Inflammation, Oxidative Stress, and the Metabolic Syndrome

a report by

Sridevi Devaraj, PhD,[1] Rajeev Goyal, MD[2] *and* Ishwarlal Jialal, MD, PhD[3]

1. Associate Professor; 2. Research Fellow; 3. Director and Professor, The Laboratory for Atherosclerosis and Metabolic Research, Department of Medical Pathology and Laboratory Medicine, University of California, Davis Medical Center and Veterans Affairs Medical Centre, Mather

DOI: 10.17925/USE.2008.04.2.32

Metabolic Syndrome

The metabolic syndrome (MetS) comprises a cluster of metabolic abnormalities with insulin resistance and adiposity as its central features, which confer an increased propensity to diabetes and cardiovascular disease (CVD). The metabolic abnormalities include central obesity, dyslipidemia (high triglycerides, low high-density lipoprotein [HDL]), hypertension, and impaired fasting glucose (IFG). Five diagnostic criteria have been identified by the Adult Treatment Panel III (ATP-III) and the presence of any three features (central obesity, dyslipidemia [high triglycerides, low HDL], hypertension, and IFG) is considered sufficient to diagnose the syndrome.[1–3] Twenty-four percent of US adults have the MetS and the prevalence increases with age (44% at 60 years of age).[4]

The Metabolic Syndrome and Cardiovascular Disease

Subjects with MetS have an increased burden of CVD.[5–9] In the Kuopio Ischemic Heart Disease study, Lakka et al.[5] convincingly showed that men with the MetS, even in the absence of baseline coronary artery disease (CAD) or diabetes, had significantly increased mortality from CAD. In the Botnia Study,[6] the MetS was defined as the presence of at least two of the following risk factors: obesity, hypertension, dyslipidemia, and microalbuminuria. Cardiovascular mortality was assessed in 3,606 subjects with a median follow-up of 6.9 years. In women and men, respectively, the MetS was seen in 10 and 15% of subjects with normal glucose tolerance (NGT), 42 and 64% of those with IFG/impaired glucose tolerance (IGT), and 78 and 84% of those with type 2 diabetes. The risk for coronary heart disease (CHD) and stroke increased three-fold in subjects with MetS ($p<0.001$), and cardiovascular mortality was increased six-fold (12 versus 2.2%; $p<0.001$). Using data from the National Health and Nutrition Examination Survey (NHANES) III, Alexander et al.[7] also reported that the MetS is very common, with 44% of the US population over 50 years of age meeting the ATP-III criteria. Those with MetS without diabetes had a higher CHD prevalence (13.9%), and those with both MetS and diabetes had the highest prevalence of CHD (19.2%) compared with those with neither. MetS was a significant univariate predictor of prevalent CHD. The Hoorn Study examined 615 men and 749 women from 50 to 75 years of age without diabetes or a history of CVD at baseline and reported that the National Cholesterol Education Program (NCEP)-ATP-III definition of MetS was associated with about a two-fold increase in age-adjusted risk for fatal CVD in men and non-fatal CVD in women. The lower but significant risks were also obtained using the World Health Organization (WHO), the American College of Endocrinology (ACE), and the European Group on Insulin Resistance (EGIR) definitions of MetS. Also, Ford et al.,[4] using the modified NCEP-ATP-III criteria on the NHANES cohort, also reported a significantly increased prevalence of MetS in the US population.

The Metabolic Syndrome and Diabetes

Besides the effect on cardiovascular morbidity and mortality, the components of the MetS have been associated with diabetes. Factor analysis was used to identify the components of the MetS in 1,918 Pima Indians.[8] Insulin resistance factor was strongly associated with diabetes in a four-year follow-up. Furthermore, body size and lipid factor predicted diabetes, whereas blood pressure factor did not.

In the West of Scotland Coronary Prevention Study (WOSCOPS),[9] MetS increased the risk for CHD events and for diabetes. MetS continued to predict CHD events in a multivariate model incorporating conventional risk factors. Subjects with four or five features of the syndrome had a 3.7-fold increase in risk for CHD and a 24.5-fold increased risk for diabetes compared with those with none. The Prospective Cardiovascular Münster (PROCAM) study[10] also reported a 2.3-fold increased incidence of CVD in subjects with the MetS, and these effects persisted after adjustment for conventional risk factors.

Therefore, overall, MetS confers an increased propensity to both diabetes and CVD. While individual components of MetS independently contribute

Sridevi Devaraj, PhD, is an Associate Professor in the Laboratory for Atherosclerosis and Metabolic Research and Director of Toxicology and Special Chemistry in the Department of Pathology at the University of California (UC), Davis Medical Center in Sacramento. Dr Devaraj is also the Laboratory Core Director of the General Clinical Research Center and Co-Director of the Translational Program at UC Davis Medical Center.

Rajeev Goyal, MD, is a Post-doctoral Research Fellow in the Laboratory for Atherosclerosis and Metabolic Research in the Department of Pathology at the University of California, Davis Medical Center in Sacramento. His research interests are atherosclerosis, diabetes, and inflammation.

