Nontraditional Risk Factors and Biomarkers for Cardiovascular Disease: Mechanistic, Research, and Clinical Considerations for Youth : A Scientific Statement From the American Heart Association

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AHA Scientific Statement

Nontraditional Risk Factors and Biomarkers for Cardiovascular Disease: Mechanistic, Research, and Clinical Considerations for Youth

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***Abstract*—**The rapid increase in the prevalence and severity of obesity in children is likely to lower the age of onset and increase the incidence of cardiovascular disease worldwide. Understanding the pathophysiology and improving the clinical management of cardiovascular disease involve a knowledge of novel risk factors and biomarkers. The clinical and mechanistic roles of these novel biological factors during childhood are currently being investigated. The goals of this scientific statement are to present the existing knowledge and theoretical framework of nontraditional risk factors for cardiovascular disease as they relate to children and adolescents, to describe the relevance and weight of available experimental and clinical evidence and the therapeutic implications pertaining to nontraditional risk factors in the pediatric population, and to stimulate further research with a goal of developing valid and reliable approaches to identify and validate novel risk factors that will aid in the clinical evaluation and perhaps prediction of cardiovascular disease in the pediatric population. Although several biomarkers are promising, substantial research is required before nontraditional risk factors can be used to identify and reduce cardiovascular disease risk in children and adolescents. **(*Circulation*. 2011;123:2749-2769.)**

Key Words: AHA Scientific Statements ■ cardiovascular diseases ■ pediatrics ■ inflammation ■ insulin resistance ■ obesity ■ risk factors

Despite remarkable advances in cardiovascular health promotion over the past several decades, cardiovascular disease (CVD) remains the leading cause of death worldwide, with obesity being a rising contributor.[1] Although atherosclerotic disease does not become clinically apparent until adulthood, epidemiological studies and autopsy data have shown that the atherosclerotic process, as evidenced by functional and morphological changes in the heart and blood vessels, begins early in childhood.[2–8]

Increased appreciation of the effects of long-term exposure to risk factors and concern about the epidemic of pediatric obesity have prompted a new sense of urgency for primordial

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and primary prevention strategies during childhood. Concurrently, there is increased investigation into novel pathophysiological processes contributing to CVD. Research in adults suggests that markers of these newly appreciated aspects of CVD have the potential to augment clinical risk stratification by aiding in the prediction, identification, and assessment of atherosclerotic disease.[9–11] However, gaps exist in our understanding of their role during childhood. Although several biomarkers are promising, it appears that none of them is ready for routine clinical use in children. A great deal more work is needed to validate these biomarkers and to improve our understanding of the role of nontraditional risk factors for CVD in children and adolescents. The purpose of this statement is to appraise pediatric research pertaining to elements of CVD beyond traditional atherosclerotic risk factors and to consider, in parallel to the debate in the adult literature, the usefulness of these newer biological factors in the assessment and reduction of CVD risk during childhood.

What Are Disease Risk Factors and What Are Biomarkers?

The concepts of risk factor and biomarker are important foundations of this review. Disease risk factors can be defined as measurable biological characteristics of an individual that precede a well-defined outcome of that disease (eg, myocardial infarction [MI]), predict that outcome, and are directly in the biological causal path. In contrast, biomarkers are biological indicators for processes that are involved in developing a disease that may or may not be causal.[10,12] The difference between a risk factor and biomarker is subtle; a biomarker can be considered a form of risk factor that is not causal. Indeed, a biomarker can qualify to be a risk factor when it is causal, but this is not a necessary characteristic for a biomarker. Risk factors are of particular importance because they help identify asymptomatic individuals who have a greater chance of developing the disease in the future compared with the general population. It has been proposed that for a biomarker to be useful for clinicians treating CVD, it should meet at least 2 criteria: (1) There must be evidence from prospective studies, either cohort or randomized trials, in a broad range of populations demonstrating independent prediction of vascular events with significant reclassification of risk, and (2) therapies that modify this biomarker need to be available that would otherwise not be used in the at-risk individual. Standardization of the measure, low variability, high reproducibility, biological plausibility, and low cost are also crucial.[9–11] Thus, when a biomarker is not predictive or causal to a disease, it cannot be considered a risk factor, but it can still shed light on the processes involved in the development of a disease, in designing therapies to ameliorate disease, and in measuring outcomes.

Traditional Risk Factors

The concept of risk factors for CVD was first introduced in an article from the Framingham Heart Study in 1961 linking the presence of specific antecedent conditions (eg, elevated cholesterol, hypertension, diabetes mellitus, tobacco use) to future CVD.[13] The major risk factors for CVD can be classified into 2 broad categories, traditional and nontraditional.[14] The traditional risk factors are included in Table 1.[15]

Table 1. Traditional Risk Factors for CVD in Children

Constitutional
Family history of atherosclerosis
Age
Sex
Behavioral/lifestyle
Nutrition/diet
Physical inactivity
Tobacco exposure
Perinatal exposures
Physiological
Blood pressure
Lipids
Obesity
Glucose metabolism and insulin resistance
Medical diagnoses*
Diabetes mellitus (types 1 and 2)
Chronic/end-stage kidney disease

CVD indicates cardiovascular disease.

*Other medical diagnoses contribute to cardiovascular disease risk in children, as described by Kavey et al.[15] The mechanisms are still being investigated but likely operate through nontraditional risk mechanisms and therefore are not included here. They include Kawasaki disease (inflammatory, postvasculitis), chronic inflammatory conditions such as inflammatory bowel disease and systemic lupus, juvenile rheumatoid arthritis, orthostatic heart transplantation, and previous treatment for cancer (radiation and chemotherapy). Recently, sleep duration and obstructive sleep apnea have also been suggested in this category of risk factors.

Although these risk factors are insufficient to identify absolute risk, they are strongly associated with the presence of atherosclerotic vascular disease and explain 75% to 90% of events.[14,16] With conventional risk prediction models such as the Framingham Risk Score, the absence of these risk factors is associated with a very low likelihood of ever getting CVD.[17] However, although traditional risk factors are validated for the diagnosis and management of CVD in many populations,[14,16–19] characterizing these attributes does not fully explain incident CVD. Furthermore, the underlying mechanisms for the association between traditional risk factors and CVD remain elusive. Research in adults suggests that biomarkers of novel pathophysiologies contributing to CVD have the potential to augment clinical risk stratification by aiding in the prediction, identification, and assessment of atherosclerotic disease.[9–11] Understanding of these processes in adults is growing; however, gaps exist in our understanding of their roles during childhood.

CVD Risk Factors, Biomarkers, and Childhood

Central to this statement is the concept of the risk factor as it relates to the pediatric population. Several pathways lead to the acceptance of a characteristic as a CVD risk factor during childhood. One strategy is to directly relate the proposed risk factor to a "hard" outcome such as MI or all-cause mortality. Few studies have made this important connection. One example is the data reported by Franks et al[7] suggesting that

Table 2. Criteria of Evaluation of Novel Biomarkers for CVD in Children

Does the biomarker provide independent information on risk or prognosis of CVD in adults?
Does the biomarker account for a clinically significant part of CVD in adults?
Do levels of the biomarker in childhood reliably relate to adult levels (tracking)?
Do biomarker levels correlate with atherosclerosis extent by autopsy studies?
Do biomarker levels correlate with preclinical vascular findings?
Is the reference limit for the biomarker available for pediatric ages?
Is the measurement of the biomarker reliable, accurate, and reproducible?
Is the dynamic range of the biomarker useful in children?
Does the measure of the biomarker provide high sensitivity and specificity in adults for CVD events? In children for preclinical disease?
Does the biomarker test predict the true positives or true negatives in adults?
Is the biomarker responsive to a treatment or intervention?
Is the biomarker test standardized and available for practical and widespread application?
Is the biomarker test convenient and cost-effective?

