Getting the message across: mechanisms of physiological cross talk by adipose tissue

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Lee DE, Kehlenbrink S, Lee H, Hawkins M, Yudkin JS. Getting the message across: mechanisms of physiological cross talk by adipose tissue. Am J Physiol Endocrinol Metab 296: E1210–E1229, 2009. First published March 3, 2009; doi:10.1152/ajpendo.00015.2009.—Obesity is associated with resistance of skeletal muscle to insulin-mediated glucose uptake, as well as resistance of different organs and tissues to other metabolic and vascular actions of insulin. In addition, the body is exquisitely sensitive to nutrient imbalance, with energy excess or a high-fat diet rapidly increasing insulin resistance, even before noticeable changes occur in fat mass. There is a growing acceptance of the fact that, as well as acting as a storage site for surplus energy, adipose tissue is an important source of signals relevant to, inter alia, energy homeostasis, fertility, and bone turnover. It has also been widely recognized that obesity is a state of low-grade inflammation, with adipose tissue generating substantial quantities of proinflammatory molecules. At a cellular level, the understanding of the signaling pathways responsible for such alterations has been intensively investigated. What is less clear, however, is how alterations of physiology, and of signaling, within one cell or one tissue are communicated to other parts of the body. The concepts of cell signals being disseminated systemically through a circulating “endocrine” signal have been complemented by the view that local signaling may similarly occur through autocrine or paracrine mechanisms. Yet, while much elegant work has focused on the alterations in signaling that are found in obesity or energy excess, there has been less attention paid to ways in which such signals may propagate to remote organs. This review of the integrative physiology of obesity critically appraises the data and outlines a series of hypotheses as to how interorgan cross talk takes place. The hypotheses presented include the “fatty acid hypothesis,” the “portal hypothesis,” the “endocrine hypothesis,” the “inflammatory hypothesis,” the “overflow hypothesis,” a novel “vasocrine hypothesis,” and a “neural hypothesis,” and the strengths and weaknesses of each hypothesis are discussed.

adipocyte; inflammation; insulin resistance; adipocytokines; endothelium

THE GLOBAL EPIDEMIC OF OBESITY is bringing in its wake a catastrophic increase in the prevalence of metabolic diseases (273). Obesity is a major cause of insulin resistance, which has been implicated in the rising prevalence of diabetes, metabolic syndrome, and cardiovascular disease (26). But independently of increased fat mass, a positive energy balance rapidly induces systemic insulin resistance because of systemic signaling of nutrient balance by adipose tissue and perhaps liver (226). Thus, during the past 15 years, adipose tissue has been widely recognized to play a more important role than simply that of a fuel depot. It is also a vital source of information to other insulin-sensitive organs and tissues of its own mass and of the organism’s nutritional state.

The other area of knowledge that has grown exponentially over the same time frame is the recognition of obesity repre-
or may not satisfy criteria to support their role as an endocrine (systemic or portal) “adiposity signal.”

We hypothesize that adipose tissue is connected by a myriad of different forms of communication with other organs and tissues, including neural and vascular routes, and so literally sits at the “crossroads” of multiple interorgan exchanges. Additionally, interactions among different cell types within adipose tissue contribute to the overall impact of this organ. Clearly, any unifying hypotheses imputed to account for these signaling effects need to explain various complex attributes of fat and insulin resistance, including the particularly adverse effects of truncal fat on the risk of both diabetes and cardiovascular disease. Therefore, this review will examine the major putative mechanisms whereby adipose tissue is a key mediator of obesity-related organ cross talk. Seven hypotheses will be presented: 1) a “fatty acid hypothesis,” 2) a “portal hypothesis,” 3) an “endocrine hypothesis,” 4) an “inflammatory hypothesis,” 5) an “overflow hypothesis,” 6) a novel “vasocrine hypothesis,” and 7) a “neural hypothesis,” along with the strengths and pitfalls of the evidence for each hypothesis.

