Biomarkers of Obesity and Subsequent Cardiovascular Events

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Obesity is a major risk factor for cardiovascular diseases, but the mechanisms for increased cardiovascular risk in obesity are still unclear. Inflammation and increased oxidative stress are two potential mechanisms proposed to play a major role in the morbidity associated with obesity. Studies that investigate these mechanisms rely on biomarkers, but validated biomarkers for obesity-related cardiovascular outcomes are lacking. By finding optimal biomarkers, diagnostic criteria for cardiovascular diseases can be refined in the obese beyond "traditional" risk factors to identify early pathologic processes. The objective of this review is to identify potential early biomarkers resulting from obesity and associated with cardiovascular disease. Studies were initially identified through the search engine PubMed by using the keywords "obesity" and "biomarker." Subsequently, combinations of the keywords "obesity," "biomarker," "cardiovascular risk," "adipose tissue," "adipokine," "adipocytokine," and "oxidative stress" were used. The SOURCE database and Online Mendelian Inheritance in Man (OMIM) were used to obtain more information on the biomarkers. Results of the searches yielded a large number of potential biomarkers that occur in obesity and which either correlate with traditional cardiovascular risk factors or predict subsequent cardiovascular events. Several biomarkers are promising regarding their biologic properties, but they require further validation in humans.

adipose tissue; cardiovascular diseases; inflammation; obesity; oxidative stress

Abbreviations: CVD, cardiovascular disease; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; oxLDL, oxidized low density lipoprotein; PAI-1, plasminogen activator inhibitor 1; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor alpha.

The prevalence of obesity, defined as a body mass index (weight (kg)/height (m)^2) of ≥30 kg/m^2, has been rising for decades. It is estimated that 32 percent of US adults were obese and 17 percent of children aged 2–19 years were overweight (gender- and age-specific body mass index percentile ≥95) in 2003–2004 (1). On the basis of mortality data from the National Health and Nutrition Examination Survey, the excess number of deaths in 2000 attributed to obesity was 112,000 compared with a healthy body mass index of 18.5–25 kg/m^2 (2). The estimated years of life lost among Black men and White men aged 20–19 years were overweight (gender- and age-specific body mass index percentile ≥95) in 2003–2004 (1). On the basis of mortality data from the National Health and Nutrition Examination Survey, the excess number of deaths in 2000 attributed to obesity was 112,000 compared with a healthy body mass index of 18.5–25 kg/m^2 (2). The estimated years of life lost among Black men and White men aged 20 years with a body mass index of >45 kg/m^2 is 20 and 13, respectively; the corresponding figures for Black women and White women are 5 years and 8 years (3). Increased mortality attributed to obesity results partly from the propensity for increased risk of chronic diseases such as cardiovascular disease (CVD), especially if obesity began at an early age.

In adolescents, obesity is significantly associated with increased risk of CVD, with an odds ratio of 19.2 for high cumulative cardiovascular risk (4). Increased risk of CVD in obesity strongly correlates with traditional risk factors such as diabetes, hypertension, and hyperlipidemia (5, 6). Such traditional risk factors are validated in many populations (7–10) for the diagnosis and management of CVD. The underlying mechanisms for the association of obesity and traditional risk factors with CVD are still not fully known. One opportunity for elucidating these mechanisms most likely involves identifying other biomarkers that result from...
obesity and that independently enhance future susceptibility for CVD. Such nontraditional biomarkers will further refine risk assessment and aid in CVD prevention. Ultimately, nontraditional biomarkers may prove useful in translational medicine for improving the prediction of CVD in the obese.

Examples of emerging risk factors proposed to predict atherosclerosis, a pathologic process associated with CVD, include lipoprotein(a), increased homocysteine, increased inflammatory markers, and prothrombotic factors (6, 11). Their predictive value in the population and in comparison with standard lipid screening is still debatable. Of these risk factors, only a few, such as C-reactive protein, an inflammatory marker, are extensively studied and shown to be variably associated with obesity or cardiovascular endpoints (12–14). There is consistent awareness of the need for other biomarkers that occur after the development of obesity and that may predict later CVD onset. Such biomarkers for early detection have yet to be identified.

Different mechanisms are implicated in the link between obesity and CVD. For example, the fetal origin of metabolic risk (15), epigenetic gene regulation (16), and the “pup in a cup” model (17) are potential causes of increased CVD risk in obesity. This review focuses on two other mechanisms of current interest: inflammation and increased oxidative stress. Both can play a role in promoting CVD, such as increased endothelial dysfunction, an early predictor of cardiovascular injury in obese individuals (18). Notably, both mechanisms are associated with the accumulation of fat that occurs in obesity.

Abnormal fat accumulation is associated with inflammatory changes, including recruitment of macrophages (19) and activation of endothelial cells (19), which promotes vascular disease (20). Indeed, several molecules produced by adipocytes (adipocytokines) are associated with increased cardiovascular risk (21–25) partly through increased expression of inflammatory genes (26) and induction of systemic inflammation (27). Increased secretion of inflammatory molecules can be modified by weight loss (28), thus indicating the dynamic influence of adipose tissue in inflammation. Accumulation of adipose tissue also leads to increased oxidative stress (29) partly via the oxidant effects of free fatty acids (29). Leukocytes derived from obese individuals and healthy individuals infused with free fatty acids suffer from increased oxidative stress (30, 31).