CVD indicates cardiovascular disease.

Adapted from Ridker et al,[9] Vasan,[10] and Hlatky et al.[11] Copyright © American Heart Association, Inc.

although obesity, glucose intolerance, and hypertension are associated with increased rates of non–injury-related premature death, childhood hypercholesterolemia was not a major predictor for premature death. A second pathway, frequently followed, is to associate a risk factor with autopsy evidence of atherosclerosis or with surrogate markers of CVD such as carotid intima-media thickness. Support for such a relationship is even stronger if treatment of that risk factor improves the surrogate outcome or retards its progression. Lipid levels in patients with familial hypercholesterolemia and carotid intima-media thickness would fit this category. A third route to acceptance for the relationship between a risk factor and CVD is to demonstrate tracking from childhood to adulthood of a risk factor that is clearly related to hard adverse outcomes in adulthood. General principles have been suggested for evaluating whether a particular biomarker might qualify as a risk factor.[10,12] In Table 2, we suggest criteria defining a good biomarker relevant to exposures during childhood.

To better understand the role of novel processes contributing to CVD in childhood, this statement begins by discussing adipocyte dysfunction, which produces endoplasmic reticulum (ER) stress, oxidative stress, inflammatory signals, and prothrombotic factors (Table 3). We review adipokines, which orchestrate organism-wide communication of these processes, intercellular communications, and organ crosstalk. For all topics, available data on the regulation of these processes and their role in the pediatric age range are discussed. Along the way, we attempt to highlight gaps in our understanding of these processes as they relate to children, and we end by exploring new frontiers and establishing clinical relevance.

Adipocyte Dysfunction

The metabolic and pathophysiological consequences of excess adiposity appear central to the pathway to CVD. Energy imbalance causes excessive circulating glucose and triglycerides, leading to adipocyte hypertrophy and hyperplasia and to a variety of resultant stresses and inflammatory processes within adipose tissue (Figure 1). If hypertrophy and hyperplasia are insufficient to absorb excess circulating nutrients, the capacity of the adipocyte to store triglycerides and glucose is overwhelmed, leading to adipocyte dysfunction.[140,141] Adipocyte dysfunction is typified by local inflammation, characterized by infiltration of inflammatory cells, and elevated proinflammatory cytokines that activate additional inflammatory pathways.[142–144] This alteration in the composition of secreted products from adipocytes contributes to both local and systemic insulin resistance in part through activation of the action of c-Jun terminal kinase on insulin receptors.[145–148] The lipotoxicity of excess free fatty acids that can no longer be accommodated is manifest at the cellular level in part as ER dysfunction. Several critical functions go awry in this state of ER stress, including lipogenesis, the creation of lipid droplets from triglycerides and lipid metabolism, the regulation of circulating cholesterol, and key lipoproteins.[149] In a state of stress, the ER produces more protein than usual, yet the mechanisms that regulate the quality of these proteins (such as chaperone proteins) are unable to keep up with the increase in production. Large numbers of poorly functioning proteins literally clog the cell, impairing normal functionality. Compensatory mechanisms exist such as the unfolded protein response, in which protein synthesis is reduced and the dysfunctional proteins are cleared. However, the unfolded protein response can itself be overwhelmed by persistent or extreme nutrient excess, and the cell may ultimately shut down.[150] This inability of the cell to process excess nutrients is hypothesized to lead to persistent elevations of circulating triglycerides at the level of the organism, a putative clinical manifestation of adipocyte dysfunction.[151] Adipocyte dysfunction is described in greater detail by de Ferranti and Mozaffarian,[147] and a detailed representation of adipocyte dysfunction is shown in Figure 2.

Adipocyte dysfunction is described primarily by in vitro and animal models; few human studies are reported, and no work directly examines adipocyte dysfunction in children. In 1 study of fat samples from overweight adults, researchers found modest correlations between levels of chaperone proteins, so important to ER functioning, and body mass index (BMI).[152] Some studies propose connections between perinatal exposures and adipocyte dysfunction. The suggestion is that either excessive or inadequate nutrient supply at critical points during fetal development may increase the propensity for fat cells to store lipids and/or lead to adipogenesis, which in turn affects risk for later obesity.[151]

Summary: Adipocyte dysfunction is an important physiological process underpinning substantial CVD processes. However, adipocyte function cannot currently be easily measured and is not associated closely with CVD outcomes, and our understanding of adipocyte function during childhood is minimal.

Table 3. Novel Biomarkers Related to Inflammation, Oxidative Stress, and Insulin Resistance in Children

		Action		
Biomarker	Biological Process	Dysfunctional	Protective	Reference*
Adiponectin	↓ Insulin resistance			20–45
	↓ Oxidative stress		√√√√√√	
	↓ Inflammation			
CRP	↑ Inflammation	√√√√√√		46–73
F2-isoprostanes	↑ Oxidative stress	√√		26,74,75
Fibrinogen	↑ Thrombosis			76–99
	↑ Inflammation	√√√√		
Ghrelin	↑ Satiety		√√	21,100–103
IL-6/TNF-α	↑ Inflammation	√√√√		52,53,68,104–110
Leptin	↑ Satiety	√√√√√ (indirect)	√ √ √ (direct)	20,21,111–117
	↑ Inflammation			
PAI-1 and tPA	↑ Thrombosis	√√√		82,84–91,94,114,118–123
D-dimer	↑ Thrombosis	√√√		52,82,86,90,91,120
	↓ Oxidative stress			
PPARs	↓ Inflammation	√ √	√	124–126
	↓ Insulin resistance			
RBP4	↑ Insulin resistance	√√√		127–134
Resistin	↑ Insulin resistance	√√		104,116,134–138
VCAM and ICAM	↑ Oxidative stress			107,108,139
	↑ Inflammation	√√		

CRP indicates C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; PAI-1, plasminogen activator inhibitor-1; tPA, tissue-type plasminogen activator; PPAR, peroxisome proliferator–activated receptor; RBP4, retinol binding protein 4; VCAM, vascular cell adhesion molecule; and ICAM, intercellular adhesion molecule.

The number of checkmarks indicates how extensively these markers have been studied in children.

*Because of space limitations, we are unable to include all references for a specific biomarker in this article. Only a selection of relevant references has been included.

Adipokines

One role of adipose tissue is as a secretory organ, producing a range of bioactive proteins collectively called adipokines (Figures 1 and 3, and Table 3). Dysregulated production of adipokines participates in the pathogenesis of obesity-associated comorbidities, including abnormal lipid and glucose metabolism, altered satiety, increased inflammation, disordered hemostasis and angiogenesis, elevated blood pressure, and cardiovascular function. These adipokines communicate both within adipose tissue and between adipose and other organ systems, and may serve as the common intercellular denominator mediating the development of CVD in the obese state. Here, we discuss some of the more functionally prominent adipokines and their role in modulating insulin resistance, inflammation, and oxidative stress.

