Vascular biology of metabolic syndrome

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The metabolic syndrome is a constellation of risk factors comprising atherogenic dyslipidemia (low high-density lipoprotein and high triglycerides levels), elevated blood pressure, elevated plasma glucose, a prothrombotic state, and a proinflammatory state accompanied by an increased risk for cardiovascular disease and type 2 diabetes mellitus. The adipose tissue of obese humans contains increased numbers of macrophages, and once activated, these macrophages are responsible for the expression of most of the tissue’s tumor necrosis factor (TNF)-α and interleukin (IL)-6. Chronic inflammation associated with visceral obesity induces altered lipoprotein metabolism and insulin resistance in the liver. Adipocytes secrete a variety of hormones, cytokines, growth factors, and other bioactive substances, conceptualized as adipocytokines, including plasminogen activator inhibitor 1 (PAI-1), TNF-α, leptin, and adiponectin. The dysregulation of these adipocytokines contributes to the pathogenesis of obesity. Adipose tissue-resident macrophages and adipocytes in the adipose tissue combined with the consequences of hyperglycemia, altered lipoproteins, and hyperinsulinemia in the vasculature and within organ microcirculation lead to dysfunctional endothelia and a proinflammatory state. Metabolic syndrome thus represents a combination of synergistic vascular pathologies that lead to an accelerated atherogenic state that compromises the ability of the patient to satisfactorily respond to humoral, cellular, and mechanical stresses. (J Vasc Surg 2011;54:819-31.)

Clinical Relevance: The incidence of metabolic syndrome is rapidly approaching epidemic levels. Cardiovascular morbidity and mortality increases 1.5-fold to 3-fold in the presence of the metabolic syndrome. Metabolic syndrome is a constellation of risk factors of metabolic origin characterized by hyperinsulinemia, low glucose tolerance, and truncal obesity. The syndrome is associated with atherogenic dyslipidemia (low high-density lipoprotein and high triglycerides levels), elevated blood pressure, elevated plasma glucose, and a prothrombotic and a proinflammatory state that act synergistically to produce a proinflammatory prothrombotic state in the vascular patient. Identifying the patient with metabolic syndrome and understanding its biology is key to developing preventive interventions and therapeutic strategies.

The metabolic syndrome is a constellation of risk factors of metabolic origin that are accompanied by an increased risk for cardiovascular disease and type 2 diabetes mellitus. Insulin resistance, which results in this risk-factor clustering, may contribute to many of the untoward outcomes attributed to the metabolic syndrome.1 The clinical risk factors are atherogenic dyslipidemia, defined as low high-density lipoprotein (HDL) and high triglycerides levels, elevated blood pressure and plasma glucose levels, a prothrombotic state, and a proinflammatory state (Fig 1). The metabolic syndrome is also characterized by hyperinsulinemia, low glucose tolerance, and truncal obesity. Lipid profile abnormalities, such as elevated serum triglyceride and reduced HDL levels, have been found in most studies of intermittent claudication,2 and there is a strong inverse relationship between HDL levels and claudication severity.3 An estimated 50% of diabetic patients have evidence of peripheral artery disease.4 However, a study of 100 consecutive nondiabetic patients attending a vascular clinic showed abnormal results for glucose tolerance tests in 40%.5 This was despite all 40 patients having random or fasting blood glucose levels that were within normal reference ranges.

Two definitions of metabolic syndrome predominate in the literature, the National Cholesterol Education Program (NCEP) and the World Health Organization (WHO; Table 1). Metabolic syndrome is defined as the presence of three or more of the following: (1) waist circumference ≥88 cm in women and ≥102 cm in men; (2) fasting triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides; (3) HDL-cholesterol <50 mg/dL in women and <40 mg/dL in men or drug treatment for reduced HDL-cholesterol; (4) blood pressure ≥130/85 mm Hg or use of blood pressure-lowering medication; and (5) fasting glucose ≥100 mg/dL or use of glucose-lowering medication.6 If waist circumference is not available, a body mass index (BMI) >30 kg/m² can be used as a determinant for abdominal obesity.7

The prevalence of metabolic syndrome in the adult population in developing countries is 22% to 39% and varies depending on the definition used and on ethnicity.8-10 The WHO or NCEP definitions (Table 1) both associate metabolic syndrome with future coronary heart disease events and type 2 diabetes. Both definitions will predict cardiovascular mortality, whereas the NCEP definition can predict all-cause morbidity.
Adipose tissue possesses a relatively dense network of blood capillaries, ensuring adequate exposure to nutrients and oxygen. WAT varies in its vascularity, both between depots and within the tissue. The balance between angiogenesis and hypoxia has a significant impact on the modulation of “good” vs “bad” tissue expansion, thereby implicating the local microvasculature as a key modulator of the systemic impact of adipose depots.

The adipose tissue of obese humans contains increased numbers of macrophages, and once activated, these macrophages secrete a range of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1. The macrophages resident in the adipose tissue are responsible for the expression of most of the tissue’s TNF-α and IL-6. The expression of macrophage markers in human adipose tissue is high in subjects with obesity and insulin resistance, and can be correlated with the expression of TNF-α and IL-6.

Recent studies suggest that macrophages infiltrate adipose tissue as part of a scavenger function in response to adipocyte necrosis. Immunohistologic studies of human adipose tissue have demonstrated that most of the macrophages in adipose tissue surround dead adipocytes and form a syncytium, often referred to as a “crown-like structure.”