Oxidative stress in turn may promote metabolic complications such as insulin resistance and induces a proinflammatory state via deregulation of adipocytokines (29). Thus, oxidative stress is both induced by and adversely impacts adipose tissue function. Consequently, biomarkers for oxidative stress may play a role in obesity-induced CVD.

Figure 1 is a schematic representation of potential biomarkers that result from obesity.

The purpose of this review is to identify novel biomarkers for inflammation and oxidative stress that can be used for early detection of obesity-related CVD risk. Our aim is to assess which biomarkers warrant further investigation and eventual clinical use for predicting CVD. Nontraditional biomarkers have the potential to aid in understanding the mechanisms for the effect of obesity on cardiovascular outcomes independent of traditional risk factors for CVD (7–10).

We performed a literature review of biomarkers associated with obesity and their potential role in cardiovascular risk.

**MATERIALS AND METHODS**

Articles eligible for inclusion met the following criteria: 1) they discussed nontraditional biomarkers associated with obesity, and 2) they studied biomarkers associated with CVD and CVD risk factors. Since the focus of this review is on nontraditional biomarkers in obesity, the following biomarkers were excluded: total cholesterol, fasting blood glucose, blood pressure, high density lipoprotein cholesterol, triglycerides, and smoking. CVD outcomes comprised cerebrovascular disease (cerebral embolism, thrombosis, and hemorrhage), peripheral arterial disease, coronary heart disease or coronary artery disease, and myocardial infarction. Research papers in the English language published as of mid-2006 were reviewed. Studies were initially identified through the search engine PubMed (32) by using the keywords “obesity” and “biomarker.” Subsequently, combinations of the keywords “obesity,” “biomarker,” “cardiovascular risk,” “adipose tissue,” “adipokine,” “adipocytokine,” and “oxidative stress” were used. Emphasis was placed on biomarkers for inflammation and oxidative stress. The SOURCE database (33) was assessed to gain more insight into the adipocytokines. Online Mendelian Inheritance in Man (34) was used to obtain more information on various genes and phenotypes of interest. Further information was obtained by examining reference lists of relevant articles.

**RESULTS**

**Adipocytokines and inflammatory biomarkers**

In addition to energy storage and regulation of endocrine function, adipocytokines can alter inflammatory responses, and they can promote endothelial injury and dysfunction, all of which predispose to atherosclerosis (35). Abdominal (visceral) distribution of adipose tissue is thought to have more detrimental effects than generalized obesity. In fact, visceral obesity is significantly associated with abnormal cytokine secretion and adverse metabolic risk factors (13, 36, 37). Adipocytokines can play a role in the observed link between obesity and its associated morbidities, such as coronary artery disease and insulin resistance (21, 38, 39); this possibility is still under debate (39). This section of the review briefly describes major factors synthesized by adipose tissues that may play a role in obesity-related CVD.

**Adipocyte differentiation-related protein.** In vitro studies. Adipocyte differentiation-related protein, also referred to as adipophilin, is an adipose-tissue-specific membrane protein. Its mRNA levels are markedly augmented during early adipocyte differentiation in mice (40). The significance of adipocyte differentiation-related protein in obesity lies in its ability to promote the storage of triglycerides (41) and, consequently, fatty liver in humans and mice (42).

**Interpretation.** There is a deficiency of human and animal studies that investigate the association of this protein with obesity-related morbidity in humans.
Adiponutrin. In vitro studies. This membrane-bound protein functions in triacylglycerol hydrolysis. It is mainly expressed in adipose tissue, and it may play a role in energy homeostasis (33) and membrane trafficking of other adipose proteins (43). As of completion of this review, no Online Mendelian Inheritance in Man information had been published.

In vivo studies. Adiponutrin mRNA expression is increased during early stages of adipocyte differentiation, in mice subjected to starvation and then fed a high-carbohydrate diet, and in genetically obese rats (43).

Human studies. Average adiponutrin expression levels in subcutaneous adipose tissue of humans were not statistically different between obese and nonobese individuals (44). Expression levels, however, were extremely responsive to changes in acute and 21-day energy intake. Adiponutrin did not correlate with adiponectin or leptin mRNA levels, and it showed a negative correlation with fasting glucose and a positive association with insulin sensitivity, while no correlation was observed with triglycerides or other clinical parameters (44). Although these results suggest a role in glucose homeostasis, this latter study was restricted to Caucasian females with a small sample size; thus, further studies in humans are needed.

Interpretation. Given the fact that adiponutrin is exclusive to adipose tissue and is modulated by nutrient intake, it may be beneficial for use in studies of energy homeostasis. Adiponutrin is responsive to obesity in mice (43), implying
a possible role in adipogenesis and/or maintenance of a differentiated state. Adiponutrin does not appear to be a measure of adiposity, and studies are lacking regarding its effect on long-term disease risk in humans. Lack of secretion into the plasma does not make it suitable for epidemiologic studies because it may not be easy to measure in the field.

**Adiponectin.** In vitro studies. The adiponectin gene, or ACDC, ACRP 30, ADIPQ, and APM1, is exclusively expressed in adipose tissue and encodes a protein similar to complement factor C1q and some collagen molecules. It is highly expressed during adipocyte differentiation (45). Adiponectin levels may be influenced by increased oxidative stress, as demonstrated by inhibition of adiponectin expression and secretion in adipocytes in response to glucose oxidase exposure (46).