Leptin

Leptin is a satiety factor produced predominantly in adipocytes[153] that is instrumental in appetite regulation and metabolism at the level of the hypothalamus via melanocortin receptors.[111,112] In animal models leptin levels are higher with higher fat mass, theoretically acting as a regulator to decrease appetite in the presence of excess body weight and to increase energy expenditure. In humans there are reports of individuals congenitally deficient in leptin who present with insatiable appetite and extremely early onset of obesity; the administration of subcutaneous recombinant leptin restores normal appetite regulation and reduces fat mass in affected children.[113] Paradoxically, most obese humans have high circulating levels of leptin as a result of what has been characterized as leptin resistance, a lack of appropriate diminishing of appetite or decrease in fat mass despite relatively high leptin levels. This phenomenon of leptin resistance may already be present in obese children.[20,21,114–117,154] Children with higher leptin and lower adiponectin (discussed below) have greater CVD risk factors, regardless of weight status,[155] although not all studies support these findings.[156]

Summary: Leptin has systemic effects on appetite and metabolism and is one of the most studied adipokines. Assessment for leptin deficiency is performed only in severe infantile-onset obesity with a familial distribution, and clinical trials of subcutaneous administration of leptin have not resulted in an effective treatment for obesity. However, its relationship with inflammatory factors is intriguing, and a better understanding of the relationship between leptin and CVD risk factors in children is needed.

Adiponectin

Adiponectin is an adipose-specific hormone that has anti-inflammatory and insulin-sensitizing properties and, in contrast to other adipokines, is protective against obesity

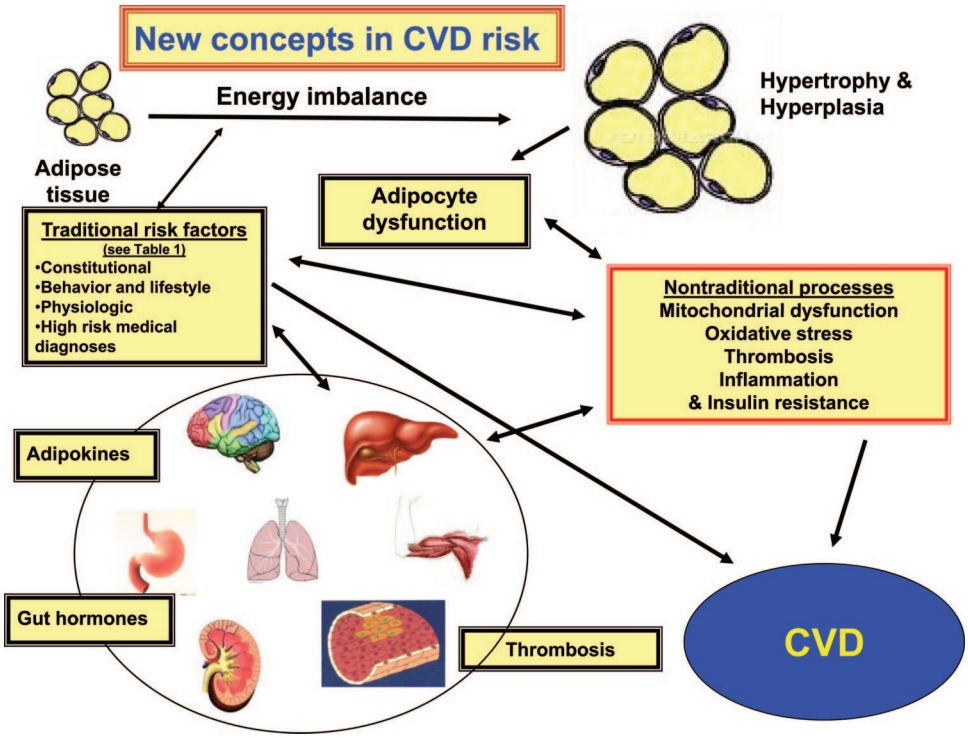

Figure 1. Overall conceptual framework of new concepts in the pathophysiology of cardiovascular disease (CVD). This schematic includes major processes involved in CVD that develop early in the course of obesity and adipocyte dysfunction. Energy imbalance in an obesogenic environment leads to hyperplasia and hypertrophy of the adipose tissue, resulting in adipocyte dysfunction. This is manifest as oxidative stress and mitochondrial dysfunction, the altered secretion of adipokines, inflammation, and insulin resistance at the cellular level. All of these processes act on diverse organs in the body to contribute to the development of CVD events. The nontraditional risk factors/biomarkers related to inflammation, oxidative stress, and insulin resistance may also interact with traditional risk factors, leading to CVD. See Table 1 for details on traditional risk factors and Table 3 for selected nontraditional risk factors/biomarkers relevant to the development of CVD in childhood. Although not noted explicitly here, these processes are clearly influenced by their environment and genetic milieu.

and obesity-related disorders.[157–159] It circulates in the blood in 3 different molecular weights (low, medium, and high), with the high-molecular-weight form appearing to be more pathogenic.[160] Although putative adiponectin receptors are widespread in peripheral organs, including muscle (receptor 1) and brain, it is uncertain whether adiponectin acts exclusively through these targets. Its secretion from adipocytes is regulated by peroxisomal proliferator-activated receptor (PPAR)-γ.[160] Thiazolidinediones, agents used to treat type 2 diabetes mellitus (T2DM) by improving insulin sensitivity, act in part by increasing levels of adiponectin.[22] In adult cross-sectional studies, adiponectin has been found to have independent negative associations with obesity, hyperinsulinemia and insulin resistance, metabolic syndrome, visceral adiposity, T2DM, and coronary artery disease.[5,23–26] Adult weight loss through dieting or bariatric surgery is also associated with increased adiponectin.[27]

Similar to adult data, observational pediatric studies in children have shown an inverse correlation between plasma adiponectin concentrations, adiposity, and insulin resistance[21,28–31]; hepatic fat by magnetic resonance imaging[32]; and carotid intima-media thickness.[33] Longitudinal decreases in adiponectin are seen with increasing adiposity,[21,29–31] and adiponectin levels predict T2DM in obese children.[30] Some adolescent studies demonstrate an inverse relationship between adiponectin and C-reactive protein (CRP), even after adjustment for pubertal status, insulin resistance by oral glucose tolerance test, and BMI.[35,36] This suggests a potential regulatory interaction between CRP and adiponectin that is independent of insulin sensitivity. Supporting this, adiponectin knockout mouse models have higher CRP expression; this relationship holds true for adult humans.[37] Several studies, although small, have corroborated not only the role of adiponectin in modulating insulin resistance in children[33,36,38–41] but also its linear relationship with other risk factors for CVD. A few studies have also suggested that high molecular weight rather than total adiponectin better reflects metabolic abnormalities associated with childhood obesity.[42–45] Most pediatric data on adipokines are observational. However, intervention studies have shown increases in adiponectin levels with or without weight reduction and increasing physical fitness.[31,36,38,161–164] The increase in adiponectin levels was mostly related to improvements in insulin resistance and other inflammatory factors in children.

Summary: In children, higher adiponectin correlates with leanness and lower CRP, lower carotid intima-media thickness, and insulin sensitivity (particularly high-molecular-weight adiponectin). It is promising that adiponectin is favorably modifiable by simple lifestyle changes, and using the levels of total adiponectin or high-molecular-weight adiponectin