There is evidence suggesting that the vascular network forms before the mature lipid-carrying adipocytes reside in the area, thus providing access of secreted cytokines and adipokines to the circulation. Despite this great angiogenic potential, the rapidly expanding fat pad still experiences hypoxia and necrosis. The induction of adipocyte hypoxia in vitro results in the expression of a number of inflammatory cytokines.

The adipose tissue expandability hypothesis states that a failure in the capacity for adipose tissue expansion, rather than obesity per se, is the key factor linking positive energy balance and type 2 diabetes. Each individual possesses a maximum capacity for adipose expansion, which is determined by genetic and environmental factors. Once the limit of adipose tissue expansion is reached, adipose tissue ceases to store energy efficiently and lipids begin to accumulate in other tissues. Ectopic lipid accumulation in nonadipocyte cells induces insulin resistance, apoptosis, and inflammation.

Adipocytes are potent sources of such adipokines, which mediate many parts of the biology encountered. The two types of adipokotkines are (1) adipose tissue-specific bioactive substances (true adipokines) and (2) adipokines that are abundantly secreted from adipose tissue but are not specific for adipose tissue.
**Table I. Definitions of metabolic syndrome**

<table>
<thead>
<tr>
<th>Variable</th>
<th>NCEP ATP III</th>
<th>WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolutely required</td>
<td>None</td>
<td>Insulin resistance (IGT, IFG, T2D, or other evidence of IR)</td>
</tr>
<tr>
<td>Criteria</td>
<td>Any 3 of the 5 criteria below:</td>
<td>IR or diabetes, plus 2 of the 5 criteria below:</td>
</tr>
<tr>
<td>Obesity</td>
<td>Waist circumference: ≥40 in (M), ≥35 in (F)</td>
<td>Waist/hip ratio: &gt;0.90 (M), &gt;0.85 (F); or BMI ≥30 kg/m²</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>Fasting glucose 100 mg/dL or on therapy</td>
<td>Triglycerides ≥150 mg/dL, or on therapy</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Triglycerides ≥150 mg/dL, or on therapy</td>
<td>IR already required</td>
</tr>
<tr>
<td>Dyslipidemia (second, separate criteria)</td>
<td>HDL-C &lt;40 (M), ≤50 mg/dL (F); or on therapy</td>
<td>Triglycerides ≥150 mg/dL, or HDL-C &lt;40 mg/dL (M), &lt;45 mg/dL (F)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>&gt;130 mm Hg systolic or &gt;85 mm Hg diastolic; or on therapy</td>
<td>≥140/90 mm Hg</td>
</tr>
</tbody>
</table>

ATP, Adult Treatment Panel III; BMI, body mass index; F, female; HDL-C, high-density lipoprotein cholesterol; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; IR, insulin resistance; M, male; NCEP, National Cholesterol Education Program; T2D, type 2 diabetes; WHO, World Health Organization.

**Lipoprotein metabolism.** Obesity, an increased mass of activated adipocytes and their linked changes in lipoproteins, is a major component in the biology of metabolic syndrome. Chronic inflammation associated with visceral obesity induces altered lipoprotein metabolism and insulin resistance in the liver. Abnormalities in the transport of lipoprotein diminish the catabolism of very low-density lipoprotein (VLDL) and increase the catabolism of HDL, which creates insulin resistance. This process is associated with a lower concentration of the adipokine adiponectin (vide infra) that in turn regulates the catabolism of VLDL and HDL, consequently increasing the flow of fatty acids from the adipose tissue to the liver and muscles.

Accumulation of lipid metabolites within nonadipose tissues can induce chronic inflammation by promoting macrophage infiltration and activation and tissue damage. Oxidized and glycerated lipoproteins, free fatty acids (FFAs), free cholesterol, triacylglycerols, diacylglycerols, and ceramides induce cellular dysfunction through their proinflammatory and proapoptotic properties. Emerging evidence also suggests that macrophage activation by lipid metabolites and further modulation by lipid signaling represents a common pathogenic mechanism underlying lipotoxicity in atherosclerosis, obesity-associated insulin resistance, liver steatosis, and chronic kidney disease. Lipid derivatives, through modulation of macrophage function, promote plaque instability in the arterial wall, impair insulin responsiveness, and contribute to inflammatory liver, muscle, and kidney disease.

In metabolic syndrome, the regulation of fat storage and energy supply by adipose tissue is impaired, leading to elevated plasma FFA levels, excessive metabolism of FFAs, and high levels of FFA metabolites in nonadipose tissue. FFAs and their metabolites act as metabolic mediators of insulin resistance. It has been proposed that insulin resistance occurs in adipose tissue before muscle insulin resistance. In adipose tissue, insulin resistance of obesity is characterized by an inadequate insulin action in the fed state that resembles conditions in the normal fasting state. This results in the release of FFAs into the circulation to deliver energy to skeletal muscle. Thus, postprandial FFAs are directly transported to the skeletal muscles and the liver, where energy is not needed. FFAs and their metabolites also act as signaling molecules interacting with insulin signaling and have direct effects on glucose transport. Reducing ectopic fat in the skeletal muscle of diabetic patients might therefore be a promising target for diabetes therapy and might partly explain the action of thiazolidinedione, a drug used to treat diabetes.

