

Pediatric Metabolic Syndrome: pathophysiology and laboratory assessment

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ABSTRACT

Pediatric overweight and obesity is an emerging public health priority as rates have rapidly increased worldwide. Obesity is often clustered with other metabolic abnormalities including hypertension, dyslipidemia, and insulin resistance, leading to increased risk of cardiovascular disease. This cluster of risk factors, termed the metabolic syndrome, has traditionally been reported in adults. However, with the increased prevalence of pediatric obesity, the metabolic syndrome is now evident in children and adolescents. This complex cluster of risk factors is the result of the pathological interplay between several organs including adipose tissue, muscle, liver, and intestine with a common antecedent – insulin resistance. The association of the metabolic syndrome with several systemic alterations that involve numerous organs and tissues adds to the complexity and challenge of diagnosing the metabolic syndrome and identifying useful clinical indicators of the disease. The complex physiology of growing and developing children and adolescents further adds to the difficulties in standardizing laboratory assessment, diagnosis, and prognosis for the diverse pediatric population. However, establishing a consensus definition is critical to identifying and managing children and adolescents at high risk of

developing the metabolic syndrome. As a result, the examination of novel metabolic syndrome biomarkers which can detect these metabolic abnormalities early with high specificity and sensitivity in the pediatric population has been of interest. Understanding this complex cluster of risk factors in the pediatric population is critical to ensure that this is not the first generation where children have a shorter life expectancy than their parents. This review will discuss the pathophysiology, consensus definitions and laboratory assessment of pediatric metabolic syndrome as well as potential novel biomarkers.



INTRODUCTION

The worldwide prevalence of pediatric overweight and obesity combined has risen by 47.1% between 1980 and 2013 (1). This alarming increase in pediatric obesity has become a global public health burden, evident by the World Health Organization (WHO) Health Assembly endorsement for the Comprehensive Implementation Plan on Maternal, Infant, and Young Child, Nutrition, which consisted of six global nutrition targets to be achieved by 2025, including “Target 4: no increase in childhood overweight” (2). Obesity is the most important risk factor for cardiovascular disease (CVD) and is often clustered with additional metabolic abnormalities including hypertension, dyslipidemia, and insulin resistance (3). These CVD risk factors tend to cluster, not only in adults, but more recently in children (4). This common cluster of major determinants of CVD led to the definition of what is known as the metabolic syndrome (MetS). The current paradigm of MetS was established by Reaven and colleagues (5) in 1988, originally termed Syndrome X. Reaven described MetS as the interrelation between insulin resistance, hypertension, type 2 diabetes (T2D), and CVD. Although this

syndrome was not defined until the late 1980s, the relationship between obesity, hypertriglyceridemia, and hypertension was first recognized in the early 1980s (6). This was followed by the description of the central roles of insulin resistance and abdominal obesity in MetS in the late 1980s to early 1990s (7). Clinical definitions of MetS have been extremely variable, however almost all definitions require a partial combination of the following five elements: elevated triglycerides (TGs), reduced high-density lipoprotein cholesterol (HDL-C), increased blood pressure, elevated fasting plasma glucose, and increased waist circumference (3). Although MetS was once thought to be an adult-onset disease, this clustering of metabolic disorders is becoming increasingly prevalent in children and adolescents, making it a public health priority in the pediatric population as well. This review will discuss what is currently known about the underlying pathophysiology of pediatric MetS, particularly in regards to the major organs involved. Additionally, the difficulty in defining pediatric MetS, current definitions and laboratory assessment to define and monitor pediatric MetS, and potential novel biomarkers will be discussed.

In the Third National Health and Nutrition Examination Survey (NHANES III), conducted between 1988 and 1994 in the US, the prevalence of MetS in adolescents aged 12-19 years was 4%, increasing to 28.7% among strictly obese adolescents (8). A more recent analysis of NHANES data from 1999-2002, demonstrates that MetS prevalence in obese adolescents has since increased to 44% (9). If current trends continue, the World Health Organization (WHO) predicts that 70 million infants and young children will be overweight or obese by 2025. The prevalence of MetS directly increases with the degree of obesity and each component of the syndrome worsens with increasing obesity, independent of age, sex, and pubertal status (3).

Childhood obesity is also an early risk factor for adult morbidity and mortality (10,11) and 85% of obese children become obese adults (10,12). It is important to detect MetS early in childhood and adolescence to prevent further health complications in adulthood and minimize the global socio-economic burden of CVD and T2D. Unless action is taken, diabetes experts agree that this is the first generation where children may have a shorter life expectancy than their parents (13).