Ishwarlal Jialal, MD, PhD, is the Robert E Stowell Endowed Chair in Experimental Pathology, Director of the Laboratory for Atherosclerosis and Metabolic Research, and Professor of Internal Medicine and Pathology at the University of California, Davis Medical Center. Dr Jialal's research has been funded by the National Institutes of Health (NIH), the American Diabetes Association (ADA), the Juvenile Diabetes Research Foundation (JDRF), and the American Heart Association (AHA).

E: ijialal@ucdavis.edu

to increased cardiovascular risk in concert, they do not explain the increased propensity of vascular disease in subjects with MetS, and the precise mechanisms for this increased propensity remain to be elucidated. Inflammation is pivotal in all phases of atherosclerosis from foam cell formation to culmination in acute coronary syndromes. Furthermore, several lines of evidence demonstrate that diabetes is a pro-inflammatory state. It appears that low-grade chronic inflammation is a central feature of MetS and could contribute to increased risk for both CVD and diabetes in MetS.

Inflammation and the Metabolic Syndrome

A large number of investigators have consistently reported that the MetS is associated with low-grade systemic inflammation.

Pro-inflammatory Cytokines and the Metabolic Syndrome

Cytokines, in particular interleukin (IL)-1, tumor necrosis factor (TNF), and IL-6, are the main inducers of the acute phase response (APR). Several lines of evidence indicate that IL-6 is the central mediator of the inflammatory response.[11–14] IL-6 has been shown to directly impair insulin signaling in mouse hepatocytes and 3T3 adipocytes via decreased activation of insulin receptor substrate 1 (IRS-1) and PI3 kinase as well as impaired insulin-induced glycogenesis in liver cells. IL-6 knockout mice have an impaired inflammatory response.[11–14] IL-1 and TNF can induce IL-6. IL-1 knockout mice do not develop appreciable IL-6 and also have a diminished inflammatory response.[11–14] In addition, IL-6 subcutaneously administered to humans induces an acute inflammatory response.[11–14] Two important acute-phase proteins, C-reactive protein (CRP) and fibrinogen, have IL-6 response elements in the promoter regions of their genes; the presence of IL-6, therefore, may facilitate the production of CRP and fibrinogen. In fact, IL-6 is believed to be the main driver of CRP release from hepatocytes.[11–14] IL-6 plasma levels are significantly increased in murine and human insulin resistance and obesity and baseline IL-6 levels independently predict future cerebrovascular events (CVEs).[11–14]

Pickup et al.[15] have shown increased levels of IL-6 in subjects with more than two features of the MetS. Furthermore, we have also shown that in type 2 diabetes both CRP and monocyte release of IL-6 are significantly increased compared with patients without diabetes controls.[16] In a study on a group of 58 Pima Indians without diabetes, employing the hyperinsulinemic euglycemic clamp to assess insulin action, the results indicated that plasma IL-6 was positively related to adiposity and negatively related to insulin sensitivity. The investigators concluded that the relationship between IL-6 and insulin action appeared to be mediated through adiposity. Thus, IL-6 levels are closely related to features of the MetS.

IL-6 is an important adipocyte signaling molecule.[17] Adipose tissue IL-6 expression and circulating IL-6 concentrations are positively correlated with obesity, IGT, and insulin resistance.[11–14] Both expression and circulating levels decrease with weight loss. Furthermore, plasma IL-6 concentrations predict the development of type 2 diabetes and CVD. It is released from visceral and subcutaneous fat subsequent to sympathetic nervous system (SNS) activation (e.g. with stress). Approximately 25–30% of systemic IL-6 is derived from adipose tissue. IL-6 and free fatty acid fluxes are the pathogenic transmitters of the effects of obesity, the latter, together with corticosteroids amplifying insulin resistance thereby playing a role in the pathogenesis of MetS.

TNF is another pro-inflammatory cytokine secreted by monocyte-macrophages and endothelial cells, and also to a large extent by adipocytes.[18–21] Several studies have shown that levels of TNF are an important regulator of insulin sensitivity and that neutralization of TNF improves insulin sensitivity in leptin-receptor-deficient Zucker (fa/fa) rats. In human subjects, TNF messenger RNA (mRNA) and protein positively correlate with body adiposity and decrease in obese subjects with weight loss; however, insulin sensitivity was not studied.[18–21] Obesity is one of the major features of the MetS. Adipose tissue expression of TNF is increased in obese rodents and humans and is positively correlated with adiposity, and insulin resistance TNF levels also strongly correlate with the body mass index (BMI).[18–21] TNF is overexpressed in adipose and muscle tissues of obese individuals compared with tissues from lean individuals.[18–21]

IL-10 is a potent anti-inflammatory cytokine. In the Leiden 85-Plus study, subjects who developed diabetes and had more than three features of the MetS had lower levels of lipopolysaccharide (LPS)-stimulated whole blood release of IL-10 than those who did not.[22] In another study, Esposito et al.[23] examined the relationship of IL-10 to the MetS in obese women. As a group, obese women had higher circulating levels of IL-6, CRP, and IL-10 than non-obese women. In both obese and non-obese women, IL-10 levels were lower in those with than in those without the MetS. These results show that circulating levels of the anti-inflammatory cytokine IL-10 are elevated in obese women and that low IL-10 levels are associated with the MetS.