### Insulin Resistance in Various Target Organs

To consider the systemic effects of fat on insulin action, the concept of insulin resistance in individual target organs needs to be defined. The term insulin resistance generally refers to resistance to the metabolic effects of insulin, including the suppressive effects of insulin on endogenous glucose production (primarily in liver) and the stimulatory effects of insulin on peripheral glucose uptake and glycogen synthesis (predominantly in skeletal muscle). In short, a reduction of insulin-mediated peripheral glucose uptake produces an elevation of glucose concentrations predominantly in the postprandial state, whereas insensitivity of the suppression of hepatic glucose output to insulin is responsible for fasting hyperglycemia. Of note, mitochondrial density and activity in skeletal muscle and liver may contribute importantly to insulin sensitivity in different tissues (42, 126). Insulin resistance in adipose tissue is characterized by a decreased suppression of adipose tissue lipolysis by insulin, resulting in elevated circulating nonesterified fatty acid (NEFA) levels. Indeed, the suppressive effect of insulin on NEFA levels is impaired in obese insulin-resistant individuals (33, 112) and in type 2 diabetes mellitus (T2DM) (90). In addition to this, it has been shown that impaired glucose uptake into fat resulted in both hepatic and skeletal glucose uptake predominantly in the postprandial state, whereas insensitivity of the suppression of hepatic glucose output to insulin is responsible for fasting hyperglycemia. Of note, mitochondrial density and activity in skeletal muscle and liver may contribute importantly to insulin sensitivity in different tissues (42, 126). Insulin resistance in adipose tissue is characterized by a decreased suppression of adipose tissue lipolysis by insulin, resulting in elevated circulating nonesterified fatty acid (NEFA) levels. Indeed, the suppressive effect of insulin on NEFA levels is impaired in obese insulin-resistant individuals (33, 112) and in type 2 diabetes mellitus (T2DM) (90). In addition to this, it has been shown that impaired glucose uptake into fat resulted in both hepatic and skeletal glucose uptake predominantly in the postprandial state, whereas insensitivity of the suppression of hepatic glucose output to insulin is responsible for fasting hyperglycemia. Of note, mitochondrial density and activity in skeletal muscle and liver may contribute importantly to insulin sensitivity in different tissues (42, 126). Insulin resistance in adipose tissue is characterized by a decreased suppression of adipose tissue lipolysis by insulin, resulting in elevated circulating nonesterified fatty acid (NEFA) levels. Indeed, the suppressive effect of insulin on NEFA levels is impaired in obese insulin-resistant individuals (33, 112) and in type 2 diabetes mellitus (T2DM) (90). In addition to this, it has been shown that impaired glucose uptake into fat resulted in both hepatic and skeletal muscle insulin resistance (1). Furthermore, selective overexpression of the insulin-responsive glucose transporter GLUT4 in adipose tissue both increased adipose tissue mass and improved whole body insulin sensitivity (235, 255). These findings suggest that adipose tissue provides a “sink” to protect other tissues from toxic effects of excessive NEFA, and/or that the increased fat mass generated in this model produced factor(s) promoting insulin sensitivity. This evidence of cross talk between adipose tissue and other organs indicates that adipose tissue serves not only as a target organ of insulin but can modulate insulin’s effects on other target tissues, which will be the focus of this article.

More recently, it has become clear that insulin activates intracellular signaling pathways in a variety of other tissues, including vascular endothelium and nervous tissue. Since both tissues may coordinate the body’s response to insulin in obesity and other insulin-resistant states, the relevance of insulin resistance in these tissues will be presented briefly.

Normally, the vascular endothelial cell is sensitive to various actions of insulin, which include stimulating the expression and activation of endothelial nitric oxide synthase (eNOS) (171), with consequent vasodilatation (13, 243). In obesity, impaired insulin-mediated vasodilatation is a recognized precursor of atherothrombotic vascular disease (7, 244). Whether insulin-mediated vasodilatation in vessels supplying skeletal muscle plays any role in increasing insulin-dependent glucose uptake remains disputed (48, 241, 278). However, insulin clearly has a substantially greater impact on capillary recruitment to increase nutritive tissue perfusion than it has on total limb blood flow (48, 51). Such recruitment, probably through diversion of flow from nonnutritive circuits (205), has a rapid timescale and a dose-response relationship in the physiological concentration range, making it likely to represent an important physiological mechanism (48, 286). Bergman and his group have shown how important interstitial space accessibility is to insulin’s mechanism of action, not only in muscle and adipose tissue (40, 277) but, curiously, also for its liver effect (206). Furthermore, this group has recently shown that a high-fat diet reduces the access of insulin to target tissues, potentially by inhibiting capillary recruitment (65).