PATHOPHYSIOLOGY: UNDERSTANDING THE COMPLEX CLUSTER

The etiology of MetS is incompletely understood; however, insulin resistance is thought to be central to the development of MetS and play a role in the pathogenesis of its individual metabolic components. The World Health Organization (WHO) hypothesizes that the association and clustering of T2D, hypertension, dyslipidemia, and CVD arises from a common antecedent - insulin resistance (14). Insulin resistance is the decreased tissue response to insulin-mediated cellular actions.

Although hyperglycemia, the primary complication of insulin resistance, can result in substantial morbidity in T2D, CVD is the leading cause of death in T2D patients, mainly due to lipid abnormalities (15). This phenomenon is well-supported by results of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, in which attempts to tightly control glucose did not lead to an improvement in mortality (16,17). Insulin elicits peripheral effects on several organ systems, including adipose tissue, muscle, liver, and intestine. Therefore, in insulin resistant states, metabolic dysfunction across several organs occurs, together creating this observed interplay of several concurrent metabolic abnormalities.

Lipid partitioning and inflammation

It is widely accepted that obesity and the concomitant development of inflammation are the major components of insulin resistance (18). In obesity, adipose tissue storage capacity becomes saturated and insulin suppression of adipose tissue lipolysis is diminished (19). As a result, plasma free fatty acid (FFA) levels increase and this excess lipid can be stored in sites other than conventional subcutaneous adipose depots, including intraabdominal (visceral) adipose compartments and insulin-responsive tissues (i.e. muscle and liver). This altered lipid partitioning can shift the balance between adipocytokines, producing more inflammatory cytokines (i.e. TNF- α and IL-6) and fewer anti-inflammatory peptides (i.e. adiponectin). In addition to inflammatory effects of obesity, the increased FFA flux results in several metabolic dysfunctions. When the subcutaneous fat depot reaches its storage capacity and lipid is shunted to ectopic tissues (i.e. liver and muscle), peripheral insulin resistance occurs (20).

Derivatives of fatty acids (e.g. long chain fatty acyl-CoA and DAG) in hepatocytes and myocytes may alter the insulin signal transduction pathway, leading to this observed decrease in insulin sensitivity. Several studies support this theory, as lipid content in liver and muscle is increased in obese and T2D subjects and is a strong predictor of insulin resistance (21). Furthermore, obese adolescents with a high visceral to subcutaneous fat ratio demonstrate a markedly adverse metabolic phenotype of severe insulin resistance and alterations in glucose and lipid metabolism (22). Taken together, obesity results in increased inflammatory markers and FFA flux, subsequently reducing the insulin sensitivity of several organs (i.e. adipose tissue, muscle, liver, intestine). Insulin resistance across several organs results in the MetS phenotype which

includes dyslipidemia, subsequently increasing CVD risk by affecting endothelial function and the vascular system (23).

Adipose tissue insulin resistance and FFA flux

Adipose tissue enlargement (i.e. obesity) leads to a proinflammatory state in the cells, with reduced secretion of adiponectin and increased secretion of several inflammatory cytokines and chemokines (24). One of these chemokines, monocyte chemoattractant protein-1 (MCP-1), plays an important role in recruiting macrophages into adipose tissue (24). Macrophages infiltrate adipose tissue and contribute to adipocyte hypertrophy and further cytokine release (24,25). These cytokines can affect insulin action in other tissues, such as liver and muscle, but can also lead to local insulin resistance. Insulin inhibits lipolysis in adipose tissue, and therefore in insulin resistance, lipolysis is accelerated, leading to increased FFA release into the circulation (3). Therefore, insulin resistance further supports the proinflammatory state of obesity because its anti-lipolytic and anti-inflammatory effects are negated.

Muscle insulin resistance and glucose intolerance

Increased plasma FFAs, due to reduced insulin suppression of adipose tissue lipolysis, disrupt insulin-mediated glucose uptake by skeletal muscle, facilitating development of hyperglycemia (26). Insulin resistance in skeletal muscle may promote atherogenic dyslipidemia by diverting ingested carbohydrate towards hepatic *de novo* lipogenesis (DNL), rather than muscle glycogen storage (23). Young, lean, insulin-sensitive subjects store most of their ingested energy in liver and muscle glycogen, while young, lean insulin-resistant subjects have dysfunctional muscle glycogen synthesis and divert more of their ingested energy into hepatic DNL (27). This results in increased plasma TGs, lower

HDL-C, and increased hepatic TG synthesis (27). Mouse studies further support these findings as muscle-specific inactivation of the insulin receptor gene results in increased plasma TGs and increased adiposity as a result of muscle-specific insulin resistance (28).