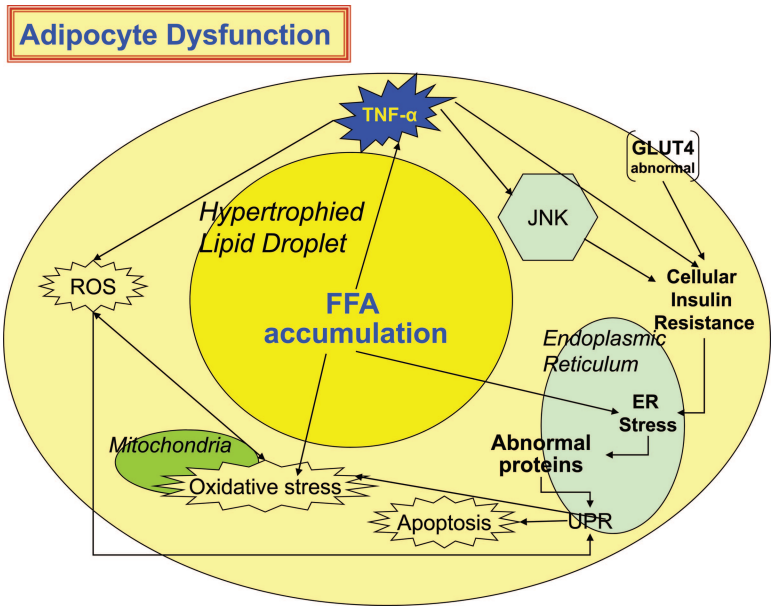

Figure 2. This figure outlines some aspects of adipocyte dysfunction due to nutrient excess. Excess lipid accumulation leads to increased endoplasmic reticulum (ER) activity, which ultimately can overwhelm the capacity of the ER to properly fold nascent proteins. The unfolded protein response (UPR) attempts to compensate for this. However, if the process proceeds unchecked, apoptosis may result. Endoplasmic reticulum stress can lead to oxidative stress in the mitochondrion, as does the presence of excess free fatty acids (FFAs). Oxidative stress produces reactive oxygen species (ROS). Tumor necrosis factor (TNF)-α production is stimulated by FFAs, which in turn acts on c-Jun N-terminal kinases (JNK) to contribute to cellular insulin resistance. Reprinted from de Ferranti and Mozaffarian[147] with permission of the publisher. Copyright © 2008, American Association for Clinical Chemistry, Inc.

as a biomarker for insulin sensitivity and/or as a risk factor for CVD is gaining support. However, the additive value of adiponectin levels remains unclear, and it is not currently measured clinically in children or adults.

Retinol Binding Protein 4

Retinol binding protein 4 (RBP4) is gaining recognition as an adipokine that may play an important role in obesity and insulin resistance.[165,166] Originally characterized as a transport protein for retinol to the tissues,[100,167] RBP4 is now recognized as a mediator of insulin resistance and T2DM because of its relationship with glucose transporter 4.[168,169] Circulating RBP4 concentrations are elevated in mouse models of obesity and insulin resistance, and RBP4 gene deletion in mice increases insulin sensitivity.[168] Humans with RBP4 mutations seem to have a predisposition to T2DM.[170] Although RBP4 and insulin resistance are related, there seems to be no agreement about causality.[127–131,167–169,171–175]

There are only a handful of studies on RBP4 in the pediatric population, mostly cross-sectional[128,132–134,176] or involving short-term interventions.[132] As in adults, several researchers reported increased levels of RBP4 in obese compared with lean children that are independent of sex and age.[128,131–134,176–178] Changes in RBP4 in children over 3 years of follow-up showed that increases were associated with worsening insulin resistance, independent of BMI.[178] Goodman et al[179] provided longitudinal data on the role of RBP4 in modulating insulin resistance and suggested that the change in insulin resistance in non-Hispanic black adoles-

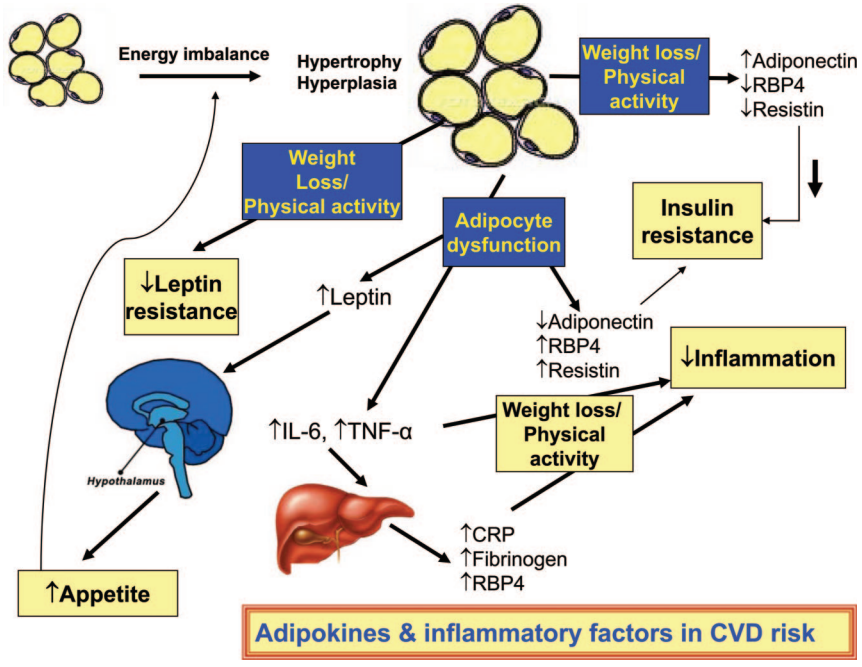

Figure 3. Adipokines and inflammatory factors in cardiovascular disease (CVD) risk. Alterations in selected nontraditional risk factors/biomarkers and their response to lifestyle-based interventions are described here. The potential impacts of these changes on distant organs are also shown. RBP4 indicates retinol binding protein 4; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; and CRP, C-reactive protein.

cents was related to RBP4 and was dependent on the initial RBP4 level. Alterations in RBP4 may also be associated with lipid metabolism,[180] inflammation,[132,181] hepatic function,[177,182] and renal function.[183] These relationships between RBP4 and other traditional and nontraditional risk factors for CVD, such as inflammatory factors[132,133,181] and/or oxidative stress, are yet to be confirmed in larger populations, and causality has not been established. There are few pediatric intervention studies on RBP4. Short-term physical activity–based lifestyle intervention showed a reduction in RBP4 in adolescents without weight loss,[132] and a similar year-long trial inducing substantial weight loss produced sustained reductions in RBP4.[178] The reduction in RBP4 levels was related to concomitant reductions in insulin resistance.

Summary: In children and adults, RBP4 is related to important CVD risk factors (insulin resistance and obesity); however, it is unclear whether RBP4 is an important bystander or central to a causal pathway. At present, determination of RBP4 levels is limited mainly to research settings, but the stability of the plasma levels enhances its potential to become useful as a biomarker for insulin resistance and a predictor of diabetes mellitus and possibly CVD.

Resistin

Resistin is an adipokine that belongs to a unique family of cysteine-rich C-terminal domain proteins called resistin-like molecules that are expressed highly in visceral compared with subcutaneous adipose tissue. In mice, resistin antagonizes insulin action, causing glucose intolerance,[135] and resistin-deficient animals are protected from obesity, whereas elevated resistin is associated with insulin resistance.[136] However, extrapolation from animal studies to humans should be done cautiously. There are various cross-sectional studies, both for and against a role for resistin in insulin resistance. The cross-sectional Study of Inherited Risk of Coronary Atherosclerosis found that resistin levels were positively correlated with higher coronary calcium scores and correlated with higher levels of soluble tumor necrosis factor-α (TNF-α) receptor-2, lipoprotein-associated phospholipase A2, and interleukin (IL)-6.[136] Longitudinal studies are mixed; some suggest a correlation between resistin levels and changes in fat mass and/or weight loss,[137,163] but others do not.[138]

Resistin is minimally described in the pediatric age range. Yoshinaga et al[155] measured resistin in 6 to 12 year olds and found no independent association between resistin and metabolic syndrome. Li et al[104] showed a weak correlation with central obesity, adiposity, and inflammation but not with insulin resistance. Examined longitudinally, resistin levels in obese children remained unaltered after weight loss over a period of 1 year.[184] A few studies have shown beneficial effects of physical activity/exercise on resistin levels in overweight/obese adolescents that were independent of weight status,[185,186] whereas others have shown that exercise training without weight reduction does not improve resistin in overweight children.[187]

Summary: Data on resistin in the pediatric population are limited and conflicting. Whether resistin is a causative factor in CVD remains to be established, and at present, there is no clinical role for measuring this adipokine.

