diastolic function to exercise capacity in normal subjects. J Appl Physiol. 1993;74(5):2225–33.
258. Regensteiner J, Groves BM, Bauer TA, Reusch JEB, Smith SC, Wolfel EE. Recently diagnosed type 2 diabetes mellitus adversely affects cardiac function during exercise. Diabetes. 2002;51(suppl):A59.
259. Gurdal A, Kasikcioglu E, Yakal S, Bugra Z. Impact of diabetes and diastolic dysfunction on exercise capacity in normotensive patients without coronary artery disease. Diab Vasc Dis Res. 2015;12(3):181–8.
260. Oakes ND, Thalen P, Aasum E, Edgley A, Larsen T, Furler SM, et al. Cardiac metabolism in mice: tracer method developments and in vivo application revealing profound metabolic inflexibility in diabetes. Am J Physiol Endocrinol Metab. 2006;290(5):E870–81.
261. Mazumder PK, O'Neill BT, Roberts MW, Buchanan J, Yun UJ, Cooksey RC, et al. Impaired cardiac efficiency and increased fatty acid oxidation in insulin-resistant ob/ob mouse hearts. Diabetes. 2004;53(9):2366–74.

262. Carley AN, Severson DL. Fatty acid metabolism is enhanced in type 2 diabetic hearts. Biochim Biophys Acta. 2005;1734(2):112–26.
263. Peterson LR, Herrero P, Schechtman KB, Racette SB, Waggoner AD, Kisrieva-Ware Z, et al. Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. Circulation. 2004;109(18):2191–6.
264. Banerjee S, Peterson LR. Myocardial metabolism and cardiac performance in obesity and insulin resistance. Curr Cardiol Rep. 2007;9(2):143–9.
265. Mikhalkova D, Holman SR, Jiang H, Saghir M, Novak E, Coggan AR, et al. Bariatric surgery-induced cardiac and lipidomic changes in obesity-related heart failure with preserved ejection fraction. Obesity (Silver Spring). 2018;26(2):284–90.
266. Peterson LR. Obesity and insulin resistance: effects on cardiac structure, function, and substrate metabolism. Curr Hypertens Rep. 2006;8(6):451–6.
267. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Jeneson JA, Backes WH, van Echteld CJ, et al. Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. Diabetologia. 2007;50(1):113–20.
268. Cree-Green M, Gupta A, Coe GV, Baumgartner AD, Pyle L, Reusch JE, et al. Insulin resistance in type 2 diabetes youth relates to serum free fatty acids and muscle mitochondrial dysfunction. J Diabetes Complications. 2017;31(1):141–8.
269. Cree-Green M, Scalzo RL, Harrall K, Newcomer BR, Schauer IE, Huebschmann AG, et al. Supplemental oxygen improves in-vivo mitochondrial oxidative phosphorylation flux in sedentary obese adults with type 2 diabetes. Diabetes. 2018;67(7):1369–79.
270. Holloszy JO, Schultz J, Kusnierkiewicz J, Hagberg JM, Ehsani AA. Effects of exercise on glucose tolerance and insulin resistance. Brief review and some preliminary results. Acta Med Scand. 1986;711:55–65.
271. Verity LS, Ismail AH. Effects of exercise on cardiovascular disease risk in women with NIDDM. Diabetes Res Clin Pract. 1989;6(1):27–35.
272. Teran-Garcia M, Rankinen T, Rice T, Leon AS, Rao DC, Skinner JS, et al. Variations in the four and a half LIM domains 1 gene (FHL1) are associated with fasting insulin and insulin sensitivity responses to regular exercise. Diabetologia. 2007;50(9):1858–66.

Weight Loss Medications in Adolescents

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Linda L. Buckley, Daniel H. Bessesen, and Philip S. Zeitler

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 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 behavior 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, 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.

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 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

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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 in the prescription of a combination of phentermine 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 about strokes, and in 2003, ma huang, an active ingredient in the popular over-the-counter 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 outweigh the risks. Since the history of unintended consequences associated with weight loss medications may have created a state where 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 suffice it 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, the effectiveness of current medications sufficiently limited compared to currently available medications for each of the related metabolic diseases, and the current costs 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 health-care 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 cost will likely be. 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 resistant 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 conflicts 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 health-care professionals who specialize in weight management [26, 27]. This bias has been documented in a number of studies [28–30] and may be manifested through a discomfort in interacting with obese people and a distaste for the problem and its management.

In many 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.

Health-care providers sometimes think of obesity as a "lifestyle" or "quality of life" condition that does not justify medical therapy. 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 require a 30–50% 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 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 4 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 become 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: (1) accept their weight where it is, which frequently means accepting gradual progressive weight gain; or (2) attempting some form of treatment. In this context, the most important issue is whether the patient thinks that a 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 where it is. Many 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, another view would be 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. Currently, 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 endpoints, 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

Weight loss medications currently available for adults 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 weight loss medications likely will need to be used for long term to give long-term benefits [36]. Intermittent administration of medications is a reasonable strategy if the patient or physician is concerned about long-term use. However, weight is lost while medications are taken, stabilizes at a new lower level, and begins to increase almost immediately with discontinuation of the medication, followed by restoration of weight loss with reinstitution of medication. Thus, 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. This cost will be borne by the patient for the duration of therapy, which likely will be many years. Thus, 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 U.S. Food and Drug Administration (FDA) approval. There is hope that in the future, there may be increased insurance coverage, and perhaps, improved efficacy with the use of combination therapy or newer medicines acting through novel pathways. Of the medications listed below, only orlistat has been approved for use in adolescents >12 years of age.

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 $120.00 per month. Orlistat has been studied in more than 30,000 patients for up to 4 years in more than 90 controlled clinical trials in a range of patient types [8, 37]. Like sibutramine, orlistat can deliver a 5–7% weight loss above that seen

with a behavioral treatment program alone. The effectiveness of orlistat therapy given for 2 years was demonstrated in a study by Sjostrom [38]. 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 a 10% weight loss [39]. Orlistat has a number of metabolic benefits in addition to simply producing weight loss. These include a reduction in LDL cholesterol, serum triglycerides, waist circumference, and improvement in blood glucose and HbA1C in people with type 2 diabetes [40].

Orlistat has also been tested for the prevention of the development of type 2 diabetes in at-risk individuals [41]. In the Xenical in the prevention of Diabetes in Obese Subjects trial (XENDOS), high-risk individuals were randomized to either placebo or orlistat; 53% of individuals randomized to orlistat achieved a 5% weight loss or greater and 26% achieved a 10% or greater weight loss at 4 years of follow-up [41]. The 4-year incidence of type 2 diabetes in the placebo group, already receiving diet and exercise counseling, was 9%. The 4-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 [42–44]. These studies demonstrated 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 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 approved a 60-mg dose of this medication for over-the-counter (OTC) sales—the only OTC weight loss medication approved by the FDA. It is marketed by GlaxoSmithKline under the name Alli. 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 alone.

Phentermine

Phentermine is an older medication that increases 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. Data in adolescents are limited. A small retrospective chart review compared adolescents treated with phentermine 15 mg once daily for 6 months to lifestyle modification therapy [45] alone and found a −4.1% reduction in body mass index (BMI), a result similar to that reported in adults. Phentermine is chemically related to amphetamine but does not have the same addictive potential. Probably because of its low cost, phentermine is the most widely prescribed anti-obesity drug in the United States [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.

In the original studies from the 1960s, this medication was only used for 3 months and as a result, is only FDA approved for 3 months of use. Therefore, many physicians are reluctant to prescribe it. However, our current understanding of medical therapy for obesity suggests that if a medication is only used for a limited time, it will not produce a lasting change in weight. This leaves physicians in a position of needing 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 up to the discretion of the patient and physician. 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. It is important if a provider chooses to prescribe phentermine that they begin at a low dose, 8–15 mg/d, and follow blood pressure and pulse over the next several weeks. If the patient is tolerating the medication well, the dose can be increased up to 30 mg/d in 1–2 months and again the blood pressure should be monitored.

Topiramate

Topiramate is FDA approved for the treatment of epilepsy in ≥2 years of age and migraine prophylaxis in ≥12 years old and causes weight loss in adult obesity trials. The combination obesity medication phentermine/topiramate ER is FDA approved for chronic weight management in adults. A small randomized, placebo-controlled pilot clinical trial evaluating 75 mg topiramate in 30 adolescents ages 12–17 with severe obesity showed a 2% BMI reduction at 6 months, which did not reach statistical significance compared with placebo [46]. A retrospective chart review examining the effect of 75 mg topiramate once daily for at least 3 months found a 4.9% BMI reduction [47]. No studies of the combination of phentermine and topiramate have been reported in adolescents, but adult results suggest that this may be a promising approach in severely obese adolescents.