A growing body of evidence indicates that brain insulin action is required for physiological glucose homeostasis (184, 186, 190). This counters the longstanding perception of the brain as an insulin-insensitive organ (228). Although peripheral insulin at basal concentrations has been shown to stimulate global brain glucose uptake (20), this effect is markedly reduced in insulin-resistant subjects (4). Indeed, although glucose disposal occurs in an insulin-independent manner in the majority of neurons, neurons in the hypothalamus and other discrete brain areas express the insulin-responsive glucose transporter GLUT4 (138). Targeted impairments in insulin receptors either in all neurons (28) or specifically in the hypothalamus (184) rapidly cause hyperphagia and diet-dependent obesity. Central inhibition of insulin action also dramatically reduces the ability of exogenously infused insulin to blunt hepatic glucose output, suggesting an important role for hypothalamic insulin receptors in the regulation of hepatic glucose metabolism (186). Therefore, resistance to the central appetite-suppressing and metabolic effects of insulin may play a seminal role in the development of insulin resistance.

The individual hypotheses concerning mechanisms whereby adipose tissue signals its mass to insulin target tissues will now be outlined and discussed. Before exploring other possible adipose signals, a brief outline of the actions of NEFA on insulin signaling is presented. Of note, although a discussion of genetic factors is beyond the scope of this review, it is likely that genetic factors would influence an individual’s metabolic responses to a given degree of fat accumulation.

### Obesity and Insulin Resistance

**The fatty acid hypothesis.** The original mechanism proposed for the effect of NEFA on glucose uptake was that of Randle, who some 40 years ago postulated that fatty acids inhibit glucose oxidation in the tricarboxylic acid cycle by direct substrate competition in heart and diaphragm muscle (203, 204). As initially proposed by Randle, an increase in fatty acid...
availability results in an elevation of the intramitochondrial acetyl-CoA/CoA and NADH/NAD+ ratios, with subsequent inactivation of pyruvate dehydrogenase. This, in turn, causes citrate concentrations to increase, leading to inhibition of phosphofructokinase. Subsequent increases in intracellular glucose 6-phosphate concentration were predicted to inhibit hexokinase II activity, resulting in an increase in intracellular glucose concentrations and a decrease in muscle glucose uptake. Recent observations by Shulman’s group have used 13C-NMR to measure intracellular concentrations of glucose 6-phosphate and have shown substantial decreases in obese patients and those with T2DM rather than the increase predicted by Randle (239). These findings have led to the proposal of an alternative mechanism whereby increased NEFA leads to downregulation of insulin signaling in human skeletal muscle (239). Increased NEFA concentrations have been shown to lead to serine/threonine phosphorylation of insulin receptor substrates (IRS-1 and IRS-2), subsequently reducing the ability of the IRS to activate phosphatidylinositol (PI) 3-kinase and glucose transport (281). This may occur through the mediation of the serine kinase protein kinase C (PKC). Since an acute elevation of plasma fatty acids for 5 h resulted in the activation of PKC in skeletal muscle and was associated with decreased tyrosine phosphorylation of IRS-1 (89). Indeed, inactivation of PKC was protective against fat-induced insulin resistance in skeletal muscle (121) and against fatty acid-induced insulin resistance in endothelial cells (10). Additionally, overexpression of uncoupling protein-3 (UCP3) in skeletal muscle was recently shown to protect against fat-induced insulin resistance and reduce PKC activity in muscle, presumably by dissipating the mitochondrial proton gradient and promoting conversion of intramyocellular fat into thermal energy (44). These findings are all in line with elegant studies using NMR spectroscopy, showing that NEFA-induced insulin resistance occurs through the impairment of transmembrane glucose transport (210) and that this, in turn, is the consequence of reduced substrate activation downstream from the insulin receptor, IRS-1, and PI 3-kinase.