Additional Adipokines

Numerous other products are secreted by adipocytes; of particular importance in the context of CVD are inflammatory and thrombotic elements such as IL-6, TNF-α, and plasminogen activator inhibitor-1 (PAI-1). These are discussed in more detail in the Inflammation and the Hemostasis and Thrombosis sections. Other discoveries include visfatin, touted to play an important role in regulation of glycemic homoeostasis, and apelin, the function of which appears to be related to regulation of nutritional intake. The role of these and other adipokines in CVD and T2DM remains unclear, and further discussion is beyond the scope of this statement.

Mitochondrial Dysfunction and Oxidative Stress

The same nutrient excess that produces adipocyte dysfunction and ER stress can also lead to mitochondrial dysfunction and oxidative stress. Mitochondria are the major site of intracellular respiration and energy metabolism (ATP); they are necessary for regular cell functions and regulate cell growth and death. Excess circulating nutrients prompt the mitochondria to produce ATP at rapid rates via uncoupling of oxidative phosphorylation, leading to higher-than-normal production of reactive oxygen species, and reactive oxygen species are believed to be an important cause of mitochondrial DNA damage, which is intimately related to obesity-related insulin resistance.[188,189] Oxidative stress occurs when the production of reactive oxygen species and other elements can no longer be controlled by compensatory responses from the endogenous antioxidant network. The system becomes overwhelmed (redox imbalance), leading to the activation of multiple stress-sensitive signaling pathways (nuclear factor-κB, p38 mitogen-activated protein kinase, c-Jun terminal kinase/stress-activated protein kinase, protein kinase C, advanced glycation end products/receptor for advanced glycation end products, sorbitol, and others).[190,191] In vitro studies have demonstrated that exposure of adipocytes to oxidants reduces the effect of insulin on glucose transporters; this supports the hypothesis that oxidative stress induces insulin resistance at the cellular level.[192] Furthermore, in a circular effect, glucose excess resulting from insulin resistance induces oxidative stress within the cell. The combination of these forces may be a pathway by which hyperglycemia is pathophysiological.[191] Oxidative stress also seems to be important in cholesterol metabolism; oxidized low-density lipoprotein is toxic to vasculature, and in vitro studies demonstrate that high-density lipoprotein acts as an antioxidant.[193] In vitro work suggests that plaque rupture and thrombosis, both late events in atherosclerosis, are mediated by oxidative stress.[194] Increased oxidative stress underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular wall cells. The sum of this in vitro work supports the idea that oxidative stress is involved in multiple processes relevant to CVD.

Human studies of oxidative stress are limited. Correlation between antioxidant levels (vitamin C, vitamin A, lycopene) and traditional CV risk factors has been reported in the National Health and Nutrition Examination Survey data sets; however, this survey did not measure oxidative stress itself,

just nutritional intake of antioxidants, making it difficult to draw solid conclusions about that relationship.[74] Smaller studies have examined oxidative stress directly but used diverse methodologies of measurement. Adults with metabolic syndrome had nearly 4 times the level of plasma 8-isoprostanes compared with those with normal lipids and normal weight.[75] Higher plasma malondialdehyde levels were reported in Koreans with insulin resistance syndrome compared with controls, and associations were found between plasma malondialdehyde, triglyceride levels, and waist-to-hip ratio, although the correlation coefficients were weak.[195] Two relatively small studies describe the relationship between oxidative stress and CVD event rates. A case-control study demonstrated that malondialdehyde levels were higher in adults with T2DM who had an MI compared with those without MI, and compared with normal-weight controls. An interventional trial of statins in adults with T2DM suggested that statins reduced malondialdehyde levels.[196] Quantification of both reduced and oxidized concentrations of glutathione, cysteine, and methionine, the first line of defense of the cell against any rise in oxidants, as well as even more readily oxidized thiol amino acids like homocysteine and cysteinylglycine, permits comprehensive metabolic profiling and may offer more sensitive information on host responses to oxidative stress and inflammation.[197] These techniques, although promising, remain reserved for research protocols.

Little is known about oxidative stress in the pediatric age range; however, there are some observational data in children with traditional CVD risk factors. One cross-sectional study found oxidative stress to be correlated with adiposity and insulin sensitivity by euglycemic clamp, with the combination of high BMI and insulin resistance being associated with the highest F2α-isoprostane levels compared with lower BMI and less insulin resistance.[28] Another observational study of overweight children demonstrated higher measures of oxidative stress in hypertension versus normal blood pressure.[198] A third study of normal-weight, overweight, and children with metabolic abnormalities who were also overweight noted higher isoprostane levels in children with metabolic abnormalities compared with the other 2 groups, describing a dose-effect.[199]

Summary: Measures of oxidative stress are not shown to predict future CVD events in observational studies, and no interventional trials altering oxidative stress have affected CVD event rates. One barrier is the lack of an agreed-on indicator for oxidative stress; each assay represents a different aspect of the oxidative stress process. Oxidative stress is not measured clinically in adults or children, and much remains to be done before we can truly understand how oxidative stress during childhood relates to CVD. Identifying a suitable and sensitive measurement marker(s) is the key.

Inflammation

We have reviewed adipocyte dysfunction and its consequences, adipokines, ER stress, and oxidative stress as they relate to CVD. Oxidative stress and ER stress lead to the release of inflammatory cytokines, which have wide-ranging effects throughout the body, as described next. Multiple lines of evidence now point to inflammation as central to all stages of atherosclerosis, including plaque development, disruption, and thrombosis.[12,144,200] Much of the research has focused on CRP. CRP is a downstream marker of inflammation that has multiple effects, including complement binding, augmentation of expression of adhesions molecules, and decreased expression of the vasodilator endothelial nitric oxide synthase.[201] Additionally, CRP may stimulate the expression of the thrombotic factor PAI-1 and may induce oxidative stress[200,201] and the secretion of other cytokines. Adipocytes and macrophages release IL-6 and TNF-α, which stimulate the liver to produce CRP, demonstrating a direct link between adipocytes and CRP (Figure 3).

Several prospective adult epidemiological studies show that CRP levels are independent predictors of CVD events in the acute post-MI period, in near-term recurrent disease and in primary prevention populations.[202] There is a strong association between CRP and excess adiposity in humans,[203] suggesting that some of the association between CRP and cardiovascular events is related to adiposity, although other traditional CVD risk factors (smoking, insulin resistance) correlate independently with CRP levels.[202] Weight loss by lifestyle change or bariatric surgery[204] and nutritional modifications (such as a Mediterranean diet) produce a decrease in CRP.[46] Although a body of evidence supports the independence of CRP as a risk factor in adults, the precise mechanism by which CRP is related to CVD continues to be debated. Some evidence suggests that CRP may play a causative role in atherosclerotic risk (eg, inflammation seems to precede weight gain).[47] However, gene polymorphisms producing clinically relevant differences in CRP levels have not translated into differences in CVD event rates.[48] The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER), aimed at lowering CRP, demonstrated a decrease in CRP by 37%, with rosuvastatin lowering CVD event rates by $\approx$50% independently of a reduction in low-density lipoprotein levels.[49]