C-reactive Protein and the Metabolic Syndrome

The best characterized and standardized marker and mediator of inflammation and a predictor of future CVEs is CRP.[24] Numerous studies have now confirmed that CRP levels are elevated in subjects with the MetS. Yudkin et al.[25] conducted Z-score analyses in 107 patients without diabetes and found a significant correlation between inflammatory markers and several features of the MetS. CRP levels were shown to be strongly associated with insulin resistance calculated from the the Homeostasis Model Assessment (HOMA) model, blood pressure, low HDL, and triglycerides, and with levels of the pro-inflammatory cytokines, IL-6, and TNF. BMI and insulin resistance were the strongest determinants of the inflammatory state. There is a linear relationship between the number of metabolic features and increasing levels of high-sensitivity (hs)-CRP. Furthermore, Festa et al.[26] from the Insulin Resistance and Atherosclerosis Study (IRAS) showed that hsCRP was positively correlated with BMI, waist circumference, blood pressure, triglycerides, cholesterol, low-density lipoprotein (LDL) cholesterol, plasma glucose, and fasting insulin and inversely correlated with HDL cholesterol (HDL-C) and insulin sensitivity index. The strongest associations were observed between CRP levels, central adiposity, and insulin resistance.

The largest study to date that examined the association between inflammation and the MetS was the third NHANES study.[27] In a representative sample of the US population (8,570 participants >20 years of age), subjects with the MetS, defined using ATP-III criteria, were more likely than those without the syndrome to have elevated levels of markers of inflammation such as CRP, fibrinogen, and leukocyte count. Thus, there appears to be a clear relationship between the number of metabolic features and increasing hsCRP levels. In addition, we have shown that CRP levels were equivalent to the ratio of high-molecular-weight (HMW) adiponectin–CRP in predicting MetS using receptor-operated curve (ROC) analyses.[28] Furthermore, Sugiura et al.[29] have also reported that leptin (positively) and adiponectin (negatively)

were independently associated with CRP. Thus, MetS is a pro-inflammatory state characterized by increased CRP levels.

Elevated High-sensitivity C-reactive Protein Levels Predict Increased Cardiovascular Risk in Metabolic Syndrome

Evidence supporting the hypothesis that elevated CRP levels contribute to increased CV risk is now available from at least six major prospective studies, which include the Physicians' Health Study (PHS), the Women's Health Study (WHS), the Atherosclerosis Risk in Communities (ARIC), the Air Force Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TEXCAPS) in the US, and the Multinational Monitoring of Trends and Determinants in CVD (MONICA) and Reykjavik studies from Europe.[30–35]

> In an eight-year prospective follow-up of 14,719 women in the Women's Health Study, a high-sensitivity C-reactive protein level >3mg/l in patients with the metabolic syndrome predicted a greater age-adjusted relative risk for future cardiovascular events.

Taking a large-scale population cohort of the Women's Health Study (WHS), Ridker et al.[31] evaluated the potential inter-relationships between CRP, the MetS, and incident cardiovascular events. In an eight-year prospective follow-up of 14,719 women in the WHS, an hsCRP of >3mg/l in patients with MetS predicted a greater age-adjusted relative risk for future CVE. Furthermore, they reported that at all levels of severity of the MetS, CRP added prognostic information with regard to subsequent risk of incident CVE and was additive to the Framingham Risk Score. Thus, it has been proposed that hsCRP be added as a clinical criterion for MetS and for creation of an hsCRP-modified CHD risk score.[36]

In the WOSCOPS, in which 6,447 men were followed for 4.9 years, an hsCRP level >3mg/l predicted greater CVD risk in patients with the MetS in a multivariate model. In the Framingham Offspring Study, both CRP and MetS were independent predictors of new CV events, but were not additive. In an Italian study, patients with MetS and CRP >3mg/l had higher incidence of both carotid and coronary artery disease. Pischon et al. showed in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) that while MetS was a strong predictor of CHD in both men and women, CRP was additive in men only. It should be emphasized that in this study a modified definition of MetS was used as waist circumference, blood pressure, and glucose were not available at baseline. In a smaller Japanese study of 461 patients with acute myocardial infarction (AMI), CRP levels were additive to MetS in predicting future major adverse cardiac events. Furthermore, a recent investigation relating increased CRP levels and MetS in 1,044 older individuals (≥65 years of age) has also led to the conclusion that MetS is associated with low-grade systemic inflammation; the association is mainly supported by a strong independent correlation between waist circumference and high hsCRP levels. Collectively, all of these studies support the hypothesis that an increased CRP in the setting of MetS confers an increased risk of future CV events.[31–36]

Adipokines and Metabolic Syndrome

The adipose tissue was originally considered as a storage site of excess energy as triglycerides, but several studies now indicate that it is a hormonally active system.[37–41] Adipose tissue is now known to express and secrete a variety of bioactive peptides, known as adipokines, which act at both the local (autocrine/paracrine) and systemic (endocrine) level and secretes a series of adipocyte-derived biologically active molecules such as IL-6, TNF, plasminogen activator inhibitor-1 (PAI-1), leptin, adiponectin, etc. Adipose tissue excess or obesity, particularly in the visceral compartment, is associated with insulin resistance, hyperglycemia, dyslipidemia, hypertension, and pro-thrombotic and pro-inflammatory states.