Glucagon-Like 1 Receptor (GLP-1) Agonists

Exenatide and liraglutide are GLP-1 agonists that are FDA approved for type 2 diabetes mellitus in adults and are used off-label in adolescents <18 years of age. When used for the treatment of diabetes, both exenatide and liraglutide are associated with weight loss in adults and liraglutide is FDA approved for weight loss in adults. There are limited data on the efficacy of these agents for weight loss in adolescents. In pooled data from two small randomized trials of pediatric age patients with severe obesity, 3 months of treatment with exenatide plus lifestyle modification reduced BMI by 3.42% compared to lifestyle alone [48]. On the other hand, in the Ellipse study of adolescents with obesity and type 2 diabetes, BMI *Z*-score in those on liraglutide decreased by only 0.25 kg/m^2 and was not different from placebo, though the dose approved for weight loss in adults was not used [49]. Studies of liraglutide for the treatment of obesity in adolescents without diabetes are now underway, as well as for weekly and oral GLP-1 agents.

Other Agents

Lisdexamfetamine is FDA approved for the treatment of attention deficit hyperactivity disorder (ADHD) in children ≥6 years and adults and binge-eating disorder in adults. It is not approved specifically for weight loss, but weight loss in the range of 2–5 pounds is common, and this agent may be useful for younger children and those with binge-eating disorder and impulsive behavior.

Lorcaserin is a 5-hydroxytryptamine receptor agonist that acts on proopiomelanocortin neurons in the hypothalamus and is FDA approved for the chronic treatment of obesity in adults [50]. There are no studies of lorcaserin in adolescents.

The combination naltrexone/bupropion blocks opioid-mediated pro-opiomelanocortin (POMC) autoinhibition, inhibits reuptake of dopamine and noradrenaline, and has been FDA approved for the chronic treatment of obesity in adults [50]. There are no data on the efficacy of this combina-

tion for obesity in adolescents, though both have been used for other indications in the pediatric population.

Setmelanotide is the first of a pipeline of new agents targeted to specifically address mutations in the regulation of appetite. This melanocortin-4 receptor agonist is being studied for the treatment of obesity related to POMC deficiency, leptin receptor deficiency, Bardet–Biedl syndrome, Alstrom syndrome, and Prader–Willi syndrome and can result in very substantial weight loss in the appropriately chosen patient [51].

Medications That Promote Weight Gain

When seeing an overweight or obese patient, it is also important to consider whether medications the patient is taking are contributing to weight gain [52]. A number of antipsychotic agents [53], progestational agents, glucocorticoids, antidiabetic medications (sulfonylureas, thiazolidinediones, and 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 that the risks of continued administration outweigh the benefits of that medication.

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.

References

1. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. JAMA. 2005;293(15):1861–7.
2. National Heart Lung, and Blood Institute (NHLBI) and North American Association for the Study of Obesity (NAASO). Practical guide on the identification, evaluation, and treatment of overweight and obesity in adults. Bethesda: National Institutes of Health; 2000. NIH Publication No. 00-4084. 2006. Ref Type: Generic.
3. Ajani UA, Lotufo PA, Gaziano JM, et al. Body mass index and mortality among US male physicians. Ann Epidemiol. 2004;14(10):731–9.
4. Bray GA. Medical consequences of obesity. J Clin Endocrinol Metab. 2004;89(6):2583–9.
5. Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB. Years of life lost due to obesity. JAMA. 2003;289(2):187–93.
6. Folsom AR, Kushi LH, Anderson KE, et al. Associations of general and abdominal obesity with multiple health outcomes in older women: the Iowa Women's Health Study. Arch Intern Med. 2000;160(14):2117–28.
7. McTigue KM, Harris R, Hemphill B, et al. Screening and interventions for obesity in adults: summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med. 2003;139(11):933–49.
8. Li Z, Maglione M, Tu W, et al. Meta-analysis: pharmacologic treatment of obesity. Ann Intern Med. 2005;142(7):532–46.
9. Maggard MA, Shugarman LR, Suttorp M, et al. Meta-analysis: surgical treatment of obesity. Ann Intern Med. 2005;142(7):547–59.
10. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults – the evidence report. National Institutes of Health. Obes Res 1998;6 Suppl 2:51S–209S.
11. Snow V, Barry P, Fitterman N, Qaseem A, Weiss K. Pharmacologic and surgical management of obesity in primary care: a clinical practice guideline from the American College of Physicians. Ann Intern Med. 2005;142(7):525–31.
12. Haddock CK, Poston WS, Dill PL, Foreyt JP, Ericsson M. Pharmacotherapy for obesity: a quantitative analysis of four decades of published randomized clinical trials. Int J Obes Relat Metab Disord. 2002;26(2):262–73.
13. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA. 2006;295(13):1549–55.

14. Yanovski SZ. Pharmacotherapy for obesity – promise and uncertainty. N Engl J Med. 2005;353(20):2187–9.
15. Colman E. Anorectics on trial: a half century of federal regulation of prescription appetite suppressants. Ann Intern Med. 2005;143(5):380–5.
16. Bray GA. Drug treatment of obesity. Rev Endocr Metab Disord. 2001;2(4):403–18.
17. Weintraub M, Hasday JD, Mushlin AI, Lockwood DH. A double-blind clinical trial in weight control. Use of fenfluramine and phentermine alone and in combination. Arch Intern Med. 1984;144(6):1143–8.
18. Stafford RS, Radley DC. National trends in antiobesity medication use. Arch Intern Med. 2003;163(9):1046–50.
19. Abenhaim L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. N Engl J Med. 1996;335(9):609–16.
20. Jick H, Vasilakis C, Weinrauch LA, Meier CR, Jick SS, Derby LE. A population-based study of appetite-suppressant drugs and the risk of cardiac-valve regurgitation. N Engl J Med. 1998;339(11):719–24.
21. Klein S, Burke LE, Bray GA, et al. Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. Circulation. 2004;110(18):2952–67.
22. Kushner RF, Blatner DJ. Risk assessment of the overweight and obese patient. J Am Diet Assoc. 2005;105(5 Suppl 1):S53–62.
23. Jackson JE, Doescher MP, Saver BG, Hart LG. Trends in professional advice to lose weight among obese adults, 1994 to 2000. J Gen Intern Med. 2005;20(9):814–8.
24. Foster GD, Wadden TA, Makris AP, et al. Primary care physicians' attitudes about obesity and its treatment. Obes Res. 2003;11(10):1168–77.
25. Galuska DA, Will JC, Serdula MK, Ford ES. Are health care professionals advising obese patients to lose weight? JAMA. 1999;282(16):1576–8.
26. Puhl RM, Brownell KD. Confronting and coping with weight stigma: an investigation of overweight and obese adults. Obesity (Silver Spring). 2006;14(10):1802–15.
27. Puhl RM, Brownell KD. Psychosocial origins of obesity stigma: toward changing a powerful and pervasive bias. Obes Rev. 2003;4(4):213–27.
28. Schwartz MB, Chambliss HO, Brownell KD, Blair SN, Billington C. Weight bias among health professionals specializing in obesity. Obes Res. 2003;11(9):1033–9.
29. Simkin-Silverman LR, Gleason KA, King WC, et al. Predictors of weight control advice in primary care practices: patient health and psychosocial characteristics. Prev Med. 2005;40(1):71–82.
30. Teachman BA, Brownell KD. Implicit anti-fat bias among health professionals: is anyone immune? Int J Obes Relat Metab Disord. 2001;25(10):1525–31.
31. Foster GD, Wadden TA, Phelan S, Sarwer DB, Sanderson RS. Obese patients' perceptions of treatment outcomes and the factors that influence them. Arch Intern Med. 2001;161(17):2133–9.
32. Hamman RF, Wing RR, Edelstein SL, et al. Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes Care. 2006;29(9):2102–7.
33. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346(6):393–403.
34. Lindstrom J, Ilanne-Parikka P, Peltonen M, et al. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. Lancet. 2006;368(9548):1673–9.
35. Wadden TA, Berkowitz RI, Womble LG, et al. Randomized trial of lifestyle modification and pharmacotherapy for obesity. N Engl J Med. 2005;353(20):2111–20.
36. Frank A. The long-term management of obesity with continuing pharmacotherapy. Obes Res. 2004;12(11):1821–7.
37. O'Meara S, Riemsma R, Shirran L, Mather L, ter Riet G. A systematic review of the clinical effectiveness of orlistat used for the management of obesity. Obes Rev. 2004;5(1):51–68.
38. Sjostrom L, Rissanen A, Andersen T, et al. Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. European Multicentre Orlistat Study Group. Lancet. 1998;352(9123):167–72.
39. Hutton B, Fergusson D. Changes in body weight and serum lipid profile in obese patients treated with orlistat in addition to a hypocaloric diet: a systematic review of randomized clinical trials. Am J Clin Nutr. 2004;80(6):1461–8.
40. Kelley DE, Bray GA, Pi-Sunyer FX, et al. Clinical efficacy of orlistat therapy in overweight and obese patients with insulin-treated type 2 diabetes: a 1-year randomized controlled trial. Diabetes Care. 2002;25(6):1033–41.
41. Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care. 2004;27(1):155–61.
42. Ozkan B, Bereket A, Turan S, Keskin S. Addition of orlistat to conventional treatment in adolescents with severe obesity. Eur J Pediatr. 2004;163(12):738–41.
43. Maahs D, de Serna DG, Kolotkin RL, et al. Randomized, double-blind, placebo-controlled trial of orlistat for weight loss in adolescents. Endocr Pract. 2006;12(1):18–28.
44. Chanoine JP, Hampl S, Jensen C, Boldrin M, Hauptman J. Effect of orlistat on weight and body composition in obese adolescents: a randomized controlled trial. JAMA. 2005;293(23):2873–83.