In vivo studies. The protective roles of adiponectin include reduction of tissue triglyceride content and inhibition of insulin resistance in diabetic and obese mouse models (47). Adiponectin itself was reduced in obese rodents (48). Given its protective role on the vascular endothelium (49), adiponectin’s reduction in obesity may play a part in obesity-related vascular damage.

Human studies. Plasma levels of adiponectin were inversely associated with body mass index, percentage of body fat, and fasting plasma insulin in different ethnic groups (50–52), and they were increased with a 21 percent reduction in mean body mass index (53). Oxidative stress may play a role in altering adiponectin levels in obesity, since plasma adiponectin was inversely associated with increased oxidative stress as measured by urinary isoprostanes (54). Some studies signified the role of adiponectin in regulating vascular function.

Adiponectin correlated with endothelial-dependent vasodilatation and was found to have potent antiinflammatory effects on the cellular components of blood vessel walls (55, 56). Adiponectin negatively correlated with increased carotid intima-media thickness in obese children (57). Potential mechanisms for the protective role of adiponectin on endothelial cells include inhibiting tumor necrosis factor alpha (TNF-α)–induced expression of cell adhesion molecules (56) and inducing inhibitors of metalloproteinases via interleukin (IL)-10 (58). Implications of adiponectin’s protective role were demonstrated in several studies.

Adiponectin was significantly reduced in patients with coronary artery disease compared with controls matched on age and body mass index (56) after adjustment for other risk factors such as diabetes mellitus, smoking, dyslipidemia, and hypertension (59). It was also associated with a decreased risk of myocardial infarction after adjustment for traditional risk factors, but the risk was attenuated when adjusted for high density lipoprotein and low density lipoprotein cholesterol (60). This latter study was performed among only males employed in health care, which may have introduced some bias. Overall, the above results suggest a link between adiponectin regulation with oxidative stress and increased CVD risk.

Interpretation. Because adiponectin is both a marker and possibly a mediator of CVD, it has a good potential to predict adverse cardiovascular events. The precise target of adiponectin is unknown, and the nature of gender differences in its expression, as reported by some studies (52), needs to be elucidated. Methods commonly used to assess the role of adiponectin in endothelial dysfunction, such as response to reactive hyperemia, may not be specific and require further validation. Since the design of several human studies performed so far is cross-sectional or case-control, there is still a need for prospective assessment of adiponectin’s clinical utility and predictive power.

**Resistin.** In vitro studies. Resistin is expressed in adipose tissue and is regulated by nutritional status (61, 62). It is reduced during adipogenic differentiation of human-derived adipocytes (63).

In vivo studies. Although circulating resistin was reported to be increased in obesity, its expression in adipose tissue was unchanged (64) in some murine models of obesity compared with lean animals. In contrast, circulating resistin levels were higher in obese mice compared with lean controls (64).

Human studies. Serum resistin correlated with obesity in some adult studies (65, 66); in others, it did not (67). Serum resistin did not differ significantly between obese and nonobese children (68, 69) nor did it change with body mass index over time (70). There is controversy regarding the contribution of resistin to insulin resistance in obesity because some studies find an association while others do not (61, 63, 71). Plasma resistin was associated with inflammatory mediators as well as coronary calcification, an indicator of the degree of atherosclerosis (72). This study was cross-sectional, however, and the temporality of the findings could not be assessed.

Interpretation. Additional studies are needed to elucidate resistin’s biologic function and contribution, if any, to CVD.

**IL-6.** In vitro studies. IL-6 is an inflammatory marker induced by TNF-α in cultured subcutaneous adipose cells (23). IL-6 regulates lipid metabolism and C-reactive protein production, both of which are known risk factors for CVD (73). IL-6 may also promote insulin resistance. One mechanism by which IL-6 antagonizes insulin action is via inhibition of insulin-stimulated glucose transport (23).

In vivo studies. IL-6-deficient mice develop obesity that is associated with altered carbohydrate and lipid metabolism as well as leptin resistance, and which is partially reversed by IL-6 treatment (74). Intracerebroventricular injection of IL-6 increases energy expenditure in these mice (74) and decreases body weight by 8.4 percent (75), thereby demonstrating that IL-6 can act centrally in regulating energy homeostasis. IL-6 also plays a role in insulin resistance, as demonstrated by increased insulin sensitivity in IL-6-depleted mice (76).

Human studies. IL-6 is increased in obesity and correlates with insulin resistance (73, 77, 78). IL-6 levels are also responsive to weight loss (79); for example, a 30 percent reduction in fat mass is associated with a 25–30 percent decrease in IL-6 (80). Genetic studies show an association of IL-6 with body mass index and fat mass (81) as well as with insulin levels (82). Prospective studies of IL-6 in relation to CVD risk are deficient, thus hindering proper assessment of the role of IL-6 in CVD.
Interpretation. The effects of aberrant IL-6 production in obesity are not fully known but may involve alterations in inflammatory signals or lipid homeostasis.