Adiponectin

One of the most widely studied secreted adipokines is adiponectin. Adiponectin is an important link between adiposity, type 2 diabetes, and CVD. Circulating adiponectin concentrations are reduced in humans with obesity, type 2 diabetes, and CAD. Adiponectin-deficient mice exhibit diet-induced insulin resistance. In humans, low plasma adiponectin concentrations independently predict the development of type 2 diabetes and MI. Hypoadiponectinemia is also associated with MetS. Furthermore, adiponectin concentrations have been found in a number of studies to be inversely associated with systemic inflammation, as evidenced by increased concentrations of hsCRP.[42–47]

In the circulation, adiponectin is found in three major forms: as trimers (low molecular weight [LMW]), as hexamers (medium molecular weight [MMW]), and as larger multimers of 12–18 subunits (HMW). HMW adiponectin, but not the MMW form, lowers blood glucose concentrations in adiponectin-deficient mice.[42–51] Moreover, thiazolidinedione- and gastric-bypass-surgery-mediated improvements in insulin sensitivity are more closely associated with changes in HMW adiponectin than with total adiponectin concentrations. HMW adiponectin concentrations are also closely related to improvements in HDL-C following weight loss. A proteolytic cleavage product containing the globular domain of adiponectin also circulates at physiologically significant levels and has biological activity.

A strong correlation between low levels of adiponectin and increased insulin resistance has been well-established both *in vivo* and *in vitro* in animal models and in humans.[42–51] Adiponectin also improves insulin resistance *in vivo,* and patients with diabetes and obese subjects have lower levels of adiponectin compared with controls. Hypoadiponectinemia is associated with the MetS. Circulating adiponectin levels are significantly reduced in non-obese healthy first-degree relatives of patients with type 2 diabetes and correlate negatively with insulin and PAI-1, and positively with insulin sensitivity and HDL-C. In Asian Indians, plasma levels of adiponectin in subjects with IGT are a strong predictor of the development of diabetes. In a stepwise regression model of a study examining the association between CRP and adiponectin levels, hsCRP was independently associated inversely with levels of adiponectin and positively with levels of leptin. Adiponectin has been shown to improve insulin signaling and glucose uptake by skeletal muscle by activation of 5' adenosine monophosphate-activated protein kinase (AMPK)-associated signaling. However, the strongest association is with hepatic insulin sensitivity, where adiponectin is thought to increase insulin sensitivity through effects on fatty acid oxidation.

We evaluated whether hsCRP, adiponectin, or the ratio of adiponectin or its oligomers especially the HMW oligomer with hsCRP predict MetS in subjects with MetS compared with healthy controls.[28,52] One hundred and twenty-three subjects with MetS and 91 healthy controls were studied. MetS subjects had significantly higher hsCRP and lower total adiponectin and its oligomers relative to controls ($p<0.0001$). The ratio of HMW–total adiponectin and the adiponectin–CRP ratio was significantly lower in MetS subjects compared with controls ($p<0.005$). The odds ratio (OR) of MetS using the 75th percentile cut-off of CRP was 3.8 (95% confidence interval [CI] 2.1–6.8) and equivalent to either low total adiponectin (OR 2.5, 95% CI 1.3–4.5), its oligomers or the adiponectin–hsCRP ratio (OR 2.6, 95% CI 1.5– 4.8). Thus, measurements of CRP, adiponectin, or its oligomers provide robust biomarkers for predicting MetS.

Several mechanisms for adiponectin's metabolic effects have been described.[42–51] In the liver, adiponectin enhances insulin sensitivity, decreases influx of non-esterified fatty acids (NEFAs), increases fatty acid oxidation, and reduces hepatic glucose output. In muscle, adiponectin stimulates glucose use and fatty acid oxidation. Within the vascular wall, adiponectin inhibits monocyte adhesion by decreasing expression of adhesion molecules, inhibits macrophage transformation to foam cells by inhibiting expression of scavenger receptors, and decreases proliferation of migrating smooth-muscle cells in response to growth factors. In addition, adiponectin increases nitric oxide (NO) production in endothelial cells and stimulates angiogenesis. These effects are mediated via increased phosphorylation of the insulin receptor, activation of AMP-activated protein kinase, and modulation of the nuclear factor B pathway.[42–51] Adiponectin receptors (AdipoR) 1 and 2 have been identified. The biological effects of adiponectin depend not only on the relative circulating concentrations and properties of the different adiponectin isoforms but also the tissue-specific expression of the adiponectin receptor subtypes.

Taken together, these studies suggest that adiponectin is a unique adipocyte-derived hormone with antidiabetic, anti-inflammatory, and anti-atherogenic effects.