**Adipokines.** Adipocytes secrete a variety of hormones, cytokines, growth factors, and other bioactive substances, conceptualized as adipocytokines, including plasminogen activator inhibitor 1 (PAI-1), TNF-α, leptin, and adiponectin (Table II). Dysregulated production of these adipocytokines is part of the pathogenesis of metabolic syndrome. Increased productions of PAI-1 and TNF-α from accumulated fat contribute to the formation of thrombosis and insulin resistance in obesity, respectively. A lack of leptin causes metabolic syndrome. Adiponectin exerts insulin-sensitizing and antiatherogenic effects; hence, a decrease of plasma adiponectin is causative for insulin resistance and atherosclerosis in obesity.

**Leptin.** Leptin is a 167-amino acid hormone secreted largely by adipose tissue that controls food intake and energy expenditure. Circulating levels of leptin parallel fat cell stores, increasing with overfeeding and decreasing with starvation. Although the absence of leptin or a mutation in leptin receptor genes induces massive hyperphagia and obesity in humans, the prevalence of these mutations in obese humans is rare. The effects of leptin are mediated by receptors, mainly located in the central nervous system and in other tissues, including adipocytes and endothelial cells. Leptin receptor belongs to the class I family of cytokine receptors, and it engages both the signal transducer and activator of transcription-3 (STAT3) pathway and the insulin receptor substrate phosphoinositide-3 kinase pathway. STAT3 has been shown to be essential for mediating food intake, liver glucose production, and
gonadotropin secretion; however, the control of adipose tissue metabolism by leptin is STAT3-independent. In-fusion of leptin into the hypothalamus led to the suppression of lipogenesis in adipose tissue through activation of the phosphoinositide-3 kinase pathway, the sympathetic nervous system, and the engagement of the adipose tissue endocannabinoid system. Leptin modulates the T-cell immune response, stimulates proliferation of T-helper cells, and increases production of proinflammatory cytokines by regulating different immune cells.

**Adiponectin.** Adiponectin is a 30-kDa protein secreted from adipocytes. Circulating adiponectin is found in several different isoforms: trimer, low-molecular weight (hexamers), and high-molecular weight (HMW; 18-mer) forms, each with distinct biologic functions. The HMW isoform is linked to the insulin-sensitizing effects of adiponectin, whereas the central effects of adiponectin are linked to the hexamer and trimer isoforms. Adiponectin is present in cerebrospinal fluid largely in the trimer and hexamer forms.

Adiponectin increases food intake by enhancing hypothalamic 5’-adenosine monophosphate-activated protein kinase activity in fasting conditions. Circulating levels of adiponectin are decreased in obesity-induced insulin resistance. The HMW oligomer of adiponectin is inversely associated with the risk for diabetes independent of total adiponectin levels and is responsible for the association of adiponectin with traits of metabolic syndrome.

Adiponectin binds two transmembrane receptors (AdipoR1 and AdipoR2) that are ubiquitously expressed. AdipoR1 is predominantly expressed in skeletal muscle, with a preference for binding to globular adiponectin, whereas AdipoR2 is most abundant in the liver, with a preference for binding to full-length adiponectin. Adiponectin improves insulin sensitivity by increasing energy expenditure and fatty acids and by the expansion of subcutaneous adipose tissue with decreased levels of macrophage infiltration, similar to the actions of PPAR-γ agonists. Thiazolidinediones are known to increase circulating levels of adiponectin, mostly the HMW form, by twofold to threefold and improve insulin resistance by diversion of fat from ectopic sites to subcutaneous adipose tissue. Interestingly, the insulin-sensitizing effects of thiazolidinediones are significantly diminished in the absence of adiponectin, suggesting an important role of adiponectin in reduction of the lipotoxicity and inflammation associated with obesity. Adiponectin has also had vasculoprotective effects through an increase in endothelial nitric oxide (NO) production and modulation of the expression of adhesion molecules and scavenger receptors.

**Resistin.** Resistin is a 12-kDa peptide that is part of a gene family of “resistin-like molecules,” produced by resistin. Resistin is expressed by adipocytes in mice but is

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**Table II. Signaling molecules of metabolic syndrome**

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Adipokines</th>
<th>Coagulation</th>
<th>Hypertension</th>
<th>Adipocyte related</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Leptin</td>
<td>PAI-1</td>
<td>Angiotensinogen</td>
<td>Acylation stimulating protein</td>
</tr>
<tr>
<td>IL-6</td>
<td>Adiponectin</td>
<td>Tissue factor</td>
<td>Prostaglandins</td>
<td>Monobutyryl</td>
</tr>
<tr>
<td>IL-8</td>
<td>Resistin</td>
<td>TIMP-1</td>
<td>Oxygen free radicals</td>
<td>Adipin</td>
</tr>
<tr>
<td>IL-10</td>
<td>Resistin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1RA</td>
<td>Viscatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6RA</td>
<td>Adiponectin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>Chemerin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>Heparidin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB-EGF</td>
<td>RBP-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>Omentin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lean and Healthy**

- **WAT Expansion and Inflammation**
  - Platelet Function
  - Leptin
  - Angiogenic Factors
  - Adiponectin
  - MCP-1
  - TNF-α
  - IL-6
  - IL-8

**Obesity and Complication**

- Hypercoagulation
- HYPERTENSION
- HYPERGLYCEMIA
- DYSLIPIDEMIA

**Inflammation**

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Adipokines</th>
<th>Coagulation</th>
<th>Hypertension</th>
<th>Adipocyte related</th>
</tr>
</thead>
</table>
| ApoE; Apolipoprotein E; HB-EGF, heparin-binding epidermal growth factor; IGF, insulin-like growth factor; IL, interleukin; MCP, monocyte chemoattractant protein; PAI, plasminogen activator inhibitor; RBP, retinol binding protein 4; SAA, serum amyloid A; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor.