45. Ryder JR, Kaizer A, Rudser KD, Gross A, Kelly AS, Fox CK. Effect of phentermine on weight reduction in a pediatric weight management clinic. Int J Obes (Lond). 2017;41(1):90–3.
46. Fox CK, Kaizer AM, Rudser KD, et al. Meal replacements followed by topiramate for the treatment of adolescent severe obesity: a pilot randomized controlled trial. Obesity (Silver Spring). 2016;24(12):2553–61.
47. Fox CK, Marlatt KL, Rudser KD, Kelly AS. Topiramate for weight reduction in adolescents with severe obesity. Clin Pediatr (Phila). 2015;54(1):19–24.
48. Nathan BM, Rudser KD, Abuzzaha MJ, et al. Predictors of weight-loss response with glucagon-like peptide-1 receptor agonist treatment among adolescents with severe obesity. Clin Obes. 2016;6(1):73–8.
49. Tamborlane WV, Barrientos-Perez M, Fainberg U, et al. Liraglutide in children and adolescents with type 2 diabetes. N Engl J Med. 2019;381(7):637–46.
50. Srivastava G, Apovian CM. Current pharmacotherapy for obesity. Nat Rev Endocrinol. 2017;14(1):12–24.
51. Kuhnen P, Clement K, Weigand S, et al. Proopiomelanocortin deficiency-treated with a melanocortin-4 receptor agonist. N Engl M Med. 2016;375(3):240–6.
52. Ness-Abramof R, Apovian CM. Drug-induced weight gain. Drugs Today (Barc). 2005;41(8):547–55.
53. Allison DB, Mentore JL, Heo M, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiatry. 1999;156(11):1686–96.

20

Adolescent Metabolic/Bariatric Surgery: Effects on Obesity, Comorbidities, and Insulin Resistance

Daniel S. Hsia, Amy Sanghavi Shah, and Thomas Inge

Introduction

Current estimates are that 17.0% of US children and adolescents ages 2–19 years are obese and 5.8% are severely obese, defined as a body mass index (BMI) at or above 120% of the sex-specific 95th percentile based on the 2000 US Centers for Disease Control (CDC) BMI-for-age growth charts [1]. Particularly alarming has been the rapid rise in the prevalence of severe obesity among adolescents ages 12–19 years, which has risen from 5% to 9.1% over the past 15 years [1]. The high prevalence of severe obesity and data demonstrating childhood obesity tracks into adulthood suggest that US adolescents are at high risk to develop obesity-related comorbidities—such as insulin resistance, type 2 diabetes, obstructive sleep apnea, polycystic ovarian syndrome, fatty liver disease, and cardiovascular disease—by young adulthood [2, 3]. Diet, exercise, and behavior modification have limited success in providing significant and durable weight loss, while pharmacological therapy is currently limited in the pediatric population [4]. This has led to exploration of alternate strategies to treat severe obesity and its associated comorbidities. Metabolic/bariatric surgery (MBS) in the adolescent population is emerging as an effective long-term option, as it results in sustained weight loss and improves obesity-related comorbidities. The term *metabolic* is used with bariatric surgery because evidence has accrued indicating that the profound metabolic and comorbidity improvements that are seen following surgery commonly surpass that expected from body weight loss alone. This chapter will review common MBS procedures, the indications for use, and the effects of surgery on obesity, associated comorbidities, and insulin resistance in adolescents.

Indications for Operation and Procedures Used for Adolescent Metabolic/Bariatric Surgery

Body mass index criteria for MBS in adolescents have evolved over the past decade from relatively conservative cutoffs (BMI ≥ 40 kg/m^2 with severe comorbidities, or BMI ≥ 50 kg/m^2 with less severe comorbidities) to BMI and comorbidity cut points that allow consideration of surgery once the diagnosis of severe obesity is made (e.g., BMI ≥ 35 kg/m^2), congruent with

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adult guidelines [5]. The American Society for Metabolic and Bariatric Surgery (ASMBS) position statement and best-practice guidelines for the use of MBS in the adolescent population in 2012 are presented in Table 20.1 [6].

Common Types of Metabolic/ Bariatric Surgery Procedures

Data from the National Inpatient Sample database from 2004 to 2011 show that approximately 950 adolescent MBS cases were conducted per year. According to recently published data from the Patient Centered Outcomes Research Initiative Bariatric Surgery Study, which included 544 adolescent cases from participating healthcare systems, major shifts in the use of specific MBS procedure types (Fig. 20.1) have occurred. In 2004–2009, Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG), and adjustable gastric banding (AGB) accounted for 46%, 13%, and 40% of adolescent cases, respectively. In contrast, in 2014, VSG accounted for more than 80% of adolescent cases, with RYGB and AGB performed in <20% of the time [8]. Although these

Table 20.1 Current eligibility criteria for adolescent metabolic bariatric surgery

Body mass index (kg/m^2)	Comorbidities
≥35	Type 2 diabetes mellitus, moderate/severe OSA (AHI >15), pseudotumor cerebri, severe NASH
≥40	Mild OSA (AHI >5), insulin resistance, hypertension, impaired fasting glucose, dyslipidemia, impaired quality of life
Additional eligibility criteria	IV or V (unless severe comorbid disease warrants earlier MBS)
Tanner stage	≥95% estimated growth
Skeletal maturity	Demonstrates ability to understand dietary/physical changes after surgery
Lifestyle changes	Evidence of mature decision making
Psychosocial	Understands risk and benefits of surgery
	Evidence of family and social support
	Evidence that patient/family will be compliant with recommended preoperative and postoperative care (dietary, medication, etc.)