Leptin

Leptin is a 16kDa polypeptide containing 167 amino acids with structural homology to cytokines.[53–57] Adipocytes secrete leptin in direct proportion to adipose tissue mass as well as nutritional status, and this secretion is greater from subcutaneous relative to visceral adipose tissue.[53–57] Leptin expression and secretion are also regulated by a variety of other factors. For example, leptin is increased by insulin, glucocorticoids, TNF, estrogens, and cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer-binding protein, and decreased by β3-adrenergic activity, androgens, free fatty acids, growth hormone (GH), and peroxisome proliferator-activated receptor agonists.[53–57] Obesity is known to be associated with hyperleptinemia, reflecting resistance to leptin because obese subjects remain overweight despite the high circulating levels of leptin. Neither endogenously high leptin levels nor treatment with exogenous leptin is effective in ameliorating this obesity, consistent with a state of leptin resistance.[53–57] In a major prospective cohort (WOSCOPS), serum leptin levels have been independently associated with CHD.[58] In addition, leptin levels independently predict future cardiovascular events in subjects with established angiographic coronary lesions.[53–57]

With regard to leptin in MetS, in a prospective population-based survey examining 888 subjects 40–79 years of age, subjects were identified fulfilling the WHO and the National Cholesterol Education Program-ATP-III (NCEP-ATP-III) criteria for diagnosing the MetS and had higher levels of leptin compared with matched controls.[53–57]

Resistin

Another adipocyte-derived cytokine that has been recently isolated is resistin, and it has been suggested that this protein impairs glucose tolerance.[59–61] Circulating resistin levels are increased in mouse models of obesity and in obese humans, are decreased by the antidiabetic drug rosiglitazone, and are increased in diet-induced and genetic forms of obesity, and administration of antiresistin antibody has been shown to improve blood sugar and insulin action in mice with diet-induced obesity.[59–61] Similarly, resistin has been implicated in the pathogenesis of diabetic complications and diabetes.[58–60] The source of resistin is now under dispute as it may not come directly from the adipocytes, and may rather originate from inflammatory cells (macrophages) infiltrating the fat tissue. However, numerous epidemiological studies in humans have failed to provide a clear and consistent link between resistin expression in adipose tissue or circulating resistin levels and adiposity or insulin resistance.[59–61] Much further research is needed to determine the role of resistin in the MetS in humans.

Retinol-binding Protein 4

Recently, retinol-binding protein 4 (RBP4) was identified as a novel adipokine that is increased in different animal models of insulin resistance.[62–64] It increases hepatic expression of the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK), and impairs insulin signaling and action in skeletal muscle. Furthermore, circulating serum levels of RBP4 in man correlate inversely with insulin sensitivity measured with the euglycemic–hyperinsulinemic clamp technique.[62–64] It is elevated not only in subjects with obesity, type 2 diabetes, and IGT, but also in normoglycemic and insulin-resistant subjects with a strong family history of type 2 diabetes and in subjects with the MetS; however, the relationship is not always consistent across studies.[62–65] Therefore, among the adipokines, low levels of adiponectin are associated with MetS; however, more studies need to be performed to evaluate the role of leptin and resistin in the MetS.

The source of resistin is now under dispute as it may not come directly from the adipocytes, and may rather originate from inflammatory cells (macrophages) infiltrating the fat tissue.

Monocyte Chemoattractant Protein-1

Monocyte chemoattractant protein-1 (MCP-1), a chemokine that recruits monocytes to sites of inflammation, plays an important role in atherosclerosis, and deletion of MCP-1 or its receptor chemokine (C-C motif) receptor 2 (CCR2) in rodent models of atherosclerosis significantly abrogates macrophage recruitment into the lesion and results in decreased atherosclerotic lesions.[66–68] MCP-1 levels are increased in diabetes and states of insulin resistance. Obesity is associated with increased levels of

Table 1: Biomarkers of Inflammation in Metabolic Syndrome

Increased C-reactive protein
Increased interleukin 6, tumor necrosis factor
Decreased interleukin 10
Decreased adiponectin
Increased leptin
Increased serum amyloid A
Increased plasminogen activator inhibitor-1

Table 2: Biomarkers of Oxidative Stress in Metabolic Syndrome

Increased oxidized low-density lipoprotein
Increased nitrotyrosine
Increased nicotinamide adenine dinucleotide phosphate-oxidase and monocyte superoxide
Decreased levels of reduced glutathione

MCP-1 and its receptor CCR2 on monocytes. Obesity is associated with increased adipose tissue infiltration by macrophages.[66–68] Activated macrophages in the adipose tissue secrete MCP-1. Several studies indicate that MCP-1-mediated macrophage infiltration of adipose tissue may contribute to the metabolic abnormalities associated with obesity and insulin resistance. MCP-1 decreases insulin-stimulated glucose uptake and insulin-induced insulin receptor tyrosine phosphorylation, suggesting that MCP-1 directly contributes to adipose tissue insulin resistance.[66–68] Inhibition of CCR2 has been shown to improve obesity and type 2 diabetes by interfering with macrophage infiltration of the adipose tissue and decreasing resultant inflammation.

Serum Amyloid A

Serum amyloid A (SAA) is an acute-phase reactant whose level in the blood increases in response to various insults. It is expressed in the liver, but its physiological role is not well understood. In the Cholesterol and Recurrent Events (CARE) study, Ridker et al.[69] showed that levels of CRP and SAA after MI were independently associated with increased risk for recurrent coronary events. Increased levels of SAA have been reported in patients with type 2 diabetes and with IGT, and Pickup et al.[15] have shown that, like CRP, SAA levels increase with an increase in the number of features of the MetS. Insulin resistance and adiposity correlate to increased levels of SAA in subjects with type 2 diabetes.[70] Thus, emerging evidence points to increased SAA levels as an additional marker of inflammation in the MetS; however, this needs to be confirmed in future studies.