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Fig 2. The development of metabolic syndrome is a journey from lean and healthy to white adipose tissue (WAT) expansion and inflammation, which progresses to obesity and its associated complications. The expansion of WAT is associated with an angiogenic response and an increase in the infiltration of macrophages (MΦ) into the WAT. The visceral and truncal WAT alter their production of adipokines and cytokines, which leads to enhanced inflammation and a loss of insulin sensitivity (development of insulin resistance). The result of the changes in adipokines and cytokines leads to hypertension, hyperglycemia, dyslipidemia, and a hypercoagulable state. IL, Interleukin-6; MCP, monocyte chemoattractant protein; PAI, plasminogen activator inhibitor; TNF, tumor necrosis factor.
expressed by the macrophages of humans. Resistin is a 12-kDa protein that circulates as either a trimer (monomeric form of the peptide hormone) or hexamer (dimeric form of resistin). The monomeric form was shown to impair hepatic insulin action more potently than the dimerized form. In contrast, the dimerized form of resistin was more effective in antagonizing insulin-stimulated glucose uptake in adult murine cardiomyocytes.

A number of studies have examined plasma resistin levels or adipose resistin expression and have found variable associations with insulin resistance. High plasma levels of resistin correlate with proatherogenic inflammatory markers, metabolic syndrome, increased cardiovascular risk, unstable angina, and poor prognosis in coronary artery disease. Resistin increases proinflammatory markers and induces the release of endothelin 1 and the production of vascular cell adhesion molecule 1 and MCP-1. A recent study involving the Framingham offspring cohort found a significant relationship between insulin resistance and resistin; however, this relationship was considerably weaker than the relationship with adiponectin and was lost after adjustment for BMI. Resistin decreases after thiadiinediones treatment of humans, although resistin was also decreased by metformin treatment.

Retinol binding protein 4. Retinol binding protein 4 (RBP4) is a highly expressed circulating adipokine that can lead to insulin resistance. Many studies have demonstrated a positive association between RBP4 and insulin resistance or obesity, whereas others have not. There are suggestions of an age-related difference, with younger people demonstrating the relation while older persons do not. RBP4 is associated with adipose tissue macrophage markers, suggesting a link between RBP4 and inflammation within adipose tissue. RBP4 circulates bound to transthyretin, which decreases RBP4 renal clearance, and transthyretin plasma levels are increased fourfold in murine models of adipose tissue angiotensinogen secretion in the genesis of metabolic syndrome. There is an association of RBP4 and Glut4, which is presumed to play a role in fuel sensing in the adipocyte.

Visfatin. Visfatin is expressed in many cells and tissues and was previously identified as a protein involved in B-cell maturation (pre-B colony-enhancing factor). Visfatin has insulin-like functions and is predominantly found in visceral adipose tissue. Human studies noted a positive correlation between visceral adipose tissue visfatin gene expression and BMI, along with a negative correlation between BMI and subcutaneous fat visfatin, suggesting that visfatin regulation in these different depots is different and that adipose depot ratios are highly dependent on the obesity of the individuals. No difference in visfatin expression between fat depots of humans was noted, and visfatin was expressed predominantly by nonmacrophage cells in the adipose tissue stroma. Plasma visfatin can be positively associated with BMI. Visfatin can upregulate IL-6 and TNF-α in vivo and in vitro. Visfatin can increase matrix metalloproteinase-9 (MMP-9) activity in monocytes as well as TNF-α and IL-8 in peripheral blood mononuclear cells.

Tissue inhibitor of metalloprotease-1. Tissue inhibitor of metalloprotease-1 (TIMP-1), another novel candidate adipokine, is the constitutive inhibitor for the gelatinase MMP-9. The expression and secretion of TIMP-1 are upregulated by proinflammatory cytokines in obese patients and in vitro, so it is possible that TIMP-1 has a role in maintaining adipose tissue mass in obesity.

Heparin-binding epidermal-growth factor-like growth factor. Heparin-binding growth factors are a family of mitogenic proteins that have varying affinities for heparin and heparin-like molecules. They include platelet-derived growth factor, acidic fibroblast growth factor (FGF), basic FGF, VEGF, HGF, and heparin-binding epidermal growth factor (HBEGF). HBEGF is expressed in adipocytes, and its plasma levels increase with the extent of obesity. HBEGF is a 22-kDa growth factor that is mitogenic for fibroblasts and smooth muscle cells. It acts by binding to the EGF receptor and, in fact, has a greater affinity for EGF receptors on smooth muscle cells and is a more potent mitogen for smooth muscle cells than EGF itself.

Angiotensinogen. The angiotensinogen gene is detected in adipose tissue, although messenger RNA (mRNA) expression is not correlated with adiposity. Angiotensinogen is the precursor of angiotensin I, which after conversion to angiotensin II, plays a major role in blood pressure regulation. Angiotensinogen mRNA expression is increased in visceral fat, which partially explains the relationship between systemic hypertension and obesity in the metabolic syndrome. Angiotensinogen knockout mice with selective overexpression of angiotensinogen in adipose tissue develop obesity with a high-fat diet and especially hypertension, which is compatible with involvement of adipose tissue angiotensinogen secretion in the genesis of this phenotype.