IL-18. IL-18 is produced by a variety of hemopoietic and nonhemopoietic cells. It induces production of reactive oxygen species, as well as T lymphocytes (Th1 and Th2), natural killer cells, neutrophils, and intracellular adhesion molecule 1 and vascular cell adhesion molecule 1 expression on endothelial cells. IL-18 was found in human atheroma tissues, and it induced the expression of several inflammatory molecules in vascular smooth muscle cells, endothelial cells, and macrophages. Information on regulation of IL-18 synthesis, cytokine release, and mediation of its activities is still not fully known (83).

In vivo studies. IL-18-deficient mice developed obesity because of increased food intake (84). These mice also developed insulin resistance at the level of the liver, muscle, and adipose tissue because of increased glucose production. Replacement of IL-18 in the brain reduced food intake and reversed the hyperglycemia (84).

Human studies. Although IL-18 levels were significantly increased in adipocytes derived from obese individuals (85), levels were significantly reduced following weight loss in individuals with regular (86) and morbid (87) obesity. IL-18 was independently associated with the metabolic syndrome, a known risk factor for CVD, after adjustment for age, gender, body mass index, and insulin (88). In a prospective study, IL-18 was found to be associated with coronary events in males, independent of age, body mass index, inflammatory biomarkers, and classic lipid predictors (89). These findings demonstrate the effect of inflammation and immune modulation on CVD via the actions of IL-18.

Interpretation. Although IL-18 shows promise in predicting CVD, further prospective studies are required. Population-based cutoff values need to be determined.

Leptin. In vitro studies. Leptin regulates the production of cytokines such as IL-6 and TNF-α (90). Leptin also causes hypertherpy of human (91) and rat (92) smooth muscle cells. It signals via the generation of reactive oxygen species (91) as well as stimulation of p38 mitogen activated protein kinase and signal transducers and activators of transcription 3 (93).

In vivo studies. Leptin is produced by adipose tissue as well as the heart (94). By regulating energy expenditure, leptin can regulate lymphocyte survival (95, 96). Its effects on vascular function are demonstrated by its ability to inhibit acetylcholine-induced vasodilatation in animals (97). Animal models further corroborate the association of leptin with cardiovascular abnormalities such as hypertrophy of vascular smooth muscle cells (98), formation of occlusive thrombus (99), and altered myocardial contractility (100).

Human studies. Leptin is also produced by the brain, which is involved in the regulation of body weight (101). It is closely associated with obesity and risk factors for CVD such as increased systolic blood pressure (102). One mechanism by which leptin can increase CVD risk is via increased production of inflammatory markers (27, 101, 103, 104), and it plays a role in the early induction of vascular dysfunction. The latter is suggested by its association with impaired cross-sectional compliance of arteries from diabetic children (105) and impaired arterial distension in adolescents (106). A link with CVD was directly demonstrated in its independent association with hemorrhagic stroke (107) and acute myocardial infarction (108) in prospective studies. Leptin was also positively associated with future development of other CVD outcomes including death from CVD and coronary revascularization after adjustment for age, gender, smoking, prior myocardial infarction, unstable angina, blood pressure, C-reactive protein, ratio of low density lipoprotein to high density lipoprotein cholesterol, insulin resistance, fibrinogen, and number of coronary vessels with significant stenosis (109).

Interpretation. Further work is needed to elucidate the effect of leptin on endothelial cells, and more prospective studies are required. Increasing evidence for its role in modulating immune and vascular function, as well as its association with discrete CVD outcomes in humans, adds support to leptin’s ability to provide significant risk prediction with good clinical utility.

Plasminogen activator inhibitor 1 (PAI-1). In vitro studies. Cultured adipocytes from PAI-1-deficient mice show enhanced adipocyte differentiation and glucose uptake, whereas PAI-1 overexpression causes inhibition of adipocyte differentiation (110).

In vivo studies. The role of PAI-1 in obesity is reflected by animal models. Evidence from obese mice shows that TNF-α (22) and immobilization-induced stress (111) stimulate the production of PAI-1 in adipose tissues and the heart. Mice that lack PAI-1 are protected from obesity (112), whereas the inhibition of PAI-1 is associated with enhanced cardiac recovery following myocardial infarction (113) and reduced aortic wall thickening as promoted by angiotensin II and a high-salt diet (114) in mice. Paradoxically, PAI-1 inhibition was also associated with increased growth of atherosclerotic plaques in mice predisposed to atherosclerosis (115).

Human studies. Blood levels of PAI-1 correlate with body mass index and increased waist circumference (37, 116, 117) and decrease following weight loss (118, 119). In some studies, PAI-1 increased risk of recurrent myocardial infarction (120), atrial fibrillation (121), and progression of coronary atherosclerosis (122) following myocardial infarction; in other studies, it was not associated with subsequent coronary events (123). One possible mechanism for the association of PAI-1 with CVD could be through enhanced expression and release of PAI-1 from visceral adipose tissue, which is highly associated with adverse metabolic risk factors (124, 125). In fact, PAI-1 is associated with several metabolic risk factors including increased cortisol (126), high triglycerides (127), TNF-α production (21), insulin resistance (128, 129), glucose intolerance (130), low adiponectin (131), and increased oxidative stress (132). PAI-1 was also highly expressed in human atherosclerotic lesions (133). Genetic polymorphisms in PAI-1 have been linked with obesity, insulin resistance, and increased triglycerides in some studies (134, 135) but not in others (136).