Hepatic insulin resistance and fasting dyslipidemia

The liver is a main target of insulin action and plays a major role in both carbohydrate and lipid metabolism. Two key hepatic insulin actions are reducing hepatic glucose output and inhibiting secretion of very low-density lipoproteins (VLDLs). To reduce hepatic glucose output, insulin phosphorylates FoxO1, preventing it from entering the nucleus, and consequently reducing the expression of genes required for gluconeogenesis (29). Postprandial insulin release enhances hepatic VLDL production by upregulating lipogenesis via activation of the transcription factor sterol regulatory element-binding protein (SREBP-1c) (30). SREBP-1c increases transcription of genes required for FA and TG biosynthesis, resulting in increased DNL. TGs synthesized by DNL and dietary lipids are packaged with apolipoprotein B100 (apoB100) into VLDLs. Although insulin increases substrate availability for VLDL production, it also acutely reduces VLDL secretion (31). This inhibitory action is thought to be due to an increase in apoB100 degradation, the main structural protein of VLDL (31).

Insulin has key metabolic regulatory roles in the liver, thus several metabolic abnormalities can clinically manifest with hepatic insulin resistance. Diabetic dyslipidemia is one such abnormality which is characterized by hypertriglyceridemia, increased small dense LDL (sdLDL) and decreased HDL-C (32). This phenomenon is the direct result of hepatic insulin resistance which results in impaired glucose homeostasis due to reduced FoxO1-mediated phosphorylation, and

enhanced hepatic DNL due to reduced SREBP-1 activation (33). Therefore, both hyperglycemia and hypertriglyceridemia are seen in hepatic insulin resistance. In addition to enhanced DNL, substrates for VLDL synthesis are increased due to elevated FFA flux from adipose tissue and increased hepatic uptake of chylomicron remnants (CM; lipoproteins secreted from the intestine) and VLDL remnants (34,35). Increased substrate availability for VLDL production and reduced apoB degradation can lead to VLDL overproduction and hypertriglyceridemia. As a result of hypertriglyceridemia, highly atherogenic sdLDL are also produced in insulin resistant states. sdLDL are produced from the action of cholesteryl ester transfer protein (CETP), which exchanges VLDL TG for LDL cholesteryl ester (CE), creating CE-depleted, TG-enriched, LDL particles (36). These particles become sdLDL after they are lipolyzed by lipoprotein lipase (LPL) or hepatic lipase (HL) (36). CETP action is thought to also contribute to reduced HDL-C levels in insulin-resistant subjects (36).

Hepatic steatosis, one of the main detriments to the liver in response to hepatic insulin resistance, is characterized by the accumulation of excess lipid in the liver, which can progress to inflammatory steatohepatitis, fibrosis, and even cirrhosis. This spectrum of diseases is collectively termed non-alcoholic fatty liver disease (NAFLD). Progression of NAFLD can cause liver failure, leading to the need for a liver transplant, even in adolescents (37). As the prevalence of pediatric obesity increases, NAFLD has also increased in prevalence, rapidly becoming the most common cause of pediatric liver disease (37). Furthermore, a pediatric study showed that every 1 cm increase in waist circumference is associated with a 1.97 and 2.08 fold increased risk of NAFLD in males and females, respectively (34). Although the pathological link between MetS and NAFLD is incompletely understood, the theory of the “two-hit model” is

the most widely accepted (38). The first hit is insulin resistance which promotes the accumulation of hepatocyte lipid due to increased hepatic FFAs available for TG synthesis in an insulin resistant state (36). This results from insulin failing to block adipose tissue lipolysis, resulting in increased FFA release from adipose tissue. Increased circulating FFAs leads to increased FFA uptake by hepatocytes, increased TG synthesis and impaired FFA oxidation, producing excess lipid in hepatocytes (38,39). The second hit is injury from reactive oxygen species (ROS). Lipid accumulation in hepatocytes impairs the oxidative capacity of the mitochondria and can also lead directly to further ROS production (40). Increased susceptibility of hepatocytes to oxidative stress and subsequent lipid peroxidation by ROS promotes progression to non-alcoholic steatohepatitis (NASH). This is due to chemoattractants (i.e. by-products of oxidative stress and lipid peroxidation), which lead to fibrosis and the production of inflammatory cytokines (37).