Adapted from Thakkar and Michalsky [5]
OSA obstructive sleep apnea, *AHI* apnea-hypopnea index, *NASH* nonalcoholic steatohepatitis

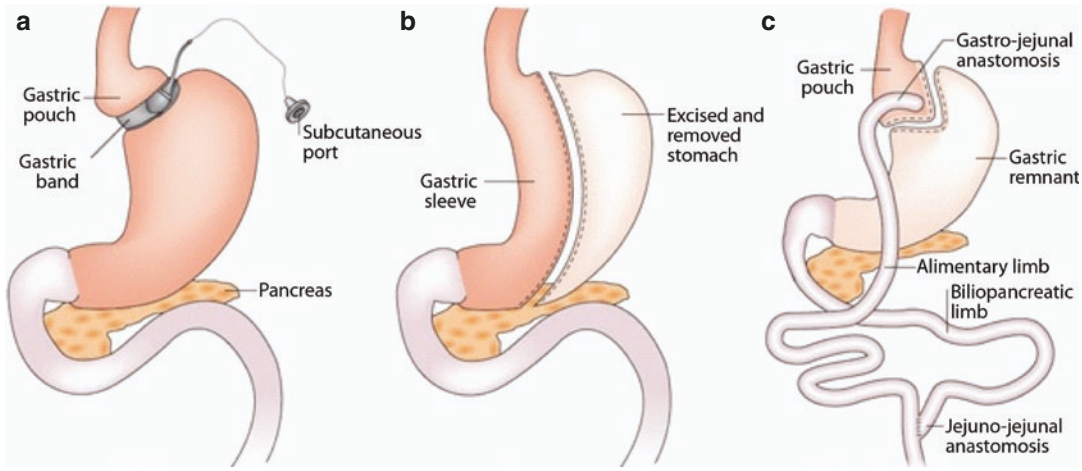

Fig. 20.1 The three most commonly performed MBS procedures. (**a**) The adjustable gastric band is placed laparoscopically around the upper stomach to restrict the transit of ingested food. (**b**) Laparoscopic sleeve gastrectomy involves separation of the greater curvature from the omentum and splenic attachments. (**c**) Roux-en-Y gastric bypass involves the rearrangement of the alimentary canal, such that ingested food bypasses most of the stomach, all of the duodenum, and a portion of the proximal jejunum. (Reprinted with permission from Miras and le Roux [7])

prevalence data provide real-world evidence of *procedure preferences*, there is no clear consensus about which MBS procedure is *most appropriate* for adolescents based on objective safety and efficacy comparisons. Each procedure has its advantages and disadvantages along with unique risks.

Adjustable Gastric Band

The AGB is a purely restrictive procedure in which a rigid, prosthetic band with an inner adjustable balloon is placed laparoscopically around the proximal stomach, usually 1–2 cm below the gastroesophageal junction, thus limiting the volume of food that can be ingested. The balloon within the band inflates with saline using a needle via a subcutaneous port anchored to the fascia of the rectus abdominis muscle to achieve a variable degree of restriction [9, 10]. Benefits of this procedure include a lack of staple lines, potential reversibility, and fewer nutritional deficits than the malabsorptive procedures. Complications include tube leaks, band migration, and erosion of the band into the stomach. Important for the long-term success of the operation, patients are required to follow up at regular intervals for band adjustment and lifestyle/nutritional counseling. Finally, while the manufacturer conducted an outcome study for this device in adolescents, these data have not been published and the use of this device in adolescents younger than 18 years of age is not approved by the US Food and Drug Administration (FDA).

Vertical Sleeve Gastrectomy

VSG was originally performed as the first step in a staged weight loss procedure for the most severely obese adults who presented the highest risks of general surgery and anesthesia [9]. VSG involves separation of the greater curvature of the stomach from the omentum and splenic attachments and the excision of approximately 75% of the stomach using a stapling device. The excision extends from approximately 5 cm proximal to the pylorus to the angle of His, leaving a narrow remnant stomach based on the lesser curve of the stomach [10]. The benefits of this procedure include the lack of a foreign body, no need for frequent adjustments as with the AGB, and fewer nutritional deficiencies than seen in other malabsorptive procedures; however, leakage from the suture line and stenosis are potential complications [9, 11]. Although originally introduced as a first-stage procedure that would ultimately entail an additional more definitive weight loss procedure, the operation appears to be a definitive bariatric operation for most who have undergone the procedure. Long-term weight loss data in large numbers of patients have not yet been published, however.

Roux-en-Y Gastric Bypass

RYGB has been widely adopted in the treatment of severe obesity in the adult population since the 1960s and was first reported for the treatment of severe adolescent obesity as early as the 1970s [5]. It has been described as a procedure with a combination of restrictive and malabsorptive effects. However, more recent investigations in human and animal models suggest that the most important effects of the operation on weight regulation involve physiologic alterations in postprandial gut hormone secretion (affecting appetite and satiety), energy expenditure, macronutrient preference, microbiota, bile acid metabolism, and micronutrient and mineral malabsorption [12]. RYGB involves the creation of a small (<30 mL) proximal gastric pouch that is divided and separated from the distal stomach and then anastomosed to a Roux limb of small bowel 75–150 cm in length [4]. The benefits of RYGB include a proven ability to induce long-term weight loss and to decrease comorbid disease [13]. However, the procedure is irreversible, causes significant change to the normal gut orientation, and carries a risk of micronutrient malnutrition if proper attention is not paid to diet and supplementation of essential nutrients.

Weight Loss Outcomes

MBS is now an established treatment for severe obesity in adults [14]. It is the only therapeutic option that results in substantial short- and

long-term durable weight loss. In 2015, Chang et al. published results of a meta-analysis that included 164 studies (37 randomized clinical trials and 127 observational studies) that comprised more than 160,000 adults [15]. Data from 12 randomized studies found the mean reduction in BMI during the first postoperative year was 13.5 kg/m^2 (95% CI 11.6–15.5), with a single study reporting a BMI reduction of 11.4 kg/m^2 at 5 years. Similar findings were reported from 57 observational studies that found a mean BMI reduction of 11.8 kg/m^2 (95% CI 9.7–13.9) 1-year postsurgery and from 10 studies with a mean BMI reduction of 14.3 kg/m^2 (95% CI 11.5–17.2) at 5 years [15]. O'Brien and colleagues pooled adult studies with follow-up of up to 10 years [16]. Percent excess weight loss for all studies ranged between 42% and 79%. Percent excess weight loss was 53.0% (range 43–55%) following RYGB at 10 years and 59% (a single study) following gastric banding [16].

Some of the most impressive long-term adult data come from the Swedish Obese Subjects (SOS) Study, an ongoing prospective, controlled intervention trial that has compared weight loss from bariatric surgery to similarly obese control participants not receiving MBS [17]. Twenty-year follow-up data are emerging. Compared to controls, MBS procedures were associated with clinically significant and durable weight loss [18]. Weight loss was greatest in the first 2 years, with some weight regain in subsequent years, and stabilization at 8–10 years. Long-term follow-up has shown that weight loss can be maintained for 15 and 20 years and a significant survival advantage has been documented in those participants who underwent MBS. The mean changes in body weight after 2, 10, 15, and 20 years were −23%, −17%, −16%, and −18% in the MBS groups and 0%, 1%, −1%, and −1% in the control group [18].

As utilization of MBS has increased for the treatment of severe obesity in adolescents, data on short- (<1 years), mid- (1–4 years), and long-term (+5 years) weight outcomes are now available. In 2013, a systematic review and meta-analysis evaluated 1-year weight outcomes after adolescent MBS [19]. In total, 637 adolescents undergoing various bariatric procedures from 23 studies were included. Mean preoperative BMI for all adolescents ranged from 38.5 to 60.2 kg/m^2 (mean 47.9 kg/m^2). At 1 year, the mean BMI loss was −13.5 kg/m^2 (95% confidence interval −15.1 to −11.9). When examined by procedure type, weight loss was greatest for RYGB (−17.2 kg/m^2, 95% CI −20.1 to −14.3), smallest for AGB (−10.5 kg/m^2, 95% CI −11.8 to −9.1), and intermediate for VSG (−14.5 kg/m^2, 95% CI −17.3 to −11.7).

The percent BMI reduction in adolescents after surgery appears similar regardless of presurgery BMI [20]. In a single-center cohort of 61 adolescents undergoing RYGB, a mean BMI reduction of 37% was found, such that the mean nadir postoperative BMI at year 1 was 31, 38, and 47 kg/m^2 for patients with starting BMIs between 40 and 54, 55 and 65, and >65 kg/m^2, respectively. Baseline BMI accounted for 67% of the variance in BMI at 1 year following surgery. These results suggest that initiating consideration of surgery soon after the diagnosis of severe obesity may be an important factor if the goal is to achieve resolution of severe obesity postsurgery.