Multiple pediatric studies have shown that elevated CRP levels correlate with CVD risk factors, including adiposity and blood pressure, supporting the importance of inflammation in the early phases of atherosclerosis.[50–58] CRP levels correlate with obesity even before the onset of puberty, as young as 3 years of age,[59] with and without other comorbidities of the metabolic syndrome.[60,61] Higher CRP values are seen in children with metabolic syndrome in the National Health and Nutrition Examination Survey data set.[62,63] Central obesity by waist circumference seems to be the driving force behind this association.[54,62] Not only is CRP correlated with CVD metabolic risk factors, but some observational studies have shown that elevated CRP levels are associated with noninvasive vascular measures, including increased carotid intima-media thickness and decreased brachial artery flow-mediated dilatation,[64] although results across studies are not consistent.[65] Further support for the role of CRP in the early phases of preclinical atherosclerosis comes from the Pathobiological Determinants of Atherosclerosis in Youth Study, which showed an independent association between serum CRP level and raised lesions in the abdominal aorta and right coronary artery that was independent of traditional coronary heart disease risk factors.[66] Interestingly, studies

have also shown that childhood CRP values predict adult CRP.[67] Lifestyle factors seem to be important correlates of CRP in cross-sectional pediatric studies. The relationship between dietary factors and CRP is not clear, but few studies have suggested that children and adolescents with higher CRP levels had significantly lower intakes of grains and vegetables.[68] CRP levels were also weakly inversely correlated with fruit, folate, and vitamin C intake, as measured by a food frequency questionnaire, even after adjusting for BMI, total energy intake, and other standard parameters.[69] Another cross-sectional study of Swiss children showed that although dietary fat correlated with CRP levels independently of BMI, intake of dietary antioxidants did not correlate with serum levels of TNF-α, IL-6, or CRP.[70] A relationship between CRP and reduced fitness in nonobese children is also reported,[71] although physical fitness and exercise have been less well studied. Several pediatric intervention studies have shown declines in CRP with or without weight loss.[56,72,73,204–206] In the presence of weight loss, modifying saturated, monounsaturated, and polyunsaturated fat intakes significantly lowered CRP concentrations in children.[207]

Summary: Inflammation is most frequently assessed with CRP because it is easily measured with relatively stable levels during the day and from day to day. There are recommendations for its use in clinical practice in adults; the Reynold score is a proposed risk prediction algorithm used by some practitioners that incorporates CRP.[208] Although numerous studies suggest that CRP is elevated in children with higher CVD risk, correlates with the progression of atherosclerotic changes, and tracks, albeit weakly, over 21 years from childhood to adulthood independently of other metabolic and conventional cardiovascular risk factors,[64] it is not yet clear whether high CRP levels during childhood and adolescence lead to an increased risk of CVD in later life. Lifestyle interventions have been shown to decrease CRP in children, and statins reduce CRP in adults. However, minimal information is available on the effect of statins on CRP in children and youth and, importantly whether lowering CRP in children per se would modify preclinical disease or CVD outcomes. There currently is no clinical role for measuring CRP routinely in children when assessing or considering therapy for CVD risk factors.

Inflammation Beyond CRP

Elements of the inflammatory process beyond CRP are likely important in CVD but are less well characterized. Major categories include (but certainly are not limited to) cytokines such as IL-6 and TNF-α, adhesion-type molecules such as the selectins and intercellular adhesion molecule (ICAM) families and immune cells. Macrophages, monocytes, and possibly adipocytes release IL-6 and TNF-α.[142,209,210] Tumor necrosis factor-α and IL-6 have important roles in insulin resistance, acting directly at the insulin receptor.[210,212] Both TNF-α and IL-6 are positively related to adiposity, particularly visceral fat, and correlate with insulin resistance and other CVD risk factors.[210,212–215] They also indirectly mediate lipolysis and augment hepatic synthesis of fatty acids, thereby increasing serum levels of fatty acids and triglycerides.[106]

Early in atherosclerotic plaque formation, leukocytes adhere to and are entrapped in the endothelial wall; this is a process mediated by inflammatory adhesion molecules such as P-selectin and ICAM-1. P-selectin levels have been shown to be higher in adults undergoing cardiac catheterization with positive angiography compared with those with negative studies.[216] Participants in the Woman's Health Study who developed CVD events had higher mean P-selectin levels at baseline compared with control subjects.[217] An adhesion molecule, ICAM-1 is involved in the binding of leukocytes to the endothelium; its expression is induced by TNF-α.[209] Levels of ICAM-1 have been associated with the risk of MI in men in the Physician's Health Study.[107,218] Adults with positive coronary angiography had higher ICAM-1 levels compared with those with negative studies independently of other CVD risk factors.[216] Other factors such as white blood cells and serum amyloid A have also emerged as additional downstream markers of inflammation. However, the data are not conclusive[58,108,139,219] and to date have not supplied additional predictive power above that of CRP, IL-6, and TNF-α.

There are few pediatric studies of inflammatory markers other than CRP. Lee et al[220] found higher levels of ICAM-1, E-selectin, and IL-6 in adolescents with metabolic syndrome compared with those without; however, this relationship disappeared after adjustment for abdominal obesity. Other studies also show that adiposity is an important contributor to inflammation; obese children had higher ICAM-1 levels compared with matched nonobese children.[109] Another study demonstrated increased levels of ICAM-1 and CRP in obese children with hypertension compared with obese control subjects.[110] Interleukin-6 levels were also measured in obese children after 9 months of follow-up; those with a decreased BMI z score had decreases in IL-6 and CRP over the course of the 9 months. Correlations between CRP, fibrinogen, and IL-6 have been reported in adolescents.[52]

Studies on the effect of therapies on inflammatory factors other than CRP are limited, except for a few studies on IL-6 and TNF-α. Although the concentration of cytokines such as IL-6 increased acutely in response to exercise,[221,222] improvement in body composition parameters (with or without weight reduction) induced by a restriction of energy intake and an increase in physical activity produced reductions in IL-6 and TNF-α levels in children and adolescents.[56,57,107,187,205,223,224] Age may be an important modulator of inflammatory marker levels. Levels of IL-6, TNF-α,[209] ICAM-1,[220] and E-selectin[76] seem to be higher in healthy children than in adults. Although data are still limited, levels of these inflammatory markers seem to decrease with increasing age, in contrast to CRP, which rises with age. Sex-related differences were also found in postpubertal children for E-selectin and vascular cell adhesion molecule-1, which were higher in obese male than in obese female children.[225] Thus, caution needs to be taken in the interpretation inflammatory marker levels in childhood; this is an important topic for further study.

Summary: Further research verifying these findings and better evaluating non-CRP inflammatory processes as they relate to CVD and CVD risk factors in childhood will be valuable.

Hemostasis and Thrombosis

Increasing evidence supports inextricable connections between CVD, inflammation, and thrombosis.[77,78,226–230] These disturbances include hypercoagulability, platelet aggregation, and hypofibrinolysis (increased PAI-1 levels). Fibrinogen, besides being the precursor for fibrin,[79] is an acute-phase reactant, a key player in the coagulation cascade, promotes atherogenesis and thrombogenesis, and is an independent risk factor for CVD.[80–84227,231–233] Fibrinolysis is mediated by plasmin, which circulates in blood as its proenzyme, plasminogen, a process mediated in part by tissue-type plasminogen activator (tPA). Activation of the fibrinolytic system results in the degradation of fibrin clots and is influenced mainly by these 2 factors, tPA and PAI-1.[229] Increased concentration of circulating PAI-1 leads to hypofibrinolysis, a state in which removal of thrombi from the vascular system is impaired. Tissue-type plasminogen activator antigen circulates in blood largely bound to PAI-1. As PAI-1 levels increase, a higher proportion of tPA is complexed with PAI-1. The enzymatically inactive PAI-1/tPA complexes have a delayed clearance compared with that of active, free tPA. Therefore, the total plasma tPA concentration increases, along with that of PAI-1,[234] because it is widely held that tPA antigen concentrations are paradoxically inversely related to fibrinolytic system activity.[234,235] D-dimer is another powerful modulator of the coagulation system; it is the primary breakdown product formed when plasmin acts on cross-linked fibrin and is considered a hemostasis marker of ongoing fibrin formation and degradation.[194,208–215,230,236–244]