Intestinal insulin resistance and postprandial dyslipidemia

In contrast to the numerous studies on insulin signaling in well-known insulin-sensitive tissues such as liver, muscle, and adipose, relatively little is known regarding intestinal insulin signaling and potential perturbations with insulin resistance (41). The intestine packages absorbed dietary fat into apoB-48-containing TG-rich lipoproteins, called chylomicrons (CMs), which transport TGs and fat-soluble vitamins to peripheral tissues (42). Similar to its actions in the liver, insulin has a key regulatory role in the production and clearance of TRLs produced from the intestine (43). Therefore, another defining feature of diabetic dyslipidemia is elevated postprandial levels of CM particles (44). The accumulation of CM particles in insulin resistance has been attributed to decreased clearance as

well as increased intestinal synthesis and secretion (45). Decreased clearance of CM and CM remnants in insulin resistance has largely been attributed to increased hepatic VLDL secretion (46), as intestinal and hepatic TRLs share common, saturable, removal mechanisms (47). Secondly, LPL activity is decreased due to diminished regulation by insulin (48), contributing to slow removal of CM and CM remnants in insulin resistance.

Although the intestine was conventionally regarded as a passive organ with respect to CM secretion, it is now evident that CM production can be actively increased in insulin resistant states (43). Insulin has been shown to directly decrease CM secretion from cultured human fetal jejunal explants (49) and to reduce CM production in healthy men following an insulin infusion (50). Mechanisms for CM overproduction in insulin resistance are unclear, yet may include increased apoB stability, increased mass and activity of microsomal triglyceride transfer protein (MTP; required for assembly of VLDLs and CMs), and enhanced DNL in the enterocyte (41,51). The inhibitory effect of insulin on CM secretion may also partly be due to its suppression of circulating FFAs (46,50,52), an effect that is blunted by insulin resistance (52) and T2D (53). Overall, human studies suggest that intestinal CM production is dysregulated in insulin resistance states, with diminished sensitivity to insulin's inhibitory effects, contributing to increased plasma CM levels. Intestinal lipoprotein production is particularly important as postprandial TG levels independently predict CVD (54). In addition, CM remnants are risk factors for atherosclerosis (55) and apoB-48 can be detected in atherosclerotic plaques (56).

The intestine is also involved in the pathogenesis of MetS through its important role as an endocrine organ. The intestine secretes several gut peptides with glucagon-like peptide 1 (GLP-1) playing a significant role in insulin secretion

and signaling. GLP-1 is secreted by ileal enteroendocrine L-cells in response to a variety of nutrient, neural, and endocrine factors (57). This hormone has several biological actions on the pancreas, nervous system, gastrointestinal system, skeletal muscle, adipose tissue, and liver. As a result of the important roles GLP-1 plays in metabolism, agonists of GLP-1, as well as inhibitors of dipeptidyl peptidase-4 (DPP-4), the main protease in GLP-1 degradation, have been successful therapeutics for T2D (58). In the pancreas, GLP-1 stimulates glucose-dependent insulin secretion, improves the capacity of β -cells to sense and respond to glucose, increases β -cell mass, and inhibits glucagon and stimulates somatostatin secretion (57). The GLP-1 receptor (GLP-1R) and nerve fibers containing GLP-1 are located in the central nervous system and therefore several studies have examined central and peripheral actions of GLP-1. Central actions of GLP-1 include satiety promotion, reduced energy intake, and consequently decreased body weight (59). Additionally, the effects of GLP-1 on the pancreas may be mediated in part by a neural mechanism (60). In the intestine GLP-1 has inhibitory effects on lipoprotein secretion, gastric acid secretion and gastric emptying, which slows the transit of nutrients from the stomach to the small intestine, contributing to the normalization of blood glucose levels (61). The effect of GLP-1 on muscle, adipose tissue, and the liver, including stimulation of glucose uptake and inhibition of hepatic glucose production, remain controversial as to whether they are independent of changes in insulin or glucagon (57).

LABORATORY ASSESSMENT OF PEDIATRIC METABOLIC SYNDROME

An adult definition of MetS cannot simply be applied for use in the pediatric population because drastic changes in blood pressure, lipid levels, as well as body size and proportion occur

with age and development. Puberty also impacts fat distribution, insulin sensitivity, and insulin secretion (62). Children develop transient physiologic insulin resistance during puberty (63), with a 25-50% decline in insulin sensitivity which recovers upon completion of pubertal development (64). The dynamic physiological changes that occur in children and adolescents has led to the lack of standardized measures in pediatrics, including measurements of central obesity (3), which is a defining feature of adult MetS. Establishing a consensus definition of MetS in the pediatric population has therefore traditionally been a challenge. However, it is important to note that the MetS is not a disease, but a cluster of metabolic disorders. Therefore, applying any set of criteria to “define” the MetS truly reduces the complex reality of this cluster of components. Each component of the MetS is a continuous variable which gradually changes. This results in a continuum between a healthy and unhealthy metabolic profile, rather than a dichotomy of healthy and unhealthy states. However, an accepted definition of pediatric MetS is important as a diagnostic and monitoring tool to ensure standardization in clinical practice as well as in research to standardize clinical trials.