Results from mid- and long-term follow-up studies in adolescents suggest that weight loss following MBS is durable (Table 20.2) [21–27]. In 2016, the 3-year outcome data for 242 adolescents from 5 US centers who participated in the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) study were published [23]. The mean age of the cohort was 17 ± 1.6 years at baseline. Among 161 participants undergoing RYGB, BMI decreased from 54 kg/m^2 at baseline to 39 kg/m^2 (28%). Among 67 participants undergoing VSG, BMI decreased from 50 kg/m^2 at baseline to 37 kg/m^2 (26%). Among the 14 undergoing AGB, the least weight loss occurred with BMI decreasing by only 8%. For all operations, the weight changes were greatest at 1 year and some weight regain was observed over the subsequent 2 years. At 3 years, only 2% of the participants who underwent gastric bypass and 4% of those

Table 20.2 Weight loss outcomes in mid- and long-term adolescent metabolic bariatric surgery studies

References	Surgery, number of subjects	Length of follow-up	BMI reduction
Olbers 2012 [21]	RYGB, $n = 81$	2 years	↓ 15.3 kg/m^2
Schmitt 2016 [22]	AGB, $n = 16$	2 years	↓ 7.6 kg/m^2
Inge 2015 [23]	RYGB, $n = 161$ VSG, $n = 67$ AGB, $n = 14$	3 years	RYGB, ↓15.1 kg/m^2 VSG, ↓13.1 kg/m^2 AGB, ↓3.8 kg/m^2
Alqahtani 2014 [24]	VSG 53	3 years	↓ 20 kg/m^2
Alqahtani 2016 [25]	VSG, Age <14 years, $n = 116$, Age ≥14–21 years, $n = 158$	5 years	Age <14 years, ↓17.3 ± 2.5 Age ≥14–21 years, ↓2.8 ± 14.6
Olbers 2017 [26]	RYGB, $n = 81$ Controls, $n = 80$	5 years	RYGB ↓13.1 kg/m^2 Controls ↓3.3 kg/m^2
Inge 2017 [27]	RYGB, $n = 58$	8 years	RYGB ↓17 ± 8 kg/m^2

BMI body mass index, *RYGB* Roux-en-Y gastric bypass, *VSG* vertical sleeve gastrectomy, *AGB* adjustable gastric banding

who underwent sleeve gastrectomy exceeded their baseline weight. At 3 years, 36% of Teen-LABS participants undergoing AGB exceeded their baseline weight.

In 2017, 5- to 12-year follow-up data from the Cincinnati adolescent cohort were published. Data from 58 adolescents undergoing RYGB were included [27]. Baseline age was 17 years (range 13–21 years) and mean BMI was 59 kg/m^2 (range 41–87 kg/m^2). All participants had severe obesity (BMI ≥ 40 kg/m^2) at baseline. In the first year following RYGB, average BMI decreased by −23 ± 6 kg/m^2, representing −39 ± 7% weight loss. At long-term follow-up (8.0 ± 1.6 years after surgery), the average BMI reduction was −17 ± 8 kg/m^2, representing −29 ± 14%; $p < 0.01$ weight loss from baseline [27]. These data demonstrate that the great majority of the weight loss effect at 1 year is durable out to 8 years on average.

Outcomes of MBS have also been compared directly to intensive lifestyle-based weight management, with severely obese participants in both groups. AMOS (Adolescent Morbid Obesity Surgery; a Swedish nationwide study) showed 81 adolescents (mean age 16.5 years) undergoing RYGB had a mean BMI decrease of −13 kg/m^2 [26]. The nonsurgical comparison group of severely obese teens experienced a mean BMI increase of 3 kg/m^2 over the 5-year study period. In contrast, when considering pediatric patients with moderate (not severe) levels of obesity, data from randomized controlled trials and meta-analyses show intensive lifestyle interventions conducted over 1 year are associated with a very modest mean BMI reduction of −0.29 to −0.63 standard deviation scores [28–32] that is sustained when the intervention has stopped over 5–10 years of follow-up [33–35]. Slightly more weight loss can be attained with weight loss drugs in pediatric patients with moderate to severe obesity. In a recent meta-analysis, treatment with sibutramine showed a mean BMI loss of −2.28 (95% CI −2.81 to −1.76), while orlistat showed a mean BMI loss of −1.67 (−3.52 to −0.18) in adolescents when starting BMI was 35–36 kg/m^2 [36]. Unfortunately, sibutramine is no longer available for use due to the documented cardiovascular adverse event profile.

One randomized control trial of MBS has been conducted in adolescents. O'Brien et al. compared conventional lifestyle interventions (diet and exercise) to gastric banding. In 50 adolescents, mean age 16.5 years, gastric banding at 2 years showed significantly greater weight loss (−34.6 kg, 95% CI, 30.2–39.0), representing an excess weight loss of 78.8% (95% CI, 66.6%–91.0%), compared to the lifestyle intervention where mean weight loss was 3.0 kg (95% CI, 2.1–8.1), representing excess weight loss of 13.2% (95% CI, 2.6%–21.0%). Randomized trials comparing RYGB or VSG to lifestyle interventions have not been conducted.

Comorbidities of Obesity and Insulin Resistance

The primary rationale for the use of MBS in adolescents is the potential to reverse serious health problems associated with severe obesity. One of the underlying metabolic abnormalities that may provide a link between obesity and multiple comorbidities is insulin resistance (IR). IR is a fundamental feature in the development of type 2 diabetes. However, the associations between IR and dyslipidemia, hypertension, polycystic ovary syndrome, fatty liver disease, and sleep apnea have also become apparent (Fig. 20.2).

Evidence suggests that there is a strong homeostatic relationship between IR and pancreatic β-cell insulin secretion, such that individuals with IR compensate by enhancing insulin secretion in the fasting state and in response to a standard carbohydrate challenge, while lean individuals who are insulin sensitive demonstrate lower insulin secretion in the same circumstances. Plotted graphically, this relationship resembles a hyperbolic curve, with variation in the population as indicated by smoothed percentile curves (Fig. 20.3) [37–39]. Clinical investigations in adolescents by Elder et al. [38] and others have shown that, compared to adolescents who are lean and insulin sensitive, those with obesity exhibit greater IR (lower insulin sensitivity) and an accentuated insulin response to intravenous glucose challenge (acute insulin response to glucose [AIRg]; Fig. 20.3). Although the pathogenesis of type 2 diabetes is multifactorial, high insulin secretion (e.g., high AIRg) in the setting of IR over time may be detrimental to β-cell function. In susceptible individuals with IR and high AIRg, progression to impaired glucose tolerance and ultimately fasting hyperglycemia is seen. Indeed, severely obese adolescents with type 2 diabetes (Fig. 20.3**,** "DM") demonstrated signs of β-cell failure with markedly impaired insulin secretion relative to their degree of IR. While the metabolic characteristics of the three cohorts (Ln, Ob, and DM) depicted in the bottom left of Fig. 20.3 are derived from a cross-sectional study of adolescents who underwent intravenous glucose tolerance testing [38], similar longitudinal studies in adult Pima Indians [40] strongly suggest that an individual's phenotype can change over time, as depicted in the dashed arrows.

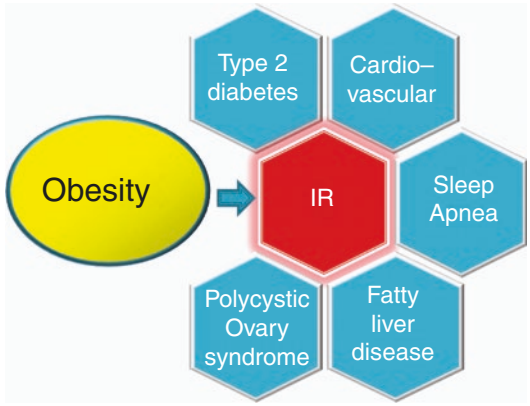

Fig. 20.2 The figure depicts the relationship between obesity and insulin resistance (IR) and graphically hypothesizes that IR may be a link between obesity and multiple comorbidities

To determine how IR and β-cell function are impacted by MBS in severely obese adolescents, we characterized the acute insulin response to glucose and insulin sensitivity using identical methods in the same clinical research unit as Elder et al. [38] The mean age of the 22 participants undergoing RYGB was 17 years, with a female-to-male ratio of 2:1. The mean BMI was 61 kg/m^2. Interestingly, at baseline (prior to surgery), the severely obese adolescents who were preparing for MBS demonstrated low insulin sensitivity and relatively high AIRg, similar to the severely obese adolescents studied by Elder et al. in 2006 (Fig. 20.3, top right insert, "B" compared to "Ob") [39]. Over the 12-month period of study, during a mean 39% weight reduction, a threefold increase in insulin sensitivity was observed, along with a 59% decrease in the AIRg (Fig. 20.3, top right insert, "3" and "12"). This decrease in AIRg has been interpreted as evidence that drastic reduction in IR after surgery