Epidemiological studies in adults have shown that PAI-1 is associated with excess cardiovascular risk and T2DM[77,229] and reduced blood fibrinolytic activity. Elevated plasma PAI-1 activity, PAI-1 antigen, and tPA antigen are associated with an increased risk of MI.[244] The increased plasma PAI-1 antigen concentrations reported in the obese subjects are accompanied by increased plasma tPA antigen. A lowered fibrinolytic state and increased plasma PAI-1 activity have both been shown to be independently associated with increased risk of future coronary heart disease events or recurrent events, respectively.[85,86,200] Furthermore, studies suggest that PAI-1 deficiency may be protective against the development of obesity in genetic and dietary models.[87]

Studies in children and adolescents have demonstrated a close relationship between fibrinogen, obesity, and physical fitness[88–90,118,119,231,233]; this in turn is implicated in cardiovascular morbidity and mortality later in life.[80,91] The underlying pathophysiological mechanisms for these alterations, however, remain elusive. Studies in children have reported that the increased plasma fibrinogen is due primarily to an increase in its fractional synthesis rate,[119,120] a phenomenon that does not occur in the elderly.[245] The role of fibrinogen fractional synthesis rate remains to be fully understood. A few studies have also shown elevated levels of D-dimer in obese versus lean children,[92,120,246,] and high levels of tPA and PAI-1 have been reported in obese children,[93,116,120,246] reflecting impairment of the fibrinolytic system. The molar ratio of PAI-1 to tPA, often considered an index of vascular fibrinolytic balance,[94] was found to be higher in obese children compared with lean control subjects in 1 study.[120] Physical activity–based interventions in children have shown consistent decreases in the levels of fibrinogen and D-dimer.[56,89,96–99,120–123,246,231] The effect on levels of PAI-1 and the fibrinolytic system, on the other hand are mixed.[97,120,246–249]

Summary: Although studies in children suggest the presence of a prothrombotic state in obese children at an early age, the role of fibrinogen, D-dimer, and PAI-1 as potential markers of CVD risk needs to be confirmed in longitudinal studies; a cause-and-effect relationship cannot be assigned at present in children.

Insulin Resistance

As discussed previously, insulin resistance is the product of multiple processes mediated at the level of the cellular mitochondria that produce ER stress and oxidative stress, adipocyte dysfunction, and the release of various adipokines. Systemic manifestation is most evident in fat, muscle, and liver. The role of insulin and/or resistance in the development of cardiovascular morbidity has been widely studied in both adults and children. A scientific statement from the American Heart Association[143] provides a detailed review of insulin resistance in children. In this statement, we have limited our discussion to a few of the prominent novel biomarkers related to insulin resistance. There is evidence to suggest that insulin, in the presence of insulin sensitivity, may be antiatherogenic in that it inhibits the inflammatory transcription factors,[250] decreases levels of early growth response gene-1 and tissue factor,[251] decreases TNF-α,[252] and stimulates nitric oxide to lower blood pressure.[253] However, hyperinsulinism driven by insulin resistance promotes cardiovascular pathology, stimulating mitogen-activated protein kinase, mitogenesis, and PAI-1 within vascular smooth muscle cells,[254] endothelin-1 production with subsequent vascular smooth muscle growth,[255] and ras-p21 in vascular smooth muscle, which promotes a cascade of other growth factors such as platelet-derived growth factor.[124] As described above, oxidative stress and adipocyte dysfunction are postulated to increase insulin resistance. The adipokines adiponectin, leptin, RBP4, and resistin have been implicated in insulin sensitivity in the pediatric population as noted above (Adipokines section).

Crosstalk at the Cellular and Molecular Levels

The interconnections between adipocyte dysfunction, ER and oxidative stress, inflammation, thrombosis, and insulin resistance are related by common transcriptional pathways. At least in mice, large networks of genes related by global transcriptional regulators control pathways involving cholesterol metabolism, mitochondrial oxidative phosphorylation, and inflammation.[125] Several factors regulate this transcription, including PPAR and liver X receptor enzymes, master modulators of the dynamic equilibrium between energy storage and expenditure.[256] Peroxisomal proliferator–activated receptors are implicated in glucose homeostasis, lipid use, and immunity/inflammation. Liver X receptors mediate cholesterol catabolism through the conversion of cholesterol

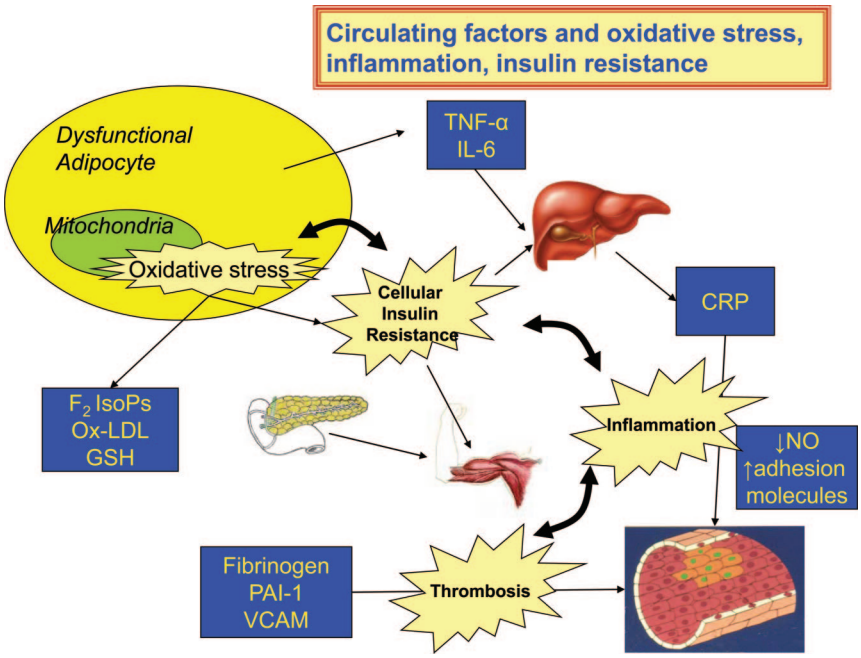

Figure 4. Circulating factors and oxidative stress, inflammation, insulin resistance. Selected circulating factors involved in crosstalk, including oxidative stress, inflammation, and insulin resistance. Processes are shown in the stars; measurable potential biomarkers and risk factors are shown in the boxes. TNF-α indicates tumor necrosis factor-α; IL-6, interleukin-6; IsoP, isoprostanes; Ox-LDL, oxidized low-density lipoprotein; GSH, growth-stimulating hormone; PAI-1, plasminogen activator inhibitor-1; VCAM, vascular cell adhesion molecule; CRP, C-reactive protein; and NO, nitric oxide.

to bile acids and the promotion of cholesterol clearance. Responding respectively to cellular lipid and cholesterol levels, these are the pathways that ultimately negotiate the critical balance between lipid utilization and lipid overload, thereby mediating cardiovascular risk.[257] Hundreds of genes are responsive to nuclear receptor activation or repression, and the resultant downstream transcription is reflected in many of the classic and atypical biomarkers associated with CVD risk, including autoimmunity and inflammation mediated by nuclear factor-κB, interferon regulatory factor receptor, protease inhibitor-3 kinases, mitogen-activated protein kinases, and toll-like receptors.[258]

Many established heart-healthy lifestyle recommendations have been specifically linked to activation of the PPAR family of receptors, including restriction of total caloric intake with adequate nutrition,[126] increased intake of fruits and vegetables with their rich antioxidant polyphenolic content,[259] lowering of dietary glycemic load,[260] and increased physical activity.[261] The interface between these lifestyle factors and gene expression may be mediated at the level of epigenetic chromatin modifications that facilitate or suppress transcriptional activity across the lifespan.[262] For example, the increasing incidence of dyslipidemias and the associated subclinical inflammation that occur with age compounded by a Western lifestyle can be attributed in part to the age-related decreases in PPAR expression.[263] Decreased PPAR activity can be causally linked to the increased inflammation associated with age and cardiovascular risk factors,[264] possibly as a result of aberrant DNA hypermethylation, a dysmetabolic process associated with inflammation and increased CVD risk.[265] Classic and atypical cardiovascular risk factors that reflect suboptimal PPAR activity both reflect present metabolic dysregulation and predict accelerated aging of the vasculature and associated heightened CVD risk.