Rapid rises in obesity trends sparked the need to understand how to distinguish between children and adolescents at high risk of health complications and those with “simple” uncomplicated obesity. Traditionally, researchers have used several different definitions (65), resulting in the prevalence of metabolic syndrome varying between 0% and 60% in the same group of children, depending on the diagnostic criteria applied (66). This drove the International Diabetes Federation (IDF) to develop a universally accepted and easy to use definition for MetS in children and adolescents in 2007 (13). This definition was created with the intention to allow preventative measures to be taken before the

child or adolescent develops T2D and/or CVD (13). The main component of the definition is waist circumference because it is an independent predictor of insulin resistance, lipid levels, and blood pressure (67,68). However, percentiles, rather than single cut-off points, must be used for this measure due to the dynamic metabolic changes that occur throughout the pediatric age range. A cut-off of the 90th percentile was chosen, as children and adolescents with a waist circumference \geq 90th percentile are more likely to have multiple CVD risk factors (13).

The IDF consensus definition of MetS in children and adolescents is shown in Table 1. The definition excludes children who are younger than 6 years because of insufficient data for this age-group (13). For children aged 6-10 years, MetS should not be diagnosed, but those with abdominal obesity should be strongly advised to reduce their weight. For children age 10-<16 years, MetS should be diagnosed for those with abdominal obesity and two or more other clinical features including elevated triglycerides, decreased HDL-C, increased blood pressure, and increased fasting plasma glucose. For adolescents older than 16 years of age, it is recommended to use the IDF adult criteria. This IDF pediatric definition provides a standard that facilitated comparisons of study results, including prevalence estimates across studies.

However, the IDF definition of pediatric MetS is not without limitations. First, this definition does not provide criteria to diagnose children under the age of 10 years. Additionally, the blood pressure cut-off used in this definition is the same as that defined for adults and is thus too high for the pediatric population. This results in blood pressure contributing to a negligible proportion of children being classified as having the MetS using this definition (69). Lastly, rather than being based on evidence from the pediatric population, the IDF consensus definition is modified from a definition created for

Table 1 IDF consensus definition of the Metabolic Syndrome in children and adolescents

Age (years)	Obesity (WC)	Triglycerides	HDL-C	Blood pressure	Glucose
6-<10	≥ 90th percentile	Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, T2DM, dyslipidemia, cardiovascular disease, hypertension and/or obesity			
10-<16	≥ 90th percentile or adult cut-off if lower	≥1.7 mmol/L (≥150 mg/dL)	<1.03 mmol/L (<40 mg/dL)	Systolic ≥130/ diastolic ≥85 mm Hg	≥5.6 mmol/L (100 mg/dL) (If ≥5.6 mmol/L [or known T2DM] recommend an OGTT)
≥ 16 (adult criteria)	Central obesity (defined as waist circumference ≥ 94cm for European men and ≥ 80cm for European women)	≥1.7 mmol/L (≥150 mg/dL)	<1.03 mmol/L (<40 mg/dL) in males and <1.29 mmol/L (<50 mg/dL) in females, or specific treatment for these lipid measurements	Systolic ≥130/ diastolic ≥85 mm Hg, or treatment of previously diagnosed hypertension	Fasting plasma glucose ≥5.6 mmol/L (100 mg/dL), or previously diagnosed type 2 diabetes

*Table adapted from (13).

WC: waist circumference; HDL-C: high-density lipoprotein cholesterol; T2DM: type 2 diabetes mellitus; OGTT: oral glucose tolerance test.

the adult population. A more recent MetS definition for European pre-pubertal children was proposed by the Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants (IDEFICS) Study which addresses these limitations.

The main factor contributing to the absence of a consensus MetS definition in children is the lack of reference values for MetS components in the

pediatric population (70). Therefore, the IDEFICS study used reference values provided by their study of European children to classify children according to the different components of the MetS (69). They propose a definition with different cut-offs to classify children requiring either close monitoring (monitoring level) or an intervention (action level) (69). Using age-, sex-, and height- (in the case of blood pressure) specific

percentiles established from the IDEFICS cohort, percentile cut-offs are defined for the MetS components (shown in Table 2 for the monitoring level). Children are classified as requiring close monitoring of the MetS if three or more of these risk factors exceed the 90th percentile defined in the IDEFICS studies (69). If three or more of these risk factors exceed the 95th percentile, defined in the IDEFICS studies, an intervention is appropriate in affected children (69). They also created a simple web application (www.ideficsstudy.eu) to more easily classify an individual by entering individual measurement values and obtaining the appropriate percentiles. As a result of using percentile cut-offs established from a pediatric population rather than arbitrary cutoffs for MetS components, the IDEFICS definition provides a more equal weight to components of the definition, allowing a more equal contribution to the overall prevalence of the MetS. However, this definition is also not without limitations. In addition to only being applicable to children and not adolescents, the percentile cut-offs for each parameter is population-specific and therefore may differ for smaller, local populations. Also,

clinically relevant, prospective outcomes related to the percentile cut-offs which would allow the assessment of disease risk in relation to defining the MetS are currently lacking.