Plasminogen Activator Inhibitor-1 and the Metabolic Syndrome

Population studies of people without diabetes have shown that features of the MetS cluster with coagulation and fibrinolytic proteins. PAI-1 is a member of the serine protease inhibitor family and is the primary inhibitor of fibrinolysis by inactivating urokinase-type and tissue-type plasminogen activator. The hemostatic abnormality that has been most consistently associated with insulin resistance is an elevated PAI-1 level.[71–77] Plasma PAI-1 levels are elevated in obesity and insulin resistance, are positively correlated with features of the MetS (inclusive of hyperinsulinemia, hypertension, high triglycerides, low HDL-C, and small LDL particles), and predict future risk for type 2 diabetes and CVD.[71–77]

The European Concerted Action on Thrombosis and Disabilities (ECAT) Angina Pectoris Study examined the associations between 14 hemostatic markers, fasting insulin levels, and variables related to the MetS (history of diabetes, BMI, systolic blood pressure, triglycerides, and HDL-C) in 1,484 patients with angina pectoris.[71–77] The Cardiovascular Health Study (CHS) investigated clustering of metabolic risk factors (lipids, body mass, insulin/glucose, and blood pressure) associated with the MetS and hemostatic factors that included markers of procoagulant activity, inflammation-sensitive proteins, vitamin-K-dependent proteins, and vitamin-K-dependent coagulation.[71–77] In the Framingham Offspring Study, men and women with NGT had significant positive associations between levels of fasting insulin and fibrinolytic proteins (PAI-1 antigen and tissue plasminogen activator [tPA]), inflammatory mediators (fibrinogen and plasma viscosity), von Willebrand factor (vWF) antigen, and factor VII antigen.[71–77] Among subjects with glucose intolerance (IFG or IGT and undiagnosed diabetes), only markers of impaired fibrinolysis (PAI-1 and tPA antigen) were significantly associated with hyperinsulinemia. In a cross-sectional study of 1,276 adults of South Asian, Chinese, European, and Native Indian ancestry from four communities in Canada, subjects with the MetS had higher levels of PAI-1 (24.2 versus 14.6U/ml; p=0.001), more atherosclerosis (maximum intimal medial thickness 0.78±0.18 versus 0.74±0.18mm; p=0.0005) and a higher prevalence of CVD (17.2 versus 7.0%; p=0.0001).[71–77]

The mature adipocyte is an important source of circulating PAI-1 in humans. Severely obese adults have two-fold higher adipose tissue PAI-1 mRNA levels and seven-fold higher plasma PAI-1 protein levels than observed in lean adults.[71–77] Thus, PAI-1 may contribute to the development of obesity and insulin resistance and may be a causal link between obesity and CVD.

In summary, the MetS is a pro-inflammatory state as evidenced by increased levels of IL-6, TNF as well as CRP, SAA and leptin, PAI-1, and lower levels of adiponectin and IL-10 (see *Table 1*), and that each of the features of the MetS in effect contribute to this pro-inflammatory state. While CRP levels are strongly related to both insulin resistance and adiposity, currently it is difficult to conclude whether low-grade inflammation induces insulin resistance and the MetS or is a consequence. A reasonable proposal is that an imbalance in favor of pro-inflammatory cytokines from adipose tissue (hypoadiponectinemia and increased IL-6) and other sources triggers CRP secretion. In turn, this can exacerbate mild insulin resistance and result in accentuation of other metabolic abnormalities that constitute the MetS.

Oxidative Stress and Metabolic Syndrome

Oxidative stress may be defined as an imbalance between production and degradation of reactive oxygen species (ROS). Several lines of evidence point to the role of increased oxidative stress in CVD. In fact, diabetes is a pro-oxidant state characterized by increased LDL oxidation, increased monocyte superoxide, increased nitrotyrosine, and urinary F2-isoprostanes. Several lines of evidence indicate that MetS may be a pro-oxidant state[78–81] (see *Table 2*). The levels of lipid peroxidation products, carbonylated proteins, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidases (NOXs), and catalase activity were found to be high, while reduced glutathione (GSH) levels were observed in MetS patients compared with healthy controls.[78–81] Oxidative stress, mainly superoxide, plays a critical role

in the pathogenesis of MetS parameters. Fortuno et al.[78–81] have reported increased mononuclear cell activation in MetS compared with control subjects. They also demonstrated increased superoxide, nitrotyrosine, and oxidized LDL in MetS compared with controls, although MetS subjects studied in this report were on various medications including statins (39%) as well as oral hyperglycemics (21%).

A diet rich in fats also produces a state of oxidative stress. Fat-overloaded patients with MetS also showed an increase in lipid peroxidation products, carbonylated proteins, and GSH and a reduction in NOX and catalase activity in comparison with healthy controls. It was also shown that fat overload increases total glutathione and GSH levels, indicating that the antioxidative system is trying to neutralize the oxidative stress but is overwhelmed.[78–81] NOXs are electron transporter enzymes that catalyze the production of superoxide from oxygen, which is the precursor of other ROS. In mammals, six NOX homologues have been identified.[78–81] Most mammalian cells express at least one NOX. Activity and expression of NOX2 in phagocytic cells was found to be increased in subjects with MetS.[78–81] MetS patients also showed increased levels of nitrotyrosine. The reaction of superoxide with nitrous oxide leads to production of peroxynitrite, a powerful oxidant molecule. The association of NOX overactivity with high nitrotyrosine levels suggests the possibility that phagocytic NOX may be involved in endothelial dysfunction in MetS.[78–81] Furthermore, patients with MetS were found to have a lower concentration of markers of NO formation, which included whole blood nitrite, plasma nitrite, and cyclic guanosine monophosphate (cGMP).[78–81]