Interpretation. The adverse effect of PAI-1 on CVD independent of traditional risk factors remains to be confirmed in longitudinal studies.
**TNF-α.** In vitro studies. TNF-α is expressed in adipocytes and is associated with obesity (137), adipocyte cell volume (138), and inhibition of glucose uptake in adipocytes from lean individuals (137). The roles of TNF-α in immune regulation are varied and include stimulation of growth factor production from adipose progenitor cells and mesenchymal stem cells (139), regulation of apoptosis (140), and regulation of cytokine expression in adipocytes (141).

In vivo studies. In vivo evidence for the role of TNF-α in immune regulation is demonstrated by its close association with the development of bronchial-associated lymphoid tissue in rats (142) as well as its role in suppressing T-cell proliferation (143). Adipose tissue production of TNF-α was increased in genetically obese mice (144, 145). TNF-α was also found to be increased in muscle (146), but not serum (146) or macrophages (147), from mice with diet-induced obesity. TNF-α was found to mediate insulin resistance in animal models of obesity (148, 149), a finding thought to occur through inhibition of insulin receptor signaling (150). It was also associated with increased visceral fat deposition and insulin resistance following partial removal of subcutaneous adipose tissue from mice (151). Notably, this insulin resistance was reversed by neutralization of TNF-α using a specific antibody (151). Studies in mice deficient for the TNF-α receptor gave conflicting results (152), which showed that TNF-α was not associated with insulin resistance.

Human studies. In some studies (36, 153), secretion of TNF-α was increased in relation to obesity and was reduced following a minimum 10 percent (154) but not 2 percent (30) weight loss. Its release into the circulation was not associated with obesity in other studies (24, 73). This discrepancy may be partly due to decreased cleavage of the membrane form of TNF-α despite increased production in mature adipocytes (145). Although some studies did not find an association between TNF-α and increased insulin resistance (155), other studies found that TNF-α was associated with glucose uptake and insulin resistance (156), partly through increased expression of IL-18 in muscle (157). In addition, TNF-α was found to be increased in nonobese Mexican Americans, who, compared with non-Hispanic Whites, are known to be more predisposed to obesity and subclinical inflammation. These differences were present independent of total adiposity and abdominal fat (158). TNF-α was also found to be associated with von Willebrand factor, a risk factor for CVD, in obese individuals (159) and with stroke severity (160).

Interpretation. Mechanisms for TNF-α’s link with CVD in obesity are still unclear.

**Biomarkers for oxidative stress**

As shown in the previous section of this review, abnormal regulation of adipocytokines in obesity plays a role in promoting CVD. One mechanism for this abnormal regulation may be through increased oxidative stress. Pharmacologic inhibition of oxidative stress increased plasma adiponectin and decreased adipose tissue expression of other adipocytokines such as PAI-1 and TNF-α in obese mice (144). Therefore, abrogating the effect of increased oxidative stress ameliorated the abnormal regulation of adipocytokines that occurs in obesity. Mice genetically predisposed to atherosclerosis exhibited larger atherosclerotic lesions when rendered vitamin E deficient, thus implying a role for increased oxidative stress in modifying risk of atherosclerosis in susceptible organisms (161). In contrast, mice that overexpressed antioxidant enzymes exhibited fewer atherosclerotic changes (162), demonstrating the beneficial effect of reduced oxidative stress. In addition, increased oxidative stress was associated with increased atherosclerosis in hypercholesterolemic rabbits that received advanced oxidation protein products, compared with controls (163). Human studies further demonstrate the role of oxidative stress in obesity.

In addition to promoting abnormal adipocytokine expression with consequent inflammation, oxidative stress itself may result from the inflammatory changes that occur in obesity, as demonstrated in other chronic inflammatory states including asthma and rheumatic diseases (164, 165). Therefore, a vicious cycle that provokes increased oxidative stress in obesity may exist. Reactive oxygen species that lead to increased oxidative stress can be generated in adipocytes (29) and in other cell types such as leukocytes (30), all of which can be a source of increased oxidative stress in obese humans.

Increased oxidative stress is independently associated with obesity measures including body mass index and waist-hip ratio (20, 166) and improves upon weight loss of at least 2 percent (30). It is also associated with several CVD risk factors including smoking, blood glucose, and hyperlipidemia (20, 166–168). Oxidative stress may also promote endothelial dysfunction, atherogenesis (169–172), and coronary heart disease independent of traditional risk factors (173). Antioxidant supplementation was found to be beneficial in reducing CVD risk in some studies but not others (174–176). Controversial findings may be due to lack of a strong effect of oxidative stress on atherosclerosis itself (177), choice of antioxidant therapy (178), confounding by other dietary and nondietary factors (178), or potentially inadequate evidence for an antioxidant effect of the therapy (179). Moreover, the mechanism for antioxidant action and the applicability of findings in specific population groups is still unclear. This evidence underscores the need to define reliable and noninvasive biomarkers of oxidative stress and to understand their relation with CVD risk factors in order to clarify the role of oxidative stress in development of adverse cardiovascular events.

**Oxidized low density lipoproteins (oxLDLs).** In vitro studies. Low density lipoproteins can be oxidized by endothelial cells and macrophages into oxLDL, which is cytotoxic and immunogenic and which may alter coagulation processes as well as gene expression in arterial walls. OxLDL is more readily taken up by macrophages that accumulate on arterial walls; this accumulation strongly contributes to the development of atherosclerotic lesions (180).