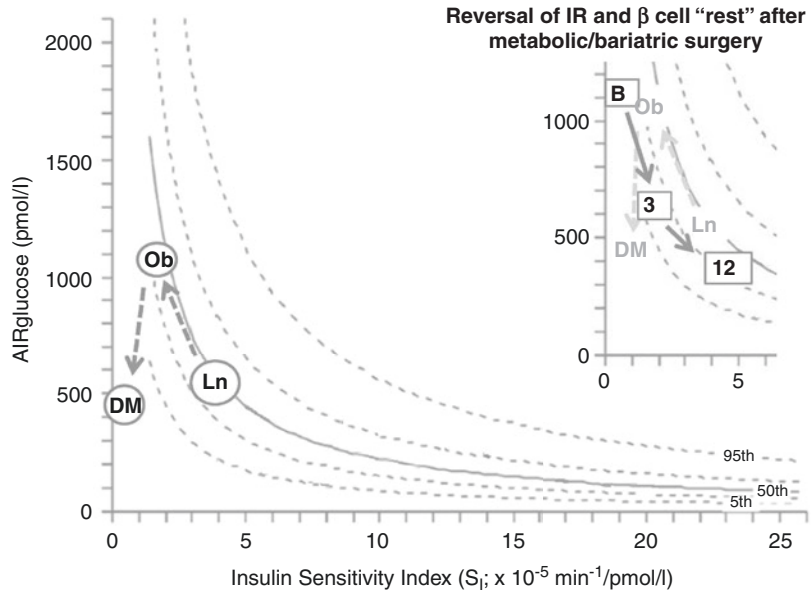

Fig. 20.3 Relationship between insulin resistance and insulin secretion in adolescents with severe obesity, diabetes, and after metabolic/bariatric surgery. The graphic uses population-based data to show the hyperbolic relationship between acute insulin response to glucose (AIRglucose) and insulin sensitivity, as described by Kahn [37]. The AIRg and insulin sensitivity data derived from lean (L; n = 13, BMI 22 kg/m^2), obese (Ob; n = 13, BMI 34 kg/m^2), and obese with type 2 diabetes (DM; n = 16, BMI 37 kg/m^2) undergoing frequently sampled intravenous glucose tolerance tests (fsIVGTT) by Elder et al. [38] were used to plot the relative positions of these cohorts on the population-based curves. The top right insert in the figure graphically depicts the metabolic phenotypes of a surgical cohort of adolescents with severe obesity who participated in longitudinal study conducted by Inge et al. [39]. Square symbols show the metabolic position of the group before ("B") and at 3 months ("3") and 12 months ("12") following RYGB surgery

can result in reversal of the exaggerated insulin secretion, a condition referred to as β-cell "rest." Graphically, this is seen as movement to a position on the homeostatic curve very similar to that of lean, healthy adolescents (compare "Ln" to "12" in Fig. 20.3). We would hypothesize that these changes in metabolic phenotype in severely obese adolescents will result in a reduction in their risk of progression to type 2 diabetes over time; however, longer-term study will be needed to support or refute this hypothesis.

In addition to these findings demonstrating correction of metabolic dysfunction in severely obese adolescents undergoing surgery, evidence of the effect of MBS on multiple other clinical comorbidities associated with adolescent obesity and insulin resistance is growing and is summarized below.

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most frequent cause of female infertility with an estimated prevalence of more than 10% in women of childbearing age [41]. Obesity-related insulin resistance and hyperinsulinemia play an important role in the hyperandrogenism seen in PCOS [42]. A meta-analysis of the impact of MBS on PCOS showed the prevalence of PCOS decreased by nearly 40% at each study endpoint, with nearly 50% improvement in menstrual irregularity and 30% improvement in hirsutism [43]. Guidelines for the use of MBS for the treatment of PCOS are limited due to the limited data concerning the amelioration of PCOS-associated symptoms such as menstrual abnormalities, hirsutism, and infertility.

That said, to the extent that PCOS is fueled by IR, there is optimism that adolescent bariatric surgery should mitigate the pathophysiology of this condition, due to the significant reduction in IR consistently observed following surgery [39].

Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is a continuum of disease ranging from hepatic steatosis alone to nonalcoholic steatohepatitis (NASH) to the most severe end of the spectrum with fibrosis and cirrhosis [44]. Obesity is a primary risk factor for NAFLD, and severely obese patients undergoing MBS may have NAFLD rates as high as 90% [44]. The key features of the pathogenesis of NAFLD are IR and dyslipidemia leading to excessive triglyceride deposition in the hepatocytes [45]. Treatment for NAFLD is limited to weight loss with some data supporting the use of vitamin E, but there are no established guidelines for optimal medical or dietary interventions specifically targeting NALFD. Given the durability of weight loss and resolution of other associated comorbidities, there has been increasing interest in the use of MBS to treat NAFLD. Manco et al. have published the only controlled trial of bariatric surgery for NASH in adolescents to date. Liver histology was examined prospectively before and 6 and 12 months after VSG ($n = 20$) and lifestyle intervention ($n = 54$). Reversal of NASH was seen in all patients who underwent surgery; however, improvement was not seen in those who did not undergo surgery. These data support current recommendations for use of MBS in adolescents with NASH with BMI values ≥ to 35 mg/m^2 [13].

Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) syndrome is characterized by frequent episodes of apnea and hypopnea due to upper airway collapse, which can result in hypertension, increased cardiovascular mortality, stroke, decreased quality of life, sleepiness, and morbidity associated with disordered sleep [46]. The prevalence of OSA in bariatric surgical candidates with a BMI ≥ 35 kg/m^2 ranges from 60% to 83% [46]. In addition, there appears to be a relationship between sleep apnea and insulin resistance, as clinically these patients have higher fasting glucose and insulin levels compared to age- and BMI-matched controls [46]. In a systematic review of 69 studies with 13,900 patients, an average of 75% of patients saw at least an improvement in their sleep apnea after undergoing MBS [47]. Combined, these results are especially important, as the rate of adherence to continuous positive airway pressure therapy (CPAP)—first-line treatment for OSA—is 29% to 83% [48]. While results in severely obese adolescents are limited, resolution of OSA after MBS is nearly 100% over the period of follow-up [49]. In addition, when critically examined by Amin et al., the amelioration of OSA following VSG in a small cohort of adolescents was apparent within weeks of the operation and occurred prior to major weight reduction [50]. These data suggest that neurophysiologic changes induced by surgery, rather than anatomic effects, could play a major role in the surgical treatment effect for OSA.

Cardiovascular Risk Factors

Obesity is associated with multiple cardiovascular (CV) risk factors and direct effects on hemodynamics and cardiovascular structure and function [51]. Increased serum concentrations of C-reactive protein and leptin seen in obese patients are independently associated with insulin resistance and cardiovascular disease [52]. Moreover, insulin resistance and diabetes are associated with intracellular lipid accumulation in the myocardium, which may lead to cell death [51]. As such, CV disease is one of the major causes of morbidity and mortality in obese patients. In a retrospective cohort study of nearly 10, 000 adults who had undergone gastric bypass surgery, with a mean follow-up of 7.1 years, long-term mortality from any cause decreased by 40% and mortality due to coronary artery disease decreased by 56% compared to a nonsurgical control group [53]. Multiple CV risk factors, such as hypertension and dyslip-

idemia, as well as cardiac structure and function have been shown to improve after MBS. Weight loss after MBS substantially improves and/or resolves hypertension in 37–53% of patients or reduces the need for antihypertensive agents in 18–36% patients over an average follow-up of 1–5 years [54]. As for dyslipidemia, a systematic review of studies with >2 years of follow-up demonstrated remission of dyslipidemia in 60.4% of patients after RYGB and 22.7% after AGB [55]. In terms of inflammatory markers, mean levels of C-reactive protein have been shown to decrease by 61.7%, from a baseline of 4.5 mg/L to 1.7 mg/L, at a mean of 34 months after MBS [10]. In another systematic review by Aggarwal et al., MBS appears to have a beneficial effect on various indices of cardiac function, including decreased left ventricular mass and improved ejection fraction [56]. Finally, after a median follow-up of 14.7 years in the SOS study, patients with higher baseline insulin values had the greatest relative benefit from MBS in terms of decreased incidence of cardiovascular events (myocardial infarction and stroke) [18]. These results indicate that the degree of insulin resistance may predict the overall improvement in cardiovascular risk after MBS in adults. Data from adolescents also suggest improvements in left ventricular mass, cardiac workload, and diastolic function 1 year following RYGB [57]. Additionally, the Teen-LABS consortium has shown remission of dyslipidemia and hypertension in 66% and 73% of adolescents, respectively, by 3 years postoperatively [23]. Similar results were also confirmed at 7 years following RYGB in adolescents, with remission of dyslipidemia and hypertension in 64% and 76% in the FABS-5 study [27]. Taken together, the 3- and 7-year outcomes suggest that the impact of adolescent bariatric surgery on reversal of risk factors for CV disease is both clinically significant and durable.