Conclusion

Emerging laboratory and clinical evidence have proved that there is a strong relationship between inflammation, especially high levels of CRP and PAI-1, low levels of adiponectin, and measures of oxidative stress and various features of MetS. The evaluation of biomarkers of inflammation and oxidative stress in MetS may help identify subjects at high risk for future diabetes and CVD. ■

Acknowledgments

Grants from National Institutes of Health (NIH) (SD and IJ) and American Diabetes Association (ADA) (IJ).

1. Reaven GM, *Annu Rev Nutr*, 2005;25: 391–406.
2. Eckel RH, Grundy SM, Zimmet PZ, *Lancet*, 2005;365(9468): 1415–28.
3. Haffner S, Cassells HB, *Diabetes Obes Metab*, 2003;5(6):359–70.
4. Ford ES, *Diabetes Care*, 2005;28(11):2745–9.
5. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, *JAMA*, 2001;285:2486–97.
6. Lakka HM, Laaksonen DE, Lakka TA, et al., *JAMA*, 2002;288(21):2709–16.
7. Alexander CM, Landsman PB, Teutsch SM, et al., *Diabetes*, 2003;52(5):1210–14.
8. Hanson RL, Imperatore G, Bennett PH, Knowler WC, *Diabetes*, 2002;51(10):3120–27.
9. Freeman D, Norrie J, Sattar N, et al. *Circulation*, 2001;103:357–62.
10. Assmann G, Nofer JR, Schulte H, *Endocrinol Metab Clin North Am*, 2004;33(2):377–92.
11. Heinrich PC, Castell JV, *Biochem J*, 1990;265:621–36.
12. Ridker PM, Hennekens CH, Buring JE, Rifai N, *N Engl J Med*, 2000;342(12): 836–43.
13. Heinrich PC, Castell JV, Andus T, *Biochem J*, 1990;265(3):621–36.
14. Vozarova B, Weyer C, Hanson K, et al., *Obes Res*, 2001;9:414–17.
15. Pickup JC, Mattock MB, Chusney GD, et al., *Diabetologia*, 1997;40:1286–92.
16. Devaraj S, Jialal I, *Free Radic Biol Med*, 2000;29(8):790–92.
17. Fasshauer M, Paschke R, *Diabetologia*, 2003;46(12): 1594–1603.
18. Moller DE, *Trends Endocrinol Metab*, 2000;11(6):212–17.
19. Hotamisligil GS, *Science*, 1993; 259:87–91.
20. Kern PA, Saghizadeh M, Ong JM, et al., *J Clin Invest*, 1995;95:2111–19.
21. Dandona P, Weinstock R, Thusu K, et al., *J Clin Endocrinol. Metab*, 1998;83:2907–10.
22. Van Exel E, Gussekloo J, de Craen AJ et al., *Diabetes*, 2002; 51(4):1088–92.
23. Esposito K, Pontillo A, Giugliano F, et al., *J Clin Endocrinol Metab*, 2003; 88(3):1055–8.
24. Jialal I, Devaraj S, *Am J Clin Pathol*, 2001;(Suppl. 116):S108–15.
25. Yudkin JS, Kumari M, Humphries SE, et al., *Atherosclerosis*, 2000;148:209–14.
26. Festa A, D'Agostino R Jr, Howard G, et al., *Circulation*, 2000;102(1):42–7.
27. Vu JD, Vu JB, Pio JR, et al., *Am J Cardiol*, 2005;96(5):655–8.
28. Devaraj S, Swarbrick MM, Singh U, et al., *Am J Clin Pathol*, 2008;129(5): 815–22.
29. Sugiura K, Tamakoshi K, Yatsuya H, et al., *Int J Cardiol*, 2008;130(2):159–64.
30. Ridker PM, Glynn RJ, Hennekens CH, *Circulation*, 1998;97(20): 2007–11.
31. Ridker PM, Buring JE, Cook NR, Rifai N, *Circulation*, 2003;107(3):391–7.
32. Ballantyne CM, Hoogeveen RC, Bang H, et al., *Arch Intern Med*, 2005;165(21):2479–84.
33. Downs JR, Clearfield M, Weis S, et al., *JAMA*, 1998;279(20): 1615–22.
34. Koenig W, Khuseyinova N, Baumert J, Meisinger C, *Clin Chem*, 2008;54(2):335–42.
35. Eiriksdottir G, Aspelund T, Bjarnadottir K, et al., *Atherosclerosis*, 2006;186(1): 222–4.
36. Ridker PM, Wilson PW, Grundy SM, *Circulation*, 2004;109:2818–25.
37. Wozniak SE, Gee LL, Wachtel MS, Frezza EE, *Dig Dis Sci*, 2008; epub ahead of print.
38. Ye J, *Int J Obes (Lond)*, 2008; epub ahead of print.