In vivo studies. Formation of oxLDL was significantly decreased in aortic segments and smooth muscle cells from mice overexpressing antioxidants. The reverse was found in mice deficient for antioxidants (181). Furthermore, these mice altered the ability of oxLDL to induce apoptosis in aortic smooth muscle cells, a cytotoxic mechanism
implicated in atherosclerosis (181). OxLDL also induced cellular damage and irregular electrical activity in ventricular myocytes from guinea pigs (182). These studies demonstrate the potential role of oxLDL in promoting CVD.

**Human studies.** No significant difference in serum oxLDL was found between obese and nonobese humans (103), but oxLDL was associated with increased waist circumference (183) and increased body mass index (184). A relation between increased oxLDL and insulin resistance was found (184), but this association could be confounded by plasma triglycerides (185). In addition to accumulation on blood vessel walls, another potential mechanism for the role of oxLDL in atherosclerosis is via leptin, since oxLDL was closely associated with plasma leptin levels (184). The role of increased oxLDL in cardiovascular outcomes was demonstrated by its association with ischemic damage in cortical lesions (186) as well as coronary heart disease (187–189). Other studies contradicted these findings, whereby antibodies to oxLDL were not associated with subsequent coronary heart disease (190).

Interpretation. The effect of oxLDL on atherosclerosis is still unclear. To our knowledge, clinical trials aimed at measuring the effectiveness of inhibition of low density lipoprotein oxidation on CVD risk are currently lacking.

**Interpretation.** Although not the sole by-product of increased oxidative stress, lipid oxidation may be vital in the pathogenesis of CVD. Hence, the search for biomarkers that reflect this process may be beneficial in predicting CVD.

**F2 isoprostanes.** F2 isoprostanes are prostaglandin-like compounds formed from the oxidation of arachidonic acid, a fatty acid found in cell membranes. Although not primary products of lipid oxidation, F2 isoprostanes possess good biomarker properties that make them suitable for use in human and animal studies, such as stability and specificity (191).

In vitro studies. F2 isoprostanes induced cell proliferation and collagen synthesis in rat hepatic cells (192). A direct role for the involvement of oxidative stress in inflammation was shown by the effect of F2 isoprostanes on human macrophages, in which F2 isoprostanes induced the expression of IL-8 and intracellular inflammatory signals (193).

In vivo studies. F2 isoprostanes are used as biomarkers for increased oxidative stress in various animal models including models of allergic lung inflammation (194), increased susceptibility to atherosclerosis (161), and obesity (29).

**Human studies.** The utility of F2 isoprostanes as biomarkers for increased oxidative stress extends to humans. F2 isoprostanes are significantly increased in obesity (20, 29, 172, 195) and following the infusion of free fatty acids, particularly in obese humans (196). Importantly, F2 isoprostanes are localized to atherosclerotic arteries (197) and show an association with CVD (195, 198, 199). In one study, an F2 isoprostane concentration of ≥131 pmol/mmol was positively associated with coronary heart disease in univariate analysis (odds ratio = 27.3, 95 percent confidence interval: 10.4, 71.4) and in two multiple logistic regression models (odds ratio = 19.3, 95 percent confidence interval: 5.4, 68.8 and odds ratio = 30.8, 95 percent confidence interval: 7.7, 124) (200).

Interpretation. Although not the sole by-product of increased oxidation stress, lipid oxidation may be vital in the pathogenesis of CVD. Hence, the search for biomarkers that reflect this process may be beneficial in predicting CVD. F2 isoprostanes are widely used to assess lipid oxidation and have demonstrated their ability to mediate adverse biologic changes in animals and humans. The specificity and sensitivity of F2 isoprostanes in identifying CVD independent of traditional risk factors still needs to be determined. More studies should be performed to validate its use and compare it with other biomarkers in the presence of disease.

**Glutathione peroxidase.** Glutathione peroxidase and its substrate glutathione are major antioxidant defense systems against increased oxidative stress.

In vitro studies. Glutathione peroxidase is expressed following adipocyte differentiation in bovine and human cells (201) and may be regulated by sex hormones (202). The cellular activity of glutathione peroxidase correlates with resistance to oxidative stress as measured by percentage of cell survival in response to oxidant damage (203).

In vivo studies. Serum glutathione peroxidase is increased in animal models of obesity (204, 205). In support of its role in preventing vascular injury mediated by oxidative stress, mice deficient for cellular glutathione peroxidase exhibit abnormal vascular and myocardial histology including endothelial dysfunction (206, 207).

**Human studies.** Glutathione peroxidase is present in most mammalian cells including the endothelium, and its expression is affected by smoking and gender (208). Glutathione peroxidase activity is low or undetectable in atherosclerotic plaques in humans (209). Importantly, in prospective studies (210, 211), risk of cardiovascular events decreased with increasing glutathione peroxidase activity independent of other risk factors.

Interpretation. Glutathione peroxidase demonstrates strong antioxidant and antiatherosclerotic properties and shows promise in its ability to protect the vascular endothelium from oxidative stress. Its validity as a predictive biomarker, however, requires further validation.

**Angiotensin-converting enzyme.** In vivo studies. Other biomarkers. Angiotensin-converting enzyme synthesizes angiotensin II, a potent vasoconstrictor. Both increase following exposure to high glucose levels and may play a role in decreasing cellular proliferation, as demonstrated in human mesothelial cells (212).