Elevated NEFA levels also impair the suppressive effects of insulin on hepatic glucose production. Various mechanisms appear to contribute to this phenomenon. NEFAs have been shown to stimulate gluconeogenesis in vitro (267), probably due to increased ATP and NADH production (266) and gluconeogenic gene expression (5). Additionally, chronic high-fat feeding in rats is associated with a higher activity ratio of hepatic glucose 6-phosphatase (G-6-Pase)/glucokinase (GK), thus favoring increased hepatic glucose output (182). Increased NEFA levels acutely induce the expression of hepatic G-6-Pase in normal rats (157). Transcriptional regulation of hepatic genes by lipids could potentially be mediated via the peroxisome proliferator-activated receptors (PPARs) (211).

In addition to total NEFA levels, certain species of NEFA and their products appear to be of considerable physiological relevance. In particular, increasing evidence appears to link tissue ceramide accumulation to insulin resistance, especially that induced by saturated fat and obesity (96). Not only did cell-permeable ceramide analogs induce insulin resistance in muscle cells, but palmitate-induced insulin resistance was prevented by inhibiting de novo ceramide synthesis (198). Recent observations have associated increased liver fat and insulin resistance with increased adipose ceramide content, further suggesting a connection between ceramide production and insulin resistance in humans (131).

Elevated concentrations of NEFA not only produce hepatic and peripheral insulin resistance (17) but also impair endothelial-dependent vasodilatation (242), perhaps through direct effects on the NOS enzyme (and thus nutritive blood flow), as well as inhibition of insulin action. The impact of tissue fat accumulation will be discussed under the overflow hypothesis.

The fatty acid hypothesis proposes, then, a central role for these molecules as the systemic signal for obesity-associated insulin resistance. Other observations on the powerful influence of visceral fat on insulin resistance, and on risk of diabetes and cardiovascular disease, have led to this model being further developed as a portal hypothesis, as will now be outlined.

**Obesity and insulin resistance: The portal hypothesis.** Although insulin resistance can occur in the absence of obesity, increased fat mass (72), particularly when distributed centrally (38, 219, 249), is a powerful contributor to this state. Obesity is also a major predictor of diabetes and cardiovascular disease, with truncal or upper body obesity being the major contributory phenotype for both conditions (209, 240). The widely accepted explanation for the deleterious effect of truncal fat is the so-called portal hypothesis (113), which suggests that the higher rates of lipolysis in visceral fat (258) expose the liver directly to high concentrations of NEFA. The consequence is hepatic insulin resistance, followed by peripheral hyperinsulinemia and insulin resistance in skeletal muscle. Bergman and colleagues have shown that a 6- to 12-wk high-fat diet in dogs, associated with a more than twofold increase in trunk adiposity, results in increased hepatic fat infiltration and insulin resistance (119, 120). There are, however, several observations that challenge the tenets of the portal hypothesis. First, an elegantly balanced study has shown that the large majority of NEFA to which the liver is exposed is generated by peripheral rather than by visceral fat, even in obese subjects, despite their proportionate increase in visceral fat (117). This observation questions the link between visceral obesity and hepatic insulin resistance and implies that NEFA concentrations in the systemic circulation should correlate more closely with insulin resistance than generally seen (11). A second observation is that, in aging rats, the removal of visceral fat leads to improved insulin sensitivity and prevents the age-related deterioration of insulin action (79). Nevertheless, the visceral fat removed in that study was epididymal and perirenal fat, both of which drain into the systemic circulation, and neither procedure changed systemic concentrations of NEFA.

There is, furthermore, evidence that adipose signaling of insulin resistance is not mediated through elevated concentrations of NEFA. Kahn and colleagues created a mouse in which insulin resistance was limited to adipose tissue by knocking out the GLUT4 glucose transporter specifically in adipocytes (1). In vitro, liver and muscle insulin actions in these mice were completely normal, but in the whole animal, insulin resistance was found in both organs, implying that there is cross talk between insulin-resistant fat and other organs. The fact that systemic NEFA concentrations were unaffected by the adipose tissue GLUT4 knockout shows that these were not likely to have been the signaling molecules (1). It is necessary, then, to explore alternative mechanisms by which adipose tissue can...
signal its own mass or its metabolic state to remote tissues and organs.