Type 2 Diabetes

There are extensive data in adults demonstrating durable diabetes remission from 5 to 15 years after MBS [58–62]. However, variable definitions of diabetes remission and inconsistencies in the control group make comparing results from study to study difficult. Regardless, these data make clear that MBS is an important tool in the armamentarium of diabetes treatment. As such, guidelines and recommendations from the second Diabetes Surgery Summit support the inclusion of MBS among antidiabetes interventions for people with type 2 diabetes and obesity [63].

RYGB and VSG have been associated with increased likelihood of diabetes remission over time compared to AGB [60]. Data from the SOS study showed that shorter diabetes duration at baseline was associated with higher diabetes remission rates in surgery patients after 2, 10, and 15 years of follow-up [58]. Older age, higher baseline HbA1c, and treatment with insulin or other diabetes medications at baseline were associated with decreased likelihood of diabetes remission over a 5-year period in another cohort [60].

There are fewer data regarding remission in adolescents, but type 2 diabetes remission rates appear to indicate similar, if not greater, metabolic effectiveness of surgery when used in adolescents compared with similar operations in adults with type 2 diabetes [64]. In the Teen-LABS study, 19 of 20 participants with type 2 diabetes at baseline remained in remission after 3 years [23]. Furthermore, in the Follow-up of Adolescent Bariatric Surgery at 5+ Years (FABS-5+) extension study, diabetes remission was seen in 7 out of 8 adolescents who had RYGB at a mean follow-up of 8 years and with no new incident diabetes cases [27]. Diabetes remission among adolescents undergoing MBS has also been compared to those receiving medical therapy. In a secondary analysis, 30 Teen-LABS participants were compared to a subset of adolescents (n = 63) who participated in the Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) study, a randomized controlled trial evaluating the efficacy of metformin alone, metformin plus rosiglitazone, and metformin plus lifestyle modification [65]. During the 2 years of follow-up, mean HbA1c concentration decreased from 6.8% (95% CI, 6.4%–7.3%) to 5.5% (95% CI, 4.7%–6.3%)

in Teen-LABS but increased from 6.4% (95% CI, 6.1%–6.7%) to 7.8% (95% CI, 7.2%–8.3%) in the TODAY study. Compared with baseline, the BMI decreased by 29% (95% CI, 24%–34%) in Teen-LABS and increased by 3.7% (95% CI, 0.8%–6.7%) in TODAY. Additionally, the prevalence of hypertension, dyslipidemia, and microalbuminuria decreased in Teen-LABS but increased in TODAY over the 2 years [65]. Thus, the understanding of the weight loss-independent beneficial effects of MBS continue to evolve and additional studies directly comparing medical to surgical treatment are needed.

Summary

In conclusion, MBS is associated with significant and durable weight loss in adolescents and adults. Additionally, MBS appears to improve obesity and insulin resistance, decreases β-cell hypersecretion associated with insulin resistance, and resolves or improves other associated comorbidities. While future work is needed to study long-term outcomes in adults after adolescent weight loss surgery, the current data support surgery as an effective treatment in adolescents with severe obesity.

References

1. Ogden CL, Carroll MD, Lawman HG, Fryar CD, Kruszon-Moran D, Kit BK, et al. Trends in obesity prevalence among children and adolescents in the United States, 1988–1994 through 2013–2014. JAMA. 2016;315(21):2292–9.
2. Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. Obes Rev. 2008;9(5):474–88.
3. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. J Pediatr. 2007;150(1):12–7.e2.
4. Kumar S, Kelly AS. Review of childhood obesity: from epidemiology, etiology, and comorbidities to clinical assessment and treatment. Mayo Clin Proc. 2017;92(2):251–65.
5. Thakkar RK, Michalsky MP. Update on bariatric surgery in adolescence. Curr Opin Pediatr. 2015;27(3):370–6.
6. Michalsky M, Reichard K, Inge T, Pratt J, Lenders C, American Society for Metabolic and Bariatric Surgery, et al. ASMBS pediatric committee best practice guidelines. Surg Obes Relat Dis. 2012;8(1):1–7.
7. Miras AD, le Roux CW. Mechanisms underlying weight loss after bariatric surgery. Nat Rev Gastroenterol Hepatol. 2013;10(10):575–84.
8. Inge TH, Coley RY, Bazzano LA, Xanthakos SA, McTigue K, Arterburn D, et al. Comparative effectiveness of bariatric procedures among adolescents: the PCORnet bariatric study. Surg Obes Relat Dis. 2018;14(9):1374–86.
9. Hsia DS, Fallon SC, Brandt ML. Adolescent bariatric surgery. Arch Pediatr Adolesc Med. 2012;166(8):757–66.
10. Beamish AJ, Olbers T, Kelly AS, Inge TH. Cardiovascular effects of bariatric surgery. Nat Rev Cardiol. 2016;13(12):730–43.
11. Stroh C, Birk D, Flade-Kuthe R, Frenken M, Herbig B, Hohne S, et al. Results of sleeve gastrectomy—data from a nationwide survey on bariatric surgery in Germany. Obes Surg. 2009;19(5):632–40.
12. Evers SS, Sandoval DA, Seeley RJ. The physiology and molecular underpinnings of the effects of bariatric surgery on obesity and diabetes. Annu Rev Physiol. 2017;79:313–34.
13. Pratt JS, Lenders CM, Dionne EA, Hoppin AG, Hsu GL, Inge TH, et al. Best practice updates for pediatric/adolescent weight loss surgery. Obesity (Silver Spring, MD). 2009;17(5):901–10.
14. Buchwald H. Consensus conference statement bariatric surgery for morbid obesity: health implications for patients, health professionals, and third-party payers. Surg Obes Relat Dis. 2005;1(3):371–81.
15. Chang SH, Stoll CR, Song J, Varela JE, Eagon CJ, Colditz GA. The effectiveness and risks of bariatric surgery: an updated systematic review and meta-analysis, 2003–2012. JAMA Surg. 2014;149(3):275–87.
16. O'Brien PE, McPhail T, Chaston TB, Dixon JB. Systematic review of medium-term weight loss after bariatric operations. Obes Surg. 2006;16(8):1032–40.
17. Sjostrom L. Review of the key results from the Swedish Obese Subjects (SOS) trial – a prospective controlled intervention study of bariatric surgery. J Intern Med. 2013 Mar;273(3):219–34.
18. Sjostrom L, Peltonen M, Jacobson P, Sjostrom CD, Karason K, Wedel H, et al. Bariatric surgery and long-term cardiovascular events. JAMA. 2012;307(1):56–65.
19. Black JA, White B, Viner RM, Simmons RK. Bariatric surgery for obese children and adolescents: a systematic review and meta-analysis. Obes Rev. 2013;14(8):634–44.
20. Inge TH, Jenkins TM, Zeller M, Dolan L, Daniels SR, Garcia VF, et al. Baseline BMI is a strong predictor of nadir BMI after adolescent gastric bypass. J Pediatr. 2010;156(1):103–8.e1.
21. Olbers T, Gronowitz E, Werling M, Marlid S, Flodmark CE, Peltonen M, et al. Two-year outcome