In addition to proposing definitions to classify children as requiring monitoring or intervention for the MetS, the IDEFICS study also developed a quantitative CVD risk score. This was established using a z-score standardization to calculate a continuous score combining the MetS components, with a higher score indicating a less-favorable metabolic profile. A study by Pandit et al. supports the use a quantitative risk score, as this study suggested that a continuous MetS score was a better tool to assess atherosclerotic risk in children than cut-offs of individual MetS components (71). Rather than dichotomizing the population into children with a healthy and unhealthy metabolic profile based on cut-offs of each MetS component, the score provides a variable that accounts for gradual changes in these components. The continuous score better reflects the complex concepts of the MetS, where risk predictors lie on continuous scale and have complex interactions. The

Table 2 IDEFICS definition of the Metabolic Syndrome in children –monitoring level

Age (years)	Obesity (WC)	Triglycerides	HDL-C	Blood pressure	Glucose
2-<11 years	≥ 90th percentile	≥ 90th percentile	≤ 10th percentile	Systolic ≥90th percentile or diastolic ≥ 90th percentile	HOMA-insulin resistance ≥ 90th percentile or fasting glucose ≥ 90th percentile

*Table adapted from (69).

WC: waist circumference; HDL-C: high-density lipoprotein cholesterol; HOMA: homeostatic model assessment.

continuous MetS score can be a useful tool in pediatric research and for evaluating interventions (69).

In addition to the parameters included in the consensus definitions of pediatric metabolic syndrome, the standard lipid profile aids in CVD risk assessment. A standard lipid profile includes fasting measurements of plasma or serum concentrations of total cholesterol, LDL-C, HDL-C, and triglycerides. Additional markers that have been added to the lipid profile in some clinical laboratories include non-HDL cholesterol, apolipoprotein B (apoB), apolipoprotein A1 (apoA1), and lipoprotein(a) (Lp(a)) (72). Non-HDL cholesterol, calculated as total cholesterol minus HDL-C, gives an indicator of the total cholesterol content of atherogenic lipoproteins. ApoB and apoA1 can also be used as alternatives to non-HDL and HDL cholesterol, respectively, where they indicate the particle number, rather than cholesterol content. Lastly, Lp(a) should only be determined in the same patient once as its concentration varies little over time.

POTENTIAL NOVEL BIOMARKERS IN LABORATORY ASSESSMENT OF PEDIATRIC METABOLIC SYNDROME

With the increasing public health burden of MetS, the identification and examination of novel biomarkers able to detect MetS and subsequently CVD risk early, with high specificity and sensitivity, is a clinical priority (73). Effective MetS biomarkers maximize the effectiveness of treatment in subjects who would benefit the most. The association of MetS with several systemic alterations that involve numerous organs and tissues adds to the complexity and challenge of identifying MetS biomarkers. A few categories of potential MetS biomarkers and nontraditional pre-analytical considerations that have recently been gaining interest will be discussed.

Adipocytokines

Recent literature has shifted the notion of adipose tissue as a nonfunctional energy storage site to an important secretory organ. Adipose tissue secretes low-molecular weight peptides, called adipocytokines, which have numerous functions including food intake regulation, glucose and lipid metabolism, and inflammation (74). More recently, studies have shown adipocytokines mediate obesity-associated metabolic disorders independently of other risk factors (75). One adipocytokine, adiponectin, is secreted primarily by the adipocyte and is actually decreased in plasma upon an increase in fat mass (76). Adiponectin has several functions including anti-inflammatory and anti-atherogenic effects, as well as insulin sensitization and lipid regulation (77). Pediatric studies have shown that plasma adiponectin concentration is inversely correlated with BMI, waist circumference (WC), fasting insulin concentration, and insulin resistance (78,79) and is 25% higher in healthy overweight youth compared to those with MetS (80). Additionally, a study of 5,088 adolescents showed that a decreased adiponectin concentration was associated with an increased risk of MetS, independent of age, BMI, WC, and total cholesterol (81).