**Obesity and insulin resistance: The endocrine hypothesis.** Adipose tissue, in addition to its role as an energy storage depot, elaborates endocrine hormones, including adiponectin, leptin, and other metabolic mediators (169, 214) (Table 1). The so-called endocrine hypothesis postulates that obesity may contribute to insulin resistance by altering the levels of these key adipose-derived circulating hormones (Fig. 1). A number of these candidate molecules are proinflammatory cytokines and will be further considered below under Obesity and insulin resistance: The inflammatory hypothesis. Of the other widely recognized adipocytokines, perhaps the most interesting candidate for systemic signaling is adiponectin. Adiponectin is produced in substantial amounts by adipose tissue. Unlike most adipose tissue products, however, it is negatively related to fat mass (227), possibly as a consequence of inhibition by TNF-α or cortisol (66, 217). In population studies, low adiponectin concentrations are associated cross-sectionally with insulin resistance and longitudinally with increased risks of diabetes and cardiovascular disease (2, 104, 147, 227). There are, nevertheless, inconsistencies among adiponectin studies which partially result from the existence of different molecular species with different biological activities; and the high-molecular-weight-to-low-molecular-weight adiponectin ratio, rather than the absolute adiponectin concentration, has been strongly associated with insulin sensitivity (192, 254). In mouse models, overexpression or infusion of adiponectin enhances insulin action and protects against endothelial damage (189); indeed, modestly increasing levels of circulating adiponectin in ob/ob mice actually improved insulin action despite inducing morbid obesity (125). Conversely, adiponectin gene knockout produces a reduction in mitochondrial content and function (47), with consequent insulin resistance. Protection from endothelial damage and the anti-inflammatory action of adiponectin appear to be mediated through a potent suppression of IKKβ activated by TNF-α or hyperglycemia (271), or reduction of oxidative stress (143). Thus the actions of this molecule may represent, reciprocally, a mediator of the systemic effects of obesity.

In addition to adiponectin, another potentially insulin-sensitizing fat-derived protein has recently been identified. Visceral adipose tissue-derived serpin (vaspin) is highly expressed in visceral adipocytes, and reduced tissue expression and serum levels are associated with worsening diabetes in an obese rat model (95). The expression of this adipose-derived protein is induced by adipogenesis (287). Administration of vaspin to obese high-fat-fed mice improved glucose tolerance and insulin sensitivity and reversed the altered expression of genes relevant to insulin resistance (95). Intriguingly, however, vaspin expression was detectable only in a minority of human adipose samples, and its expression in visceral adipose was positively associated with obesity and impaired glucose tolerance, although its expression in subcutaneous adipose was correlated with insulin sensitivity (129). The fat-derived hormone leptin also has a complex relationship to insulin sensitivity. Although the appetite centers in the brain are its main target, leptin also appears to have effects on insulin action in peripheral tissues, such as well as on blood vessels and pancreatic β-cells (214, 234). There are some indications that, as with insulin, obesity is associated with a state of peripheral leptin resistance (156). Moreover, hyperinsulinemia promotes both insulin resistance and stimulation of leptin production and secretion from adipose tissue. This may in turn enhance leptin resistance by further desensitizing the leptin signal transduction pathway, constituting a vicious cycle (234).

Other molecules that may play a role in fat mass signaling include resistin and visfatin. Resistin in mice is produced by adipocytes and induces hepatic and systemic insulin resistance (214, 260). In humans, however, its origin appears to be from macrophages (207). Despite their different cellular origins, evidence that resistin may be related to obesity-induced insulin resistance is accumulating. Increased levels of resistin are associated with insulin resistance (253) and decreased levels of resistin are associated with improved insulin sensitivity with rosiglitazone therapy (132, 122). In vitro experiments have also shown that resistin downregulates the expression of insulin receptors (27). Intriguingly, intracerebroventricular administration of resistin induces hepatic insulin resistance, suggesting that resistin may impact insulin resistance via a central mechanism (174), which is supported by the observation that resistin mRNA is present in the hypothalamus and significantly depleted in the mouse pituitary gland by food restriction (266). Visfatin has been described as a molecule originating from visceral fat but with concentrations inversely proportional to insulin sensitivity (74, 246).