CVD Risk Factors and Crosstalk Between Organs

Although the heart and vascular bed have typically been identified as the primary sites of atherosclerosis, the obesity epidemic has revealed that adipose tissue and liver play crucial roles in the development of risk factors for CVD.[266–274] It appears that some of the secreted molecules discussed above contribute to the key association of excess body fat and functional alterations in peripheral organs such as skeletal muscle, kidney, and ovary/adrenals.[8,10,19,143,144,275–280] Although an initiating factor is still elusive, development of CVD within all these tissues is a complicated cycle of lipid and glucose toxicity within stromal cells and tissue macrophages, which leads to development of oxidative and ER stress, chronic inflammation, and insulin resistance. A plethora of mechanisms have been proposed to regulate the storage of excess fuel in adipose tissue. It is expected that understanding the mechanisms underlying excess fuel storage will yield specific targets of treatment and reverse complications. The interorgan relationships and crosstalk between liver, muscle, kidney, and lung in the development of CVD have been studied extensively in adults. Similar crosstalk between CVD and related risk factors, especially adipokines and other organs such as the gut and the ovary, has also been studied.[101,102,271] The widespread expression of gastrointestinal hormones outside the gastrointestinal tract makes those hormones multifunctional regulators of general physiological interest.[102] Some of the more widely studied gut hormones include ghrelin, obestatin, peptide YY, and cholecystokinin. Although the role of these hormones in satiety and energy homeostasis is well documented, data on the potential relationship between these gut hormones and CVD are meager and conflicting,[103,154,170,282,283] but this appears to be a promising new area of investigation in light of data on the effects of bariatric surgery on human physiology. Exploring the complex interactions among the adipose tissue, brain, peripheral organs,

and other tissues (Figure 4) and the various channels of interorgan communication will continue to be a challenge. Efforts are currently underway to determine whether such effects are independent or interrelated. In this statement, we have not attempted to explore these areas in great detail.

Noninvasive measurement of end-organ function has advanced considerably. With development of cardiovascular imaging techniques, the detection of preclinical CVD in children and youth has become more feasible.[284] However, long-term studies in children assessing the relationship between preclinical CVD and novel biomarkers/risk factors are only emerging.

Search for Novel Biomarkers of CVD Using Genomics, Proteomics, and Metabolomics

Genomics, proteomics, and metabolomics seem promising as modalities for investigating new biomarkers related to CVD diagnosis, progression, and prognosis.[10] Most of the proposed biomarkers and/or risk factors are proteins and/or peptides that are integral components of all tissues. The turnover of proteins is a continuous remodeling process involving the degradation of old proteins and the synthesis of new proteins, and the delicate balance between these 2 dynamic processes determines the steady-state concentration of any protein[285–287] (eg, CRP and fibrinogen). Because of their dynamic nature and posttranslational modifications, identification and quantification of these risk factors/biomarkers are not sufficient alone to understand important functional changes. Specific measurements of protein metabolism through these individual pathways are needed to clarify why the concentration of a protein such as CRP or fibrinogen is elevated by particular genetic variations, as well as by nutritional and other environmental conditions. Along with proteomic, genomic, and metabolomic techniques, more insight into the function of these peptides and proteins is warranted.

Achieving Clinical Relevance

Although associations are emerging between nontraditional risk factors/biomarkers and noninvasive measures of early atherosclerosis in children, the clinical relevance of these associations is as yet unknown. Evidence is emerging on the measurement and normative values of some biomarkers, which must be considered across the wide developmental and maturational spectrum from birth to adulthood. The majority of current evidence is based on cross-sectional assessment; longitudinal assessment is required. Studies of biomarkers must necessarily account for a different and perhaps greater array of confounding factors present in pediatric subjects. Calibration to risk is especially challenging because atherosclerosis is subclinical. Noninvasive measures as indicators of early atherosclerosis cannot be relied on until considerable measurement issues involving validity, feasibility, accuracy, and reliability are addressed.[284] In addition, the burden of vascular pathology or dysfunction within the pediatric population may often be below the level of detection with currently available methods. Longitudinal studies, with biomarkers assessed early and repeatedly and vascular measures assessed later, are required to achieve the calibration of risk with atherosclerosis. In these types of cohort studies, atherosclerosis can be measured noninvasively in all or directly from pathological specimens obtained from a much smaller subset of members of the cohort who died.

Once longitudinal studies have defined developmental aspects of biomarkers and associations with vascular pathology or dysfunction, the nature of the relationship can be explored more fully in terms of parameters that would inform clinical utility.[288] Assuming that measurement issues have been satisfactorily addressed, the performance of the biomarker as a risk indicator can be indicated by calculation of sensitivity and specificity, positive and negative predictive values, and positive and negative likelihood ratios. The magnitude of risk indication is a key consideration driving clinical utility, particularly in the context of an important and independent contribution of the biomarker to risk assessment with conventional risk factors. In general, these multivariable models are lacking in the pediatric population. Identification of risk optimally should lead to an alteration in management that would slow or reverse the atherosclerotic process and reduce future risk of manifest clinical disease. Given the documented relationship of obesity and CVD in adults, the early signs of obesity-related alterations in subclinical inflammation, oxidative stress, and insulin resistance in children, and the beneficial effect of lifestyle-based interventions, it seems prudent to focus on prevention and/or reduction of obesity through lifestyle changes at an early age. However, evidence to support primary prevention in the pediatric population has been indirect, and given the long-term nature of the required intervention, the benefits, costs, and safety become primary concerns. Therefore, achieving clinical utility for the majority of biomarkers for use in the pediatric population is a considerable challenge.

Conclusions

Despite the advances in our understanding of nontraditional risk factors/biomarkers for CVD, the clinical utility of nontraditional risk factors/biomarkers for CVD remains limited in children because of inconsistent associations and lack of replication of findings. With the increasing connection between childhood obesity and CVD, biomarkers produced from adipose tissue and those with roles in inflammation and oxidative stress are increasingly being studied. This knowledge should eventually lead to the development of more directed therapeutic strategies to prevent CVD at an early age. It is likely that nontraditional risk factors/biomarkers could be used as a second layer of screening to follow interventions or efficacy of therapy and in predicting specific patient groups likely to benefit from targeted interventions. Currently, there are no convincing data to recommend routine testing of children for nontraditional risk factors/biomarkers. However, given the escalating prevalence of CVD risk at an early age, it is important to keep sounding the tocsin and have additional trials in this area to help decision making in this high-risk group. The remarkable advances in technology for biomarker discovery during this decade, especially in the area of proteomics and metabolomics, along with noninvasive imaging techniques, will likely facilitate the use of nontraditional risk factors/biomarkers to individualize prevention strategies and to help refashion clinical practice to respond to the dangers inherent in CVD risk in the pediatric population.

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*Modest.

†Significant.

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