Omentin. Omentin is a secretory protein that has been recently identified as a new adipokine encoded by two genes (1 and 2) that is highly and selectively expressed in visceral adipose tissue. Omentin may regulate insulin action. Omentin 1 plasma levels and adipose tissue gene expression are decreased with obesity, and they correlate positively with plasma adiponectin and HDL and negatively with waist circumference, BMI, and insulin resistance. Omentin transcripts are strongly expressed in visceral adipose tissue but poorly in subcutaneous fat. Omentin is present in the stromal vascular cells of omental adipose tissue but not in mature fat cells. Human omentin is a peptide of 313 amino acids and contains a secretory signal sequence and a fibrinogen-related domain. It is secreted in the culture medium of omental but not subcutaneous fat explants. Interestingly, it increases insulin-stimulated glucose uptake in both omental and subcutaneous adipocytes and promotes Akt phosphorylation.
volved in glucose and lipid homeostasis, including adiponectin and leptin, and alters metabolic functions in mature adipocytes.\textsuperscript{99}

**Apelin.** Apelin is an adipokine produced and secreted by adipocytes, whose plasma levels are significantly elevated by obesity and insulin in humans, which was found to be the endogenous ligand for the G protein-coupled APJ receptor. The gene that encodes the apelin receptor shares the greatest sequence identity with the angiotensin AT1 receptor.\textsuperscript{100} Apelin production is upregulated by hypoxia. Apelin has been shown to exert potent positive inotropic effects on both normal and failing myocardium. The cardiac apelin system is downregulated by angiotensin II, and its restoration is reached by treatment with angiotensin type 1 receptor blocker.

**Vaspin.** Vaspin is an adipokine recently identified as a member of the serine protease-inhibitor family.\textsuperscript{101} It is strongly expressed in visceral adipose tissue and is stimulated in mouse and human obesity.\textsuperscript{102} Its tissue expression and plasma levels are normalized in the presence of insulin or an insulin-sensitizing drug (pioglitazone).\textsuperscript{103}

**Serum amyloid A.** Serum amyloid A (SAA), an inflammatory acute-phase protein associated with systemic inflammation and atherosclerosis, is used as a predictive marker in coronary accidents or cardiovascular events. Circulating levels of SAA are significantly correlated with insulin resistance in obesity and type 2 diabetes.\textsuperscript{104} SAA is expressed in adipose tissue, and this expression is largely increased in obesity and diabetes.\textsuperscript{105, 106} Also, SAA could participate in lipoprotein metabolic alterations, promoting the linking of HDL-cholesterol to macrophages, thus reducing their cardiovascular-protective effect.

**C-reactive protein.** C-reactive protein (CRP) mRNA is detected in adipocytes. Adiponectin knockout mice show higher plasma CRP levels than wild-type mice. The strong negative correlation between adiponectin mRNA and CRP mRNA expression in human adipose tissue suggests that a decrease in adiponectin leads to a rise in CRP. CRP can inhibit insulin-evoked NO production in endothelial cells through specific inactivation of the PI3K/Akt/endothelial NO synthase pathway.\textsuperscript{107, 108} Similar to TNF-\(\alpha\), CRP simultaneously increases endothelin (ET-1) production.\textsuperscript{108}

**Thrombospondin-1 and CD36.** One of the newer adipokines is thrombospondin-1 (TSP-1), a multidomain, multifunctional glycoprotein synthesized by many cell types, which modulates cell adhesion and proliferation. TSP-1 is a major activator of TGF-\(\beta\)1 in vivo.\textsuperscript{109} As a result, TSP-1 is involved in angiogenesis, inflammation, and wound healing. CD36 is the receptor for TSP-1 and is present on platelets, mononuclear phagocytes, adipocytes, hepatocytes, myocytes, and some epithelia.\textsuperscript{110} On phagocytes, it functions as a scavenger receptor, recognizing specific oxidized phospholipids and lipoproteins. CD36 also binds long-chain fatty acids and facilitates their transport into cells, thus participating in muscle lipid utilization, adipose energy storage, and gut fat absorption, and possibly contributing to the pathogenesis of metabolic disorders, such as diabetes and obesity. The chemotactic properties of TSP-1 provide a link between TSP-1 and macrophage-mediated adipocyte inflammation. In addition, adipocyte-macrophage coculture experiments demonstrated TSP-1 gene and protein upregulation by both cell types, suggesting a feed-forward inflammatory mechanism in adipose tissue.\textsuperscript{112} TSP-1 may be an important component of inflammation and coagulation in the metabolic complications of obesity.

### INSULIN RESISTANCE AND HYPERGLYCEMIA

**Insulin resistance.** Insulin resistance is the second key biologic component of the metabolic syndrome. Chronic inflammation associated with visceral obesity induces insulin resistance in the liver.\textsuperscript{33} This chronic inflammation is characterized by the production of abnormal adipokines and cytokines such as TNF-\(\alpha\), FFA, IL-1, IL-6, leptin, and resistin. These factors inhibit insulin signaling, which in turn causes impaired suppression of glucose production by insulin in hepatocytes and leads to hyperglycemia.

An important and early complication of hepatic insulin resistance is the induction of hepatic VLDL production through changes in the rate of apolipoprotein B synthesis and degradation and de novo lipogenesis or increased FFA flux from adipose tissue into the liver. Insulin resistance also stimulates the production of CRP and PAI-1, both markers of an inflammatory state. All metabolic abnormalities related to hepatic insulin resistance have been shown to directly or indirectly promote atherosclerosis.