In vitro studies. An example of the role of angiotensin-converting enzyme in inducing metabolic risk is evident in obese mice. Inhibition of angiotensin-converting enzyme in obese mice reduces PAI-1 levels in the blood and heart and inhibits perivascular fibrosis of coronary arteries (213).

Human studies. Although plasma measurements of angiotensin-converting enzyme are reproducible in humans, they are difficult to interpret because of large interindividual variation (214). Angiotensin-converting enzyme genotype was associated with body mass index in some studies (215) but not others (216). Interestingly, the relation between body mass index and coronary heart disease was strengthened after adjustment for angiotensin-converting enzyme genotype in one study (age- and gender-adjusted odds ratio = 2.63, 95 percent confidence interval: 1.19, 5.82 from odds ratio = 2.22, 95 percent confidence interval: 1.4, 3.6) (215). An insertion/deletion polymorphism was found to influence circulating levels of angiotensin-converting enzyme (214); such polymorphisms may play a role in influencing...
susceptibility to CVD. Angiotensin-converting enzyme insertion/deletion polymorphisms in different populations were associated with CVD such as coronary heart disease and stroke in some studies (215, 217, 218) but not others (219).

**Interpretation.** Although there is some evidence for the ability of angiotensin-converting enzyme genotype to influence coronary heart disease risk, more longitudinal studies are required to assess its importance compared with other risk factors for coronary heart disease.

**Peroxisome proliferator-activated receptor gamma (PPAR-γ).** In vitro studies. PPAR-γ has adipogenic and insulin-sensitizing actions, as demonstrated by the in vivo actions of thiazolidinediones, which are PPAR-γ agonists that enhance differentiation of pluripotent stem cells to adipocytes in culture (220). PPAR-γ is also expressed in vascular cells and protects the vascular wall by inhibiting migration of both endothelial cells (221) and vascular smooth muscle cells (222).

In vivo studies. One of the first tissues to express PPAR-γ during embryogenesis is adipose tissue. The importance of PPAR-γ in cellular function is evident in mice that lack PPAR-γ activity, which die early before birth (223). Deficiency of PPAR-γ also interferes with cardiac formation and adipose tissue development (223). Because PPAR-γ is involved in adipose tissue development, it may play a role in promoting obesity under certain circumstances. In fact, PPAR-γ antagonists prevent obesity induced by a high-fat diet in mice (224). Furthermore, PPAR-γ inhibition by gene targeting causes reduced fat mass in mice (225).

Human studies. PPAR-γ is activated by fatty acids, lipoprotein-derived products, and lipid-lowering agents. It has antiatherosclerotic and antiinflammatory properties reflected by the actions of PPAR agonists commonly used to correct lipid levels and thus lower cardiovascular risk (226). Genetic polymorphisms in PPAR-γ are associated with body weight in some studies (227–229) but not others (230). PPAR-γ is also positively associated with atherosclerosis (231) and CVD (232) and is inversely associated with serum insulin (104) in obesity.

**Interpretation.** PPAR-γ has great potential to modulate lipid metabolism and immune responses in blood vessels, but its contribution to cardiovascular risk is not fully elucidated. Human studies on PPAR-γ need to be replicated to ascertain its impact on CVD risk prediction. Thus, it is still early to establish PPAR-γ polymorphisms as biomarkers for CVD in obese individuals.

**Complement factor 3.** In vitro studies. Complement factor 3 is a component of the complement system that functions in host defense mechanisms such as bactericidal activity, chemotaxis, and increased phagocytosis (233, 234). It is induced during acute inflammation by several factors such as TNF-α (235) and is regulated by proteolytic cleavage (236).

In vivo studies. The role of complement factor 3 in immune regulation is evident in complement factor 3–deficient mice, which develop decreased airway hyper-responsiveness and IL-4 production compared with controls in a mouse model of pulmonary allergy (237). Interestingly, inhibition of IL-6 in mice impairs the production of complement factor 3 as well as the development of complement factor 3–induced autoimmune myocarditis and inflammatory T-cell responses following immunization with a peptide from cardiac tissue (238). Inhibition of complement factor 3 action in mice also reduces the influx of leukocytes to venous grafts (239). Complement factor 3 is strongly associated with obesity in obese rat models (240) partly because of the actions of acylation-stimulating protein, a product of complement factor 3 cleavage derived from adipocytes. Acylation-stimulating protein stimulates glucose uptake and storage of fatty acids (241). The obesity-promoting actions of complement factor 3–derived acylation-stimulating protein may explain why complement factor 3 knockout mice develop reduced body fat and oxygen consumption because of lack of acylation-stimulating protein synthesis (241). Likewise, acylation-stimulating protein knockout mice accumulate less fat and are more sensitive to insulin action (242).