39. Rasouli N, Kern PA, *J Clin Endocrinol Metab*, 2008;93(11 Suppl. 1): S64–73.
40. Attie AD, Scherer PE, *J Lipid Res*, 2008; epub ahead of print.
41. Oda E, *Hypertens Res*, 2008;31(7):1283–91.
42. Chandran M, Phillips SA, Ciaraldi T, Henry RR, *Diabetes Care*, 2003;26: 244–50.
43. Kondo H, Shimomura I, Matsukawa YE, et al., *Diabetes*, 2002;51(7):2325–8.
44. Weyer C, Funahashi T, Tanaka S, et al., *J Clin Endocrinol Metab*, 2001; 86(5):1930–35.
45. Matsuzawa Y, Funashi T, Kihar S, Shimomura I, *Arterioscler Thromb Vasc Biol*, 2004;24: 29–33.
46. Pellme F, Smith U, Funahashi T, et al., *Diabetes*, 2003;52:1182–6.
47. Snehalatha C, Mukesh B, Simon M, et al., *Diabetes Care*, 2003;26:3226–9.
48. Lu JY, Huang KC, Chang LC, et al., *J Biomed Sci*, 2008;15(5):565–76.
49. Phillips LK, Prins JB, *Curr Hypertens Rep*, 2008;10(2):156–64.
50. Sutherland JP, McKinley B, Eckel RH, *Metab Syndr Relat Disord*, 2004;2(2):82–104.
51. Beltowski J, Jamroz-Wisniewska A, Widomska S, *Cardiovasc Hematol Disord Drug Targets*, 2008;8(1):7–46.
52. Devaraj S, Torok N, Dasu MR, et al., *Arterioscler Thromb Vasc Biol*, 2008;28(7):1368–74.
53. Beltowski J, *Atherosclerosis*, 2006; 189(1):47–60.
54. Martin SS, Qasim A, Reilly MP, *J Am Coll Cardiol*, 2008;52(15):1201–10.
55. Dubey L, Hesong Z, *Exp Clin Cardiol*, 2006;11(4):269–75.
56. Lago R, Gómez R, Lago F, et al., *Cell Immunol*, 2008;252(1–2):139–45.
57. Matarese G, Mantzoros C, La Cava A, *Curr Pharm Des*, 2007;13(36):3676–80.
58. Wallace AM, McMahon AD, Packard CJ, et al., *Circulation*, 2001;104(25):3052–6.
59. Calabrò P, Limongelli G, Pacileo G, et al., *J Cardiovasc Med (Hagerstown)*, 2008;9(5):450–60.
60. Asano T, Sakosda H, Fujishiro M, et al., *Curr Diabetes Rev*, 2006;2(4):449–54.
61. Gómez-Ambrosi J, Frühbeck G, *Curr Diabetes Rev*, 2005;1(3):227–34.
62. Bárány P, Lindholm B, Stenvinkel P, *Am J Nephrol*, 2008;29(5):447–53.
63. Kowalska I, Straczkowski M, Adamska A, et al., *J Clin Endocrinol Metab*, 2008;93(7):2786–9.
64. Lewis JG, Shand BI, Elder PA, Scott RS, *Diabetes Res Clin Pract*, 2008;80(1):e13–15.
65. Takebayashi K, Suetsugu M, Wakabayashi S, et al., *J Clin Endocrinol Metab*, 2007;92(7):2712–19.
66. Tamura Y, Sugimoto M, Murayama T, et al., *Arterioscler Thromb Vasc Biol*, 2008;28(12):2195–2201.
67. Blanco-Colio LM, Martín-Ventura JL, de Teresa E, et al., *Am Heart J*, 2007;153(5):881–8.
68. Linton MF, Fazio S, *Int J Obes Relat Metab Disord*, 2003;27 (Suppl. 3):S35–40.
69. Ridker PM, Rifai N, Pfeffer MA, et al., *Circulation*, 1998;98(9):839–44.
70. Yang RZ, Lee MJ, Hu H, et al., *PLoS Med*, 2006;3(6):e287.
71. Vague P, Juhan-Vague I, Chabert V, et al., *Metabolism*, 1989;38:913–15.
72. McGill JB, Schneider DJ, Arfken CL, et al., *Diabetes*, 1994;43:104–9.
73. Janand-Delenne B, Chagnaud C, Raccah D, et al., *Int J Obes Relat Metab Disord*, 1998;22:312–17.
74. Sakkinen PA, Wahl P, Cushman M, et al., *Am J Epidemiol*, 2000;152:897–907.
75. Juhan-Vague I, Pyke SD, Alessi MC, et al., *Circulation*, 1996;94:2057–63.
76. Festa A, D'Agostino R Jr, Mykkanen L, et al., *Arterioscler Thromb Vasc Biol*, 1999;19:605–10.
77. Meigs JB, Mittleman MA, Nathan DM, et al., *JAMA*, 2000;283:221–8.
78. Gomes VA, Casella-Filho A, Chagas AC, *Nitric Oxide*, 2008;19(4):345–50.
79. Fortuno A, Jose GS, Moreno MU, et al., *Diabetes*, 2006;55:209–15.
80. Bedard K, Krause H, *Physiol Rev*, 2007;87: 245–313.
81. Cardona F, Túnez I, Tasset I, et al., *EJCI*, 2008; 38(7),510–15.