Insulin has several direct vascular actions that contribute to vascular protection or injury, depending on the cell type. Vascular-protective effects of insulin include stimulation of endothelial cell production of the vasodilator NO. This, in turn, inhibits formation of lesions dependent on migration and proliferation of vascular smooth muscle cells, attenuates binding of inflammatory cells to the vascular wall, and inhibits thrombosis by reducing platelet adhesion and aggregation. However, insulin also promotes a host of deleterious vascular effects by stimulating the actions of various growth factors, including angiotsenin II and PAI-1.

Glucocorticoid metabolism is also abnormal in insulin-resistant states. Adipocyte-derived hormones, including adiponectin and leptin, regulate systemic insulin sensitivity in accordance to existing triglyceride reserves. Leptin levels reflect existing fat mass, and the adipokine negatively regulates insulin action in adipose tissue.

Adiponectin, on the other hand, preserves insulin sensitivity via transient increments of 5’-adenosine monophosphate-activated protein kinase activity and its circulating levels seem to reflect the adipogenic capacity of adipose tissue. Because adiponectin and insulin are synergistic, inadequate adiponectin production contributes to systemic insulin resistance. In insulin-resistant states associated with impaired PI3K-dependent insulin signaling pathways, insulin-mediated ET-1 secretion is augmented, and blockade of ET-1 receptors significantly improves insulin sensitivity and peripheral glucose uptake in the context of insulin resistance.\textsuperscript{113, 114}
Elevated blood glucose levels induce a series of alterations within the vasculature, including endothelial dysfunction, cellular proliferation, changes in extracellular matrix, and impairment of LDL receptor-mediated uptake decreasing the in vivo clearance of LDL concentrations.\textsuperscript{115-119} The presence of high glucose concentrations also increases lipoprotein oxidation.\textsuperscript{120} Elevated glucose leads to the activation of the sorbitol pathway, cellular oxidative stress, and formation of advanced glycation end products (AGE).

AGE can be processed by macrophages through a recently characterized series of high-affinity receptors: scavenger receptors types I and II, the receptor for advanced glycation end products (RAGE), oligosaccharyltransferase 48 (OST-48, AGE-R1), 80K-H phosphoprotein (AGE-R2), and galectin-3 (AGE-R3). Coupling of AGE proteins to their AGE receptor results in TNF-\(\alpha\) and IL-1 synthesis and secretion. Evidence provided by both clinical and preclinical studies regarding a central involvement of the RAGE in vascular disease continues to mount.

RAGE is upregulated as a consequence of diverse inflammatory stimuli, including hyperglycemia, oxidized LDL, and reduced shear stress. RAGE may maintain and amplify inflammatory responses in the vasculature if the ligand for the receptor is present. RAGE binding by circulating AGEs or S100 protein released by activated leukocytes results in the generation of reactive oxygen species and further activation of nuclear factor \(\kappa\B\). This leads to upregulation of adhesion molecules for circulating monocytes as well as further upregulation of RAGE itself. In addition, these reactive oxygen species may scavenge and reduce bioavailability of the labile vasodilator NO, reducing its anti-inflammatory effects and possibly compromising control of vascular tone directly.

**ENDOTHELIAL DYSFUNCTION AND INFLAMMATION**

Adipose tissue-resident macrophages and adipocytes in the adipose tissue and the consequences of hyperglycemia, altered lipoproteins, and hyperinsulinemia in the vasculature and within organ microcirculations induce dysfunctional endothelia and a proinflammatory state in metabolic syndrome.\textsuperscript{121}

**Endothelial dysfunction.** Endothelial dysfunction is an important component of the metabolic syndrome. Deficiency of endothelial-derived NO is believed to be the primary defect that links insulin resistance and endothelial dysfunction. NO deficiency results from decreased synthesis and/or release, in combination with exaggerated consumption in tissues by high levels of reactive oxygen and nitrogen species due to cellular disturbances in glucose and lipid metabolism. Insulin may stimulate endothelial NO production or may act directly on vascular smooth muscle by stimulation of the Na\(^+\)-H\(^+\) exchanger and Na\(^+\)/K\(^+\)-ATPase, leading to hyperpolarization of the cell membrane and consequent closure of voltage-gated Ca\(^{2+}\) channels.

Endothelial dysfunction contributes to impaired insulin action by altering the transcapillary passage of insulin to target tissues. Reduced expansion of the capillary network, with attenuation of microcirculatory blood flow to metabolically active tissues, contributes to the impairment of insulin-stimulated glucose and lipid metabolism. Insulin-induced vasodilation, which is mediated by the release of NO, is impaired in obese individuals who display insulin resistance, possibly due to suboptimal levels of (6R)-5,6,7,8-tetrahydrobiopterin (BH\(_4\)), the natural and essential cofactor of NO synthases (NOS), and accelerated inactivation of NO by O\(^{2-}\) within the vascular wall was observed.

A “third factor” may cause both insulin resistance and endothelial dysfunction in cardiovascular disease. Candidates include fiber type and capillary density of skeletal muscle, distribution of adiposity, and endogenous corticosteroid production. A complex interaction between endothelial dysfunction, abnormal skeletal muscle blood flow, and reduced insulin-mediated glucose uptake may be central to the link between insulin resistance, blood pressure, impaired glucose tolerance, and the risk of cardiovascular disease.