Leptin, the first identified adipocytokine, is a product of the obesity gene and is known as the "satiety hormone" because it decreases food intake and increases energy expenditure. Leptin concentration has been shown to reflect body fat mass and, as a result, can be considered a reliable marker of fat mass and energy homeostasis in non-insulin resistant individuals (82). Not only do obese individuals tend to have elevated plasma leptin concentrations, but they are also leptin-resistant, negating the beneficial effects of leptin (83). Several studies have also shown this positive association between fat mass and leptin concentration in the pediatric population (84,85). Furthermore, leptin is

positively associated with insulin resistance in pre-pubertal children after adjusting for sex, age, and BMI, and for every 1 ng/dL increase in leptin levels, the odds of MetS increase by 3%, suggesting an important role for leptin as a marker of CVD risk (86).

As a result of several studies supporting the potential roles of both adiponectin and leptin as MetS biomarkers, studies to develop normative values for adiponectin were warranted. A study in 2012 established sex-specific reference intervals (2.5th and 97.5th percentiles of concentration distribution in healthy subjects) for total adiponectin in cord blood and for each one year interval from 0-14 years of age (87). Another study of 111 healthy children aged 0-10 years provided median, 25th and 75th percentile values for leptin (88). A more recent study established age- and sex-specific reference intervals for both serum adiponectin and leptin in pre-pubertal European children (ages 3-9 years) (89). Furthermore, studies have assessed the diagnostic potential of these biomarkers in the pediatric population. One study determined an adiponectin concentration of 6.65 µg/mL as a cutoff point to identify MetS with 64% and 67% sensitivity and specificity, respectively (75). Likewise, a recent study determined a leptin level of 13.4 ng/mL as a cutoff point to identify MetS with a sensitivity and specificity of 68% and 69%, respectively (86). Although further examination of these biomarkers is needed to determine their suitability in MetS detection, extensive progress has been made in the understanding of these adipocytokines in pediatric MetS.

Microalbumin

Microalbuminuria, an increased level of urine albumin, is thought to be the renal expression of vascular endothelial damage, particularly increased vascular permeability, as evidence suggests that glomerular leaking of albumin

reflects general vascular damage (90–92). Therefore, microalbuminuria denotes preclinical atherosclerosis and can be used as an early atherosclerosis indicator (90–92). Obesity is strongly associated with the two most common causes of end-stage renal disease: diabetes and hypertension (93). Additionally, the MetS is suggested to be an independent risk factor for both chronic kidney disease and end-stage renal disease (94). Initially introduced into the criteria to define the MetS by the WHO in 1988 (14), microalbuminuria screening is now recommended to be added to the assessment of the CVD risk profile in adults (92). This is the result of well-established evidence of the relation between microalbuminuria and hypertension, central adiposity, the MetS, and CVD mortality (95). More recent studies have examined the association between microalbuminuria and obesity as well as other CVD risk factors in the pediatric population (93,96,97). A study of 150 obese children by Sanad M et al. found that obese children with microalbuminuria had a significantly higher blood pressure, triglyceride levels, LDL levels, as well as a higher prevalence of MetS, insulin resistance, and impaired fasting glucose levels, than those without microalbuminuria (93). Another study by Burgert T et al. found that 10.1% of an obese, non-diabetic pediatric cohort had a urine albumin to creatinine ratio in the microalbuminuric range (i.e. 2-20 mg/mmol), which is similar to the expected prevalence in an obese adult population (96). Even slight abnormalities in glucose metabolism may promote early vascular damage in pediatric obesity (96). Microalbuminuria has been suggested as a treatment target in adults (98,99), and now may also become an approachable treatment target in pediatric metabolic syndrome, potentially responsive to treatment (i.e. lifestyle intervention or pharmacotherapy) directed at improving insulin sensitivity and glucose tolerance (96).

Gut peptides

In contrast to the extensively studied adipocytokines, gut peptides, including GLP-1 and GLP-2, are more novel potential biomarkers that are gaining interest in parallel with the recently accepted metabolic role of the intestine. In addition to its well-known incretin action, GLP-1 also promotes satiety, inhibits gastric emptying, and regulates lipid metabolism (57). Studies have shown decreased GLP-1 secretion and blunted postprandial increase in GLP-1 in morbidly obese (83) and T2D individuals (100). This may be due to the decreased responsiveness of L-cells to nutrient intake in insulin resistant conditions (101). With the important incretin effect of GLP-1, it is evident that decreased GLP-1 secretion in an obese state would have implications on insulin action. Recent pediatric studies have shown that fasting total GLP-1 is reduced, but fasting active GLP-1 is elevated in obese compared to normal weight adolescent girls (102). Overall, GLP-1 secretion and plasma concentration in obesity remains controversial and pediatric studies of this phenomenon are extremely limited. GLP-2, encoded on the same gene and co-secreted in an equimolar amount with GLP-1, enhances intestinal lipoprotein production and nutrient absorption, as well as reduces inflammation (86). Recent studies in obese adults have shown an inverse relationship between GLP-2 secretion and insulin sensitivity, although the underlying mechanisms are still unknown (103). Studies on GLP-2 are even more scarce, particularly on obese pediatric subjects. Future studies examining the potential of GLP-1 and GLP-2 as MetS biomarkers in pediatric subjects are critical to understand their potential in laboratory assessment of pediatric MetS.