A newly described and very interesting adipocytokine is retinol-binding protein-4 (RBP4) (88, 250, 276). This molecule has been shown by Kahn’s group (276) to be produced by insulin-resistant adipose tissue, whether the insulin resistance is a consequence of glucose transporter knockout, obesity, or high-fat feeding. The gene appears to be more highly expressed in visceral than subcutaneous adipose tissue (130), and this may be influenced by single nucleotide polymorphisms in the RBP4 gene (135). This protein has been shown to inhibit insulin action in skeletal muscle and liver, and insulin sensitivity in mice is improved by a small molecule that lowers RBP4 levels through increased renal excretion (276). However, the role of this new protein in human insulin resistance has not reached a consensus (45, 80, 110), in part because of issues of assay methodology (87) and the presence of free and complexed RBP4 in the circulation (167).

Finally, the recently identified endogenous ligand of the human G protein-coupled APJ receptor apelin deserves mention here. Apelin is widely distributed in various tissues, exerting a broad range of physiological actions, including heart contractility, blood pressure, fluid homeostasis, and appetite (111, 128). It is a positive regulator of angiogenesis, is induced by hypoxia, and leads to NO-dependent vasodilation (84, 128). Furthermore, apelin has been recognized to be an endocrine regulator of AMPK synthesized and secreted by adipocytes (25). It has been shown to have a substantial glucose-lowering effect, enhancing glucose utilization in skeletal muscle and adipose tissue (25). Acute infusion of apelin in chow-fed mice drastically reduced glucose levels, stimulating peripheral glucose uptake via AMPK-, Akt-, and eNOS-dependent pathways (62). In obese and hyperinsulinemic mice and humans, plasma apelin concentrations are increased and correlate with plasma insulin levels (62), and insulin has been shown to directly influence the gene expression of apelin in adipocytes (25). Interestingly, a hypocaloric diet in obese women associated with weight loss reduced increased plasma and adipose tissue apelin expression (39). It has been hypothesized that apelin
Table 1. Potential molecular mediators of insulin action produced by adipose tissue