**Tumor necrosis factor-\(\alpha\).** Of the proinflammatory cytokines, TNF-\(\alpha\) is the best described in disturbed insulin signaling. Mice lacking TNF-\(\alpha\) or TNF-\(\alpha\) receptors are resistant to the development of obesity-induced insulin resistance.\textsuperscript{122,123} In adipose tissue, TNF-\(\alpha\) is mostly secreted by macrophages in the stromal vascular fraction. Circulating TNF-\(\alpha\) and adipose tissue TNF-\(\alpha\) gene expression are increased in insulin resistance,\textsuperscript{124} and acute infusion of TNF-\(\alpha\)-inhibited insulin-induced glucose uptake in healthy individuals.\textsuperscript{125} Neutralization of TNF-\(\alpha\) in rodents has improved insulin resistance,\textsuperscript{126} whereas attempts to neutralize TNF-\(\alpha\) in humans to improve insulin resistance have generally not been successful,\textsuperscript{127} although more recent studies have shown slight improvement in insulin resistance with TNF-\(\alpha\) inhibition.\textsuperscript{128,130} Limited effects of TNF-\(\alpha\) blockade on insulin resistance could be explained by the paracrine actions of TNF-\(\alpha\). Further investigations on the mechanisms involved in TNF-\(\alpha\) overexpression associated with obesity and molecular signals underlying TNF-\(\alpha\)-induced metabolic dysregulation are warranted. TNF-\(\alpha\) increases ET-1 secretion and inhibits insulin’s stimulating effect on endothelium-dependent vasodilation in humans.\textsuperscript{131,132}

**Interleukin-6.** IL-6 is also overexpressed in adipose tissue of the obese.\textsuperscript{124} The role of IL-6 in metabolic changes associated with obesity is unclear. Some reports show that IL-6 causes impaired insulin signaling in the liver and adipocytes by inducing ubiquitin-mediated degradation of insulin receptor substrate through suppression of cytokine signaling 1 and 3.\textsuperscript{133,134} However, effects of IL-6 on insulin sensitivity in skeletal muscle are controversial.\textsuperscript{134} Exercise that is associated with increased insulin action in skeletal muscle increases circulating IL-6 levels dramatically,\textsuperscript{135} suggesting possible anti-inflammatory roles for IL-6 in skeletal muscle. The data on the increased onset of obesity and diabetes in mice lacking IL-6 are conflicting.\textsuperscript{136,137} IL-6 also inhibits insulin-stimulated increases in
endothelial NO synthase activity and NO production in the endothelium,\textsuperscript{138} IL-1β together with IL-6 concentrations reportedly predict the risk for type 2 diabetes in humans better than either cytokine alone.\textsuperscript{139}

**Interleukin-10.** Decreased production of IL-10, an anti-inflammatory cytokine, has been associated with the development of type 2 diabetes, and IL-10 plasma levels can be positively correlated with insulin sensitivity.\textsuperscript{140,141} Lower IL-10 levels have been associated with the metabolic syndrome in obese, insulin-resistant postmenopausal women compared with women who are obese but do not satisfy the criteria for metabolic syndrome.\textsuperscript{142} IL-10 decreases IL-6–induced insulin resistance in muscle and liver in mice cotreated with IL-6 and IL-10.\textsuperscript{143} A recent study showed that IL-10 is expressed in macrophages derived from adipose tissue and that the IL-10 receptor is expressed in adipocytes and not immune or endothelial cells in fat.\textsuperscript{144} Several studies indicate that IL-10 is an anti-inflammatory factor produced by immune cells in adipose tissue that acts on adipocytes to improve insulin signaling, potentially decreasing further macrophage recruitment.

**Monocyte chemoattractant protein.** Adipocytes secrete various chemoattractants that draw monocytes from the circulation into adipose tissue. MCP-1, also known as chemokine (C-C motif) ligand 2 (CCL-2), is one of the chemoattractants that is important in the recruitment of macrophages to the adipose tissues. Moreover, obesity is associated with increased plasma levels of MCP-1 and overexpression in adipose tissue.\textsuperscript{23,145} Mice lacking MCP-1 receptor (CCR-2) have decreased adipose tissue macrophage infiltration and improved metabolic function.\textsuperscript{146,147} Other candidates might likely contribute to the recruitment of macrophages into the adipose tissue, such as macrophage inflammatory protein-1α and osteopontin.\textsuperscript{149,150} Osteopontin is an extracellular matrix protein that promotes monocyte chemotaxis, and the lack of osteopontin in mice caused improved insulin sensitivity and decreased macrophage infiltration into adipose tissue.\textsuperscript{150}

### HYPERTENSION

Essential hypertension is a complex, multifactorial, quantitative trait under polygenic control. Increased peripheral resistance due primarily to changes in vascular structure and function appear to be the fundamental hemodynamic abnormality in hypertension. These changes include arterial wall thickening and abnormal vascular tone and are due to alterations in the biology of the cellular and noncellular components of the arterial wall. Multiple interacting humoral and mechanical factors as well as oxidative stress stimulate complex signaling pathways, which modulate vascular smooth muscle cell contraction and growth.\textsuperscript{151} Hypertension is one of the commonest components of metabolic syndrome and is linked to both essential hypertension and obesity-related hypertension.\textsuperscript{152}

Excess weight gain is likely the major cause of essential hypertension, and abnormal kidney function appears to be a cause as well as a consequence of obesity hypertension. Excess renal sodium reabsorption and a hypertensive shift of pressure natriuresis play a major role in mediating increased blood pressure associated with weight gain. Activation of the renin-angiotensin and sympathetic nervous systems and physical compression of the kidneys appear to contribute to obesity-induced increases in sodium reabsorption and hypertension.