Lipoproteins and apolipoproteins

Although the standard lipid profile consists of lipids and lipoproteins, with some newly added

apolipoproteins, there are additional lipoprotein subfractions recently receiving attention for CVD risk assessment. The first parameter, remnant lipoproteins (RLPs) are metabolic products of TG-rich lipoproteins (i.e. CMs and VLDLs). A study of 1,567 women from the Framingham Heart Study showed RLP-C was an independent risk factor for CVD in women, independent of TG (55). Postprandial RLP-C was shown to be an independent predictor of insulin resistance after adjusting for age, BMI, and other lipid profiles in a study of 78 adults (104). Pediatric studies have shown that RLP-C is significantly higher in obese subjects and strongly related to insulin resistance (91). Long-term prospective studies are needed to evaluate whether children and adolescents with high RLP-C are at greater risk of developing MetS. The second parameter is apoB-48 which is a specific marker of intestinal lipoproteins (i.e. CMs). As CMs are secreted in the postprandial state, apoB-48 can subsequently be used to examine postprandial lipoprotein metabolism (91). Adult studies have shown fasting apoB-48 is elevated in subjects with MetS (105) and T2D and is significantly associated with endothelial dysfunction (106). Recent studies in pediatrics determined that fasting plasma apoB-48 concentration is 2-fold higher in obese versus normal weight subjects (107). However, pediatric data on apoB-48, particularly in the postprandial state, is needed to understand the potential of apoB-48 as a MetS biomarker.

Assessment in the postprandial state

In addition to the recent exploration of novel MetS biomarkers, emerging pre-analytical conditions that may improve both the simplicity of laboratory testing and the relevance of the laboratory test results have been examined. In clinical practice, the lipid profile is traditionally measured in a fasting state even though the postprandial state predominates over a typical

24 hour day. Therefore, the lipid and lipoprotein content of a fasting sample does not accurately reflect the daily average concentration of these parameters. Additionally, evidence is lacking that a fasting sample is superior to a postprandial sample when evaluating for CVD risk assessment, and in fact, postprandial samples seem to be more advantageous (72). Some advantages include simplification of blood sampling for patients, particularly pediatrics, improving patient compliance with lipid testing, and decreasing the volume burden on laboratories in the morning. Several studies have found that postprandial lipid and lipoprotein measurements suffice for CVD risk screening, and in some cases are even better predictors (72). As MetS is a cluster of CVD risk factors, postprandial measurements may be more relevant for clinical guidelines. For example, a meta-analysis including over 300,000 individuals found that postprandial non-HDL cholesterol and calculated LDL-C were superior to fasting measurements for predicting CVD risk (108). Furthermore, the novel MetS biomarkers discussed here are more relevant following nutrient ingestion. For example, GLP-1 and GLP-2 concentrations are much more relevant in the postprandial state, as their concentrations in the fasting state are very low and their secretion is stimulated upon nutrient ingestion (95). Additionally, approximately 80% of the postprandial increase of TG is due to the increase in TG of RLPs (109) and apoB-48 is a marker of CMs (i.e. lipoproteins secreted from the intestine following a meal). Therefore, if MetS components lead to an alteration in these biomarkers, this change would be apparent in the postprandial, rather than fasting state.

CONCLUDING REMARKS

The clustering of CVD risk factors, termed the metabolic syndrome, is present in both adults and children. MetS is primarily driven by excess adipose tissue and subsequent insulin

resistance. Insulin resistance manifests in several organs, including the muscle, liver, and intestine, and as a result is associated with several systemic complications including hypertension, dyslipidemia, and impaired glucose tolerance. The interplay of metabolic dysfunction in several organ systems leads to the development of atherosclerosis and consequent CVD complications. Defining MetS in the pediatric population has been controversial due to the difficulties of generalizing both a diverse syndrome and a diverse population. However, establishing a consensus definition is critical for identification and management of youth at a higher risk of developing CVD. As a result, the examination of novel MetS biomarkers in the pediatric population has been of interest to identify pediatric subjects with obesity-related metabolic complications early before CVD complications manifest.

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