<table>
<thead>
<tr>
<th>Cellular Origin</th>
<th>Depot Specificity</th>
<th>Plasma Levels in Obesity or Insulin-Resistant States</th>
<th>Effect on Insulin Action</th>
<th>Effect on Inflammation</th>
<th>Effect on Vascular Endothelium</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA (90, 112, 204)</td>
<td>A</td>
<td>None</td>
<td>Increased during fasting and in insulin-resistant states</td>
<td>–</td>
<td>Pro-inflammatory</td>
<td>Impairs endothelial-dependent vasodilation</td>
</tr>
<tr>
<td>Adiponectin (189, 227, 271)</td>
<td>A</td>
<td>SAT =&gt; VAT (Expression)</td>
<td>Reduced</td>
<td>+</td>
<td>Anti-inflammatory</td>
<td>Protects against endothelial damage</td>
</tr>
<tr>
<td>Leptin (34, 68, 69, 270)</td>
<td>A</td>
<td>None</td>
<td>Increased</td>
<td>+</td>
<td>Suggested pro-inflammatory action in specific circumstances (273)</td>
<td>Not well established Established adiposity signal to the brain</td>
</tr>
<tr>
<td>TNF-α (148, 194, 215, 245, 269)</td>
<td>M</td>
<td>Not well defined</td>
<td>Increased</td>
<td>–</td>
<td>Pro-inflammatory</td>
<td>Inhibits endothelial nitric oxide production, induces adhesion molecule expression</td>
</tr>
<tr>
<td>IL-6 (15, 60, 93, 106, 137, 216)</td>
<td>A</td>
<td>VAT =&gt; SAT (portal vein concentration exceeds that of radial artery)</td>
<td>Increased</td>
<td>– and + (different effects on different tissues; refer to text)</td>
<td>Pro-inflammatory</td>
<td>Potential inhibition of vasodilatation and capillary recruitment</td>
</tr>
<tr>
<td>PAI-1 (6, 16, 57, 73, 145)</td>
<td>M</td>
<td>Not well defined</td>
<td>Increased</td>
<td>–</td>
<td>Pro-inflammatory</td>
<td>Prothrombotic</td>
</tr>
<tr>
<td>Resistin (132, 174, 253)</td>
<td>A (rodents) and M (humans)</td>
<td>Not defined</td>
<td>Increased</td>
<td>–</td>
<td>Pro-inflammatory?</td>
<td>Not well defined Intracerebroventricular administration of resistin induces hepatic insulin resistance</td>
</tr>
<tr>
<td>RBP4 (88, 130, 276)</td>
<td>A</td>
<td>Not defined</td>
<td>Increased</td>
<td>–</td>
<td>Pro-inflammatory</td>
<td>Not defined</td>
</tr>
<tr>
<td>MCP-1 (22, 53, 61)</td>
<td>A</td>
<td>Not defined</td>
<td>Increased</td>
<td>–</td>
<td>Pro-inflammatory</td>
<td>Not defined</td>
</tr>
<tr>
<td>Visfatin (78, 285)</td>
<td>A</td>
<td>Produced from both depots; however, different role depending on the depot</td>
<td>Increased (visceral visfatin) decreased (subcutaneous visfatin)</td>
<td>– (Visceral visfatin) + (subcutaneous visfatin)</td>
<td>Pro-inflammatory (visceral visfatin)</td>
<td>Not defined</td>
</tr>
<tr>
<td>Vaspin (95, 129, 279)</td>
<td>A</td>
<td>VAT =&gt; SAT</td>
<td>Increased; however, this relationship seems abrogated in the presence of T2DM</td>
<td>+</td>
<td>Not well defined</td>
<td>Not defined</td>
</tr>
<tr>
<td>Thrombomodulin-1 (259)</td>
<td>A</td>
<td>Unknown</td>
<td>Up (gene expression in adipose tissue)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Apelin (25, 39, 62)</td>
<td>A</td>
<td>Unknown</td>
<td>Increased</td>
<td>+</td>
<td>Unknown</td>
<td>Endothelium-dependent vasodilation by triggering NO release</td>
</tr>
<tr>
<td>Osteopontin (85, 175, 181)</td>
<td>M</td>
<td>Unknown</td>
<td>Increased in obesity, independently of insulin resistance</td>
<td>–</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Lipocalin-2 (251, 262, 275)</td>
<td>A, M</td>
<td>Unknown</td>
<td>Increased</td>
<td>–</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Nos. in parentheses are references. A, adipocyte; M, adipose tissue macrophage or stromal vascular fraction; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; +, associated with improving insulin sensitivity; –, associated with worsening insulin sensitivity.
homeostasis may also be impaired in and contribute to obesity-associated disorders, potentially by developing adipose tissue mass via angiogenesis and/or preadipocyte proliferation (25). It remains, however, that the exact role of apelin in glucose homeostasis has yet to be elucidated.

Although this section has documented single candidates responsible for adipose tissue cross talk, insights into the mechanisms of homeostasis in human physiology would make it more likely that multiple, rather than single, cross talk mechanisms exist. As such, it is likely that different metabolic perturbations in adipose tissue are associated with a variety of alterations of adipocytokine expression and secretion, with a plethora of different signals necessary to overcome physiological homeostasis. This general understanding may also apply to the inflammatory hypothesis, where a role is proposed for proinflammatory molecules in inducing insulin resistance. And these inflammatory signals may operate via either systemic (endocrine) or local (paracrine, portal, or “vasocrine”) routes.