### COAGULATION

Fibrinolytic dysfunction mediates the increased risk of coronary artery disease in individuals with the metabolic syndrome.\textsuperscript{153} Adipose tissue induces thrombocyte activation by the production of adipokines, some of which, such as leptin and adiponectin, directly interfere with platelet function. Increased adipose tissue mass induces insulin resistance and systemic low-grade inflammation, also affecting platelet function. Adipose tissue directly impairs fibrinolysis by the production of PAI-1 and possibly thrombin-activatable fibrinolysis inhibitor.\textsuperscript{154} Adipose tissue may contribute to enhanced coagulation by direct tissue factor production, but hypercoagulability is likely to be primarily caused by altered hepatic synthesis of the coagulation factors fibrinogen, factor VII, factor VIII, and tissue factor, by releasing FFAs and proinflammatory cytokines (TNF-α, IL-1β, and IL-6) into the portal circulation and by inducing hepatic insulin resistance. Adipose tissue dysfunction could thus play a causal role in the prothrombotic state observed in obesity by directly and indirectly affecting hemostasis, coagulation, and fibrinolysis.\textsuperscript{155}

In type 2 diabetes, there are increased levels of fibrinogen and PAI-1, favoring both thrombosis and defective dissolution of clots once formed. Platelets in type 2 diabetic individuals adhere to vascular endothelium and aggregate more readily than those in healthy people. Loss of sensitivity to the normal homeostatic restraints exercised by prostacyclin (PGI\textsubscript{2}) and NO generated by the vascular endothelium present as the major defect in platelet function. Insulin is a natural antagonist of platelet hyperactivity. It sensitizes the platelet to PGI\textsubscript{2} and enhances endothelial generation of PGI\textsubscript{2} and NO. Thus, the defects in insulin action in diabetes create a milieu of disordered platelet activity conducive to macrovascular and microvascular events.\textsuperscript{156} Patients with type 2 diabetes and abdominal fat patterning displayed higher plasma activities of clotting factors VII and VIII as well as increased plasma levels of fibrinogen and von Willebrand factor antigen compared not only with healthy normal weight controls but also with diabetic patients at normal body weight.\textsuperscript{157,158}

PAI-1 is elevated in individuals with the metabolic complications of obesity and is expressed in the stromal fraction of adipose tissue, including endothelial cells.\textsuperscript{159,163} PAI-1 inhibits both tissue-type plasminogen activator and urokinase-type plasminogen activator through its serine protease inhibitor function and thus contributes to a prothrombotic state.\textsuperscript{164} PAI-1 gene expression is controlled by TGF-β, which combines with phosphorylated Smad and binds to the PAI-1 promoter.\textsuperscript{165} A second pathway is by way of TSP-1. TSP-1 is expressed in adipocytes\textsuperscript{112} and inhibits angiogenesis, cell proliferation, and wound healing.\textsuperscript{111,166} TSP-1 is
a major activator of TGF-β R1, and PAI-1 activation by TSP-1 has been described. A recent study demonstrated TSP-1 expression largely by adipocytes compared with the stromal vascular fraction of adipose tissue, suggesting that TSP-1 is a true adipokine. TSP-1 expression was increased in obese, insulin-resistant individuals, was associated with plasma PAI-1 levels, and was positively associated with adipose tissue macrophage markers. In addition, TSP-1 expression was decreased by treatment of subjects or adipocytes with the PPAR-γ agonist, pioglitazone.

**Modeling metabolic syndrome.** The ideal model for metabolic syndrome should be obese, hypertensive, insulin resistant, and have the appropriate dyslipidemia. There is at present no perfect animal model of this human disease. The most common models to study metabolic syndrome are mice that can be based on one of three strategies: obese mouse strains that mimic metabolic syndrome, mice fed high-fat diets to induce metabolic syndrome, or gene knockout mice that mimic metabolic syndrome. Insulin resistance in mice with differential susceptibility to diabetes and metabolic syndrome is preceded by differences in the inflammatory response of adipose tissue. This phenomenon may serve as an early indicator of disease and contribute to disease susceptibility and progression.

The LepRdb/db, LepRdh/db, and Aβ/β mice are the three most commonly used spontaneously mutant obese mouse models. They display insulin resistance and may develop diabetes, depending on the background strain. In addition, Aβ/β mice have intact leptin signaling and display a delayed onset obesity that can be amplified by being fed a high-fat diet, making them a good model for human obesity. All three models appear to be ideal for metabolic syndrome. Obese mouse models such as Aβ/β, LepRdb/db, and LepRdh/db have increased total plasma cholesterol levels; however, this is the result of increased HDL rather than increased VLDL and LDL levels. The increase in HDL makes these mice resistant to atherosclerotic lesion formation. These lipid changes do not mirror those seen in metabolic syndrome. Hypertensive mice do not make good models unless they are bred with obese mice. Care should thus be taken to choose the mouse model most appropriate for the scientific or mechanistic mechanism to be tested under metabolic syndrome conditions.

**CONCLUSIONS**

Metabolic syndrome represents a combination of synergistic vascular pathologies that lead to an accelerated atherogenic state that compromises the ability of the patient to satisfactorily respond to humoral, cellular, and mechanical stresses. Delineating the contribution of insulin resistance and the role of the adipokines remains a focus of current research. Defining key signaling pathways may provide opportunities for new therapeutic targets and interventions. It represents a complex pathology that will require better definition before the development of satisfactory therapeutic interventions.

**AUTHOR CONTRIBUTIONS**

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Analysis and interpretation: MD, DV
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Critical revision of the article: MD, DV
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