**Human studies.** Complement factor 3 is associated with obesity in children in the presence of normal lipid profiles (243). The role of complement factor 3 in fat deposition and immune regulation is also demonstrated in complement factor 3–deficient humans, who develop partial lipodystrophy (fat loss) in the upper body (244) as well as immunologic abnormalities (245). Complement factor 3 was significantly correlated with mean body mass index in a cross-sectional study of coronary heart disease patients (246) as well as with metabolic risk factors for CVD such as insulin levels and triglycerides (247, 248). In addition, complement factor 3 was a significant predictor of coronary heart disease after adjustment for age and gender alone (odds ratio = 3.13, 95 percent confidence interval: 1.35, 7.3); after adjustment for age, gender, smoking, total cholesterol, and use of statins (odds ratio = 3.5, 95 percent confidence interval: 1.27, 9.62); and after adjustment for age, log C-reactive protein, and fibrinogen (odds ratio = 2.74, 95 percent confidence interval: 1.03, 7.26). Levels of complement factor 3 of ≥1.6 g/liter were associated with coronary heart disease after adjustment for age, gender, use of statins, systolic blood pressure, total cholesterol, and smoking (odds ratio = 1.89, 95 percent confidence interval: 1.01, 3.58) (246). Complement factor 3 was also better than C-reactive protein in predicting risk of atrial fibrillation (249) and coronary artery disease (250).

**Interpretation.** Evidence from animal models and humans indicates that complement factor 3 can promote fat deposition and is associated with common risk factors for CVD. Its utility in predicting CVD is growing stronger and in some cases supersedes C-reactive protein. Further population-based studies are required to determine its sensitivity and specificity in predicting CVD in obese individuals.

**Monocyte chemoattractant protein 1 (MCP-1).** In vitro studies. MCP-1 plays a role in the recruitment of monocytes to sites of injury and infection (251). Metabolic and inflammatory mediators such as insulin and TNF-α induce MCP-1 expression in murine adipocytes (252).

In vivo studies. MCP-1 is increased in adipose tissue and plasma of obese rodents (252, 253) and can modify expression of adipose genes involved in glucose and fat metabolism (252). Mice that overexpress MCP-1 in adipose tissue develop insulin resistance and glucose intolerance, whereas MCP-1 knockout mice fed a high-fat diet are resistant to these conditions compared with wild-type mice.

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fed the same diet (253). The role of MCP-1 in inflammation and atherosclerosis is demonstrated in some studies. MCP-1 is expressed by vascular endothelial cells and infiltrating macrophages during the early regenerative stages following ischemic injury (254). It localizes to sites of arterial damage in experimental atherosclerosis (255). Deletion of MCP-1 in mice susceptible to atherosclerosis results in a substantial reduction in the formation of atherosclerotic lesions (256).

Human studies. MCP-1 is present in macrophages and endothelial cells of atherosclerotic lesions (257) and is positively associated with obesity and coronary artery disease (258) in humans.

Clinical implications. Studies point to the potential association of MCP-1 with atherosclerosis, fat accumulation, and adverse cardiovascular risk, but prospective evidence for the role of MCP-1 in predicting CVD is still lacking.

CONCLUSIONS

Biomarkers associated with obesity may prove useful for early identification of susceptible individuals, and they may add more value to the attributable risk of developing overt CVD (259, 260). Biomarkers produced from adipose tissue, and those with roles in inflammation and oxidative stress, are increasingly being studied in humans. While some are investigated more than others, the majority lack consistency in their associations with both obesity and CVD.

The actions of biomarkers such as IL-18, angiotensin-converting enzyme genotype, F2 isoprostanes, oxLDL, and glutathione peroxidase are promising. Rigorous prospective evaluation and comparisons with traditional risk factors are required to enable us to determine their predictive power in the population. In addition, population-based cutoff values need to be addressed. Adiponectin is both a biomarker and possibly a mediator of CVD. The precise target of adiponectin is unknown, however, and the reason for gender differences in its expression as reported by some studies (52) needs to be elucidated. Since several human studies performed so far are cross-sectional or case-control, there is still a need for prospective assessment of adiponectin’s clinical utility and predictive power. Measurement methods for TNF-α, as well as site of adipose tissue biopsy, create variability in results across different studies. PAI-1 is an interesting biomarker in obesity because it is related to thrombosis, which can lead to vascular ischemia, but the active form of PAI-1 is unstable and has a half-life of 30 minutes (261). Despite its significance as an independent risk factor in many studies, its predictive power can be surpassed by insulin resistance and possibly other major risk factors. Evidence is increasing for a role of leptin and PPAR-γ in modulating immune responses and lipid metabolism; they may provide significant risk prediction with good clinical utility, but their contribution to cardiovascular risk has not been fully elucidated.

Although promising, the clinical utility of nontraditional biomarkers for obesity-related CVD is still limited mainly by lack of replication of findings and temporal associations. Obesity is an established inflammatory condition. More evidence is needed to demonstrate that oxidative stress is indeed a clinical correlate of obesity and just as strong as inflammation in promoting CVD. Part of the answer to this question lies in the choice of biomarker as well as in teasing out the reasons for lack of effect of antioxidant supplementation in intervention studies. It may be too early for clinicians to utilize some of the above biomarkers, but efforts must continue to validate their use in human populations in order to refine disease classification and personalize treatment modalities. A good example of an emerging biomarker is C-reactive protein, which is now widely used in predicting progression of chronic inflammatory conditions in clinical settings. However, C-reactive protein is not specific to obesity. There is a growing clinical need for biomarkers that are specific to obesity and that predict CVD risk. Studies that determine the therapeutic implications of these biomarkers beyond the prediction of metabolic risk factors or arterial damage are needed. Such nontraditional biomarkers may prove useful in predicting CVD risk, particularly in susceptible subgroups.

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