**Obesity and insulin resistance: The inflammatory hypothesis.** Inflammatory signaling: the role of molecular signals. The inflammatory hypothesis proposes that proinflammatory molecules generated from fat, or in the obese state, may induce insulin resistance (Fig. 1). This hypothesis shares characteristics with the endocrine hypothesis in that the molecules responsible may act via an endocrine route. However, we are categorizing this as a separate entity, considering that these molecules, which are principally inflammatory in nature, are produced mainly by adipose tissue macrophages as opposed to an adipocyte source for the molecular candidates for the endocrine hypothesis (Table 1). The concept that inflammation may play a role in causing insulin resistance stems from the observation that high doses of salicylates are able to lower blood glucose concentrations (12, 103). Furthermore, it is now clear that obesity is a chronic low-grade inflammatory state. This concept was initially proposed by the pioneering work of Hotamisligil et al. (99), who first demonstrated that TNF-α, one of the primary inflammatory cytokines, was expressed in adipose tissue, that its expression was induced in obese states, and that its increased expression could contribute to systemic insulin resistance (99). Subsequent studies, which included an evaluation of TNF-α-null and TNF-α receptor-null mice, have largely supported this hypothesis (170). Moreover, recent studies using gene microarray techniques found increased levels of multiple proinflammatory gene expression in adipose tissue from obese rodents compared with lean counterparts (264, 272). The fact that these effects appear to precede the development of insulin resistance during high-fat feeding further supports the notion that adipose-derived inflammatory factors may have a causative role in the development of obesity- and high-fat diet-induced insulin resistance. Perhaps the most compelling evidence comes from studies where the IKKβ and nuclear factor-κB (NF-κB) pathways are activated in the liver by high-fat feeding in mice and obese Zucker rats, in parallel to the development of insulin insensitivity in both the liver and skeletal muscle (32). More directly, the animals were protected from high-fat diet-induced insulin resistance when IKKβ was...
lacking in the myeloid cell line (8). These observations raise the intriguing possibility that adipose-derived proinflammatory cytokines, perhaps in combination with circulating NEFA, may play a major role in fat mass signaling, a so-called inflammatory hypothesis. The inflammatory signals are produced by adipose tissue and either systemically induce insulin resistance or are first propagated from fat to liver, and then systemically, to skeletal muscle and other target tissues. The latter hypothesis is an extension of the portal and endocrine hypotheses; however, instead of predominantly NEFA-derived signals, these signals are a combination of various adipocytokines.

Increased NEFA levels also contribute to systemic inflammation and affect insulin action. Recent studies suggest that NEFA effects on inflammatory pathways are mediated via the Toll-like receptor-4 (TLR-4) (236), which has an important role in inflammation and immunity. Structural similarities between saturated fatty acids and lipopolysaccharide (LPS), the canonical bacterial antigen, have been postulated to explain the stimulatory effects of NEFA on TLR-4. Indeed, mice with a loss-of-function mutation in TLR4 are protected against the development of diet-induced obesity (257). This mechanism also appears to be relevant to vascular inflammation and insulin resistance, since disruption of TLR-4 blocks the ability of palmitate to activate IKKβ and to inhibit both insulin signal transduction and insulin-stimulated NO production (123). Additionally, NEFA induces a direct proinflammatory response in skeletal muscle, which can be prevented by one session of endurance exercise in humans (225).

Which, then, are the inflammatory adipocytokine candidates responsible for organ cross talk in obesity-induced insulin resistance? Of the adipocytokines, TNF-α has been best characterized for its action in inducing insulin resistance through inflammatory pathways, with consequent effects on IRS-1 and Akt phosphorylation (100, 199, 238) and on AMPK signaling (245). Rodent studies suggest that shedding of the membrane-bound form of TNF-α via the activity of a converting enzyme is needed for paracrine activity (233) and that cell signaling occurs via the TNFR1 receptor (140). An increase in TNF-α mRNA expression has been observed in adipose tissue from four different rodent models of obesity and diabetes, with elevated local and systemic levels of TNF-α protein (98). Interestingly, the actions of TNF-α on insulin signaling are very similar to those of NEFA. TNF-α and NEFA have both been shown to activate the c-Jun NH2-terminal kinase (JNK) and the inhibitor of NF-κ kinase (IKKβ) inflammatory pathways (265), the most likely pathways for mediating the inhibition of insulin signaling (238), as seen in both a skeletal muscle (232) and an adipocyte cell line (180). Additionally, TNF-α induces endothelial cell activation, expression of adhesion