of laparoscopic Roux-en-Y gastric bypass in adolescents with severe obesity: results from a Swedish Nationwide study (AMOS). Int J Obes (2005). 2012;36(11):1388–95.
22. Schmitt F, Riquin E, Beaumesnil M, Dinomais M, Topart P, Weil D, et al. Laparoscopic adjustable gastric banding in adolescents: results at two years including psychosocial aspects. J Pediatr Surg. 2016;51(3):403–8.
23. Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Helmrath MA, Brandt ML, et al. Weight loss and health status 3 years after bariatric surgery in adolescents. N Engl J Med. 2016;374(2):113–23.
24. Alqahtani AR, Elahmedi MO, Al Qahtani A. Co-morbidity resolution in morbidly obese children and adolescents undergoing sleeve gastrectomy. Surg Obes Relat Dis. 2014;10(5):842–50.
25. Alqahtani A, Elahmedi M, Qahtani AR. Laparoscopic sleeve gastrectomy in children younger than 14 years: refuting the concerns. Ann Surg. 2016;263(2):312–9.
26. Olbers T, Beamish AJ, Gronowitz E, Flodmark CE, Dahlgren J, Bruze G, et al. Laparoscopic Roux-en-Y gastric bypass in adolescents with severe obesity (AMOS): a prospective, 5-year, Swedish nationwide study. Lancet Diabetes Endocrinol. 2017;5:174.
27. Inge TH, Jenkins TM, Xanthakos SA, Dixon JB, Daniels SR, Zeller MH, et al. Long-term outcomes of bariatric surgery in adolescents with severe obesity (FABS-5+): a prospective follow-up analysis. Lancet Diabetes Endocrinol. 2017;5(3):165–73.
28. Reinehr T. Lifestyle intervention in childhood obesity: changes and challenges. Nat Rev Endocrinol. 2013;9(10):607–14.
29. McGovern L, Johnson JN, Paulo R, Hettinger A, Singhal V, Kamath C, et al. Clinical review: treatment of pediatric obesity: a systematic review and meta-analysis of randomized trials. J Clin Endocrinol Metab. 2008;93(12):4600–5.
30. Oude Luttikhuis H, Baur L, Jansen H, Shrewsbury VA, O'Malley C, Stolk RP, et al. Interventions for treating obesity in children. Cochrane Database Syst Rev. 2009;21(1):Cd001872.
31. Savoye M, Nowicka P, Shaw M, Yu S, Dziura J, Chavent G, et al. Long-term results of an obesity program in an ethnically diverse pediatric population. Pediatrics. 2011;127(3):402–10.
32. Muckelbauer R, Libuda L, Clausen K, Reinehr T, Kersting M. A simple dietary intervention in the school setting decreased incidence of overweight in children. Obes Facts. 2009;2(5):282–5.
33. Reinehr T, Kleber M, Lass N, Toschke AM. Body mass index patterns over 5 y in obese children motivated to participate in a 1-y lifestyle intervention: age as a predictor of long-term success. Am J Clin Nutr. 2010;91(5):1165–71.
34. Vignolo M, Rossi F, Bardazza G, Pistorio A, Parodi A, Spigno S, et al. Five-year follow-up of a cognitive-behavioural lifestyle multidisciplinary programme for childhood obesity outpatient treatment. Eur J Clin Nutr. 2008;62(9):1047–57.
35. Epstein LH, Valoski A, Wing RR, McCurley J. Ten-year follow-up of behavioral, family-based treatment for obese children. JAMA. 1990;264(19):2519–23.
36. Rajjo T, Mohammed K, Alsawas M, Ahmed AT, Farah W, Asi N, et al. Treatment of pediatric obesity: an umbrella systematic review. J Clin Endocrinol Metabol. 2017;102(3):763–75.
37. Kahn SE. Clinical review 135: the importance of beta-cell failure in the development and progression of type 2 diabetes. J Clin Endocrinol Metab. 2001;86(9):4047–58.
38. Elder DA, Prigeon RL, Wadwa RP, Dolan LM, D'Alessio DA. Beta-cell function, insulin sensitivity, and glucose tolerance in obese diabetic and nondiabetic adolescents and young adults. J Clin Endocrinol Metab. 2006;91(1):185–91.
39. Inge TH, Prigeon RL, Elder DA, Jenkins TM, Cohen RM, Xanthakos SA, et al. Insulin sensitivity and beta-cell function improve after gastric bypass in severely obese adolescents. J Pediatr. 2015;167(5):1042–8 e1.
40. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest. 1999;104(6):787–94.
41. Butterworth J, Deguara J, Borg CM. Bariatric surgery, polycystic ovary syndrome, and infertility. J Obes. 2016;2016:1871594.
42. Garcia-Romero G, Escobar-Morreale HF. Hyperandrogenism, insulin resistance and hyperinsulinemia as cardiovascular risk factors in diabetes mellitus. Curr Diabetes Rev. 2006;2(1):39–49.
43. Skubleny D, Switzer NJ, Gill RS, Dykstra M, Shi X, Sagle MA, et al. The impact of bariatric surgery on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Surg. 2016;26(1):169–76.
44. Saggin L, Gorza L, Ausoni S, Schiaffino S. Troponin I switching in the developing heart. J Biol Chem. 1989;264(27):16299–302.
45. Hafeez S, Ahmed MH. Bariatric surgery as potential treatment for nonalcoholic fatty liver disease: a future treatment by choice or by chance? J Obes. 2013;2013:839275.
46. Ashrafian H, le Roux CW, Rowland SP, Ali M, Cummin AR, Darzi A, et al. Metabolic surgery and obstructive sleep apnoea: the protective effects of bariatric procedures. Thorax. 2012;67(5):442–9.
47. Sarkhosh K, Switzer NJ, El-Hadi M, Birch DW, Shi X, Karmali S. The impact of bariatric surgery on obstructive sleep apnea: a systematic review. Obes Surg. 2013;23(3):414–23.
48. Rosenberg R, Doghramji P. Optimal treatment of obstructive sleep apnea and excessive sleepiness. Adv Ther. 2009;26(3):295–312.
49. Kalra M, Inge T. Effect of bariatric surgery on obstructive sleep apnoea in adolescents. Paediatr Respir Rev. 2006;7(4):260–7.

50. Amin R, Simakajornboon N, Szczesniak R, Inge T. Early improvement in obstructive sleep apnea and increase in orexin levels after bariatric surgery in adolescents and young adults. Surg Obes Relat Dis. 2017;13(1):95–100.
51. Lee GK, Cha YM. Cardiovascular benefits of bariatric surgery. Trends Cardiovasc Med. 2016;26(3):280–9.
52. Romero-Corral A, Sierra-Johnson J, Lopez-Jimenez F, Thomas RJ, Singh P, Hoffmann M, et al. Relationships between leptin and C-reactive protein with cardiovascular disease in the adult general population. Nat Clin Pract Cardiovasc Med. 2008;5(7):418–25.
53. Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, et al. Long-term mortality after gastric bypass surgery. N Engl J Med. 2007;357(8):753–61.
54. Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP. Cardiovascular benefits of bariatric surgery in morbidly obese patients. Obes Rev. 2011;12(7):515–24.
55. Puzziferri N, Roshek TB 3rd, Mayo HG, Gallagher R, Belle SH, Livingston EH. Long-term follow-up after bariatric surgery: a systematic review. JAMA. 2014;312(9):934–42.
56. Aggarwal R, Harling L, Efthimiou E, Darzi A, Athanasiou T, Ashrafian H. The effects of bariatric surgery on cardiac structure and function: a systematic review of cardiac imaging outcomes. Obes Surg. 2016;26(5):1030–40.
57. Ippisch HM, Inge TH, Daniels SR, Wang B, Khoury PR, Witt SA, et al. Reversibility of cardiac abnormalities in morbidly obese adolescents. J Am Coll Cardiol. 2008;51(14):1342–8.
58. Sjostrom L, Peltonen M, Jacobson P, Ahlin S, Andersson-Assarsson J, Anveden A, et al. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. JAMA. 2014;311(22):2297–304.
59. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, et al. Bariatric surgery versus intensive medical therapy for diabetes – 5-year outcomes. N Engl J Med. 2017;376(7):641–51.
60. Dicker D, Yahalom R, Comaneshter DS, Vinker S. Long-term outcomes of three types of bariatric surgery on obesity and type 2 diabetes control and remission. Obes Surg. 2016;26(8):1814–20.
61. Pories WJ, Swanson MS, MacDonald KG, Long SB, Morris PG, Brown BM, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. Ann Surg. 1995;222(3):339–50; discussion 50–2.
62. Adams TD, Davidson LE, Litwin SE, Kolotkin RL, LaMonte MJ, Pendleton RC, et al. Health benefits of gastric bypass surgery after 6 years. JAMA. 2012;308(11):1122–31.
63. Rubino F, Nathan DM, Eckel RH, Schauer PR, Alberti KG, Zimmet PZ, et al. Metabolic surgery in the treatment algorithm for type 2 diabetes: a joint statement by international diabetes organizations. Diabetes Care. 2016;39(6):861–77.
64. Shah AS, D'Alessio D, Ford-Adams ME, Desai AP, Inge TH. Bariatric surgery: a potential treatment for type 2 diabetes in youth. Diabetes Care. 2016;39(6):934–40.
65. Inge TH, Laffel LM, Jenkins TM, Marcus MD, Leibel NI, Brandt ML, et al. Comparison of surgical and medical therapy for type 2 diabetes in severely obese adolescents. JAMA Pediatr. 2018;172(5):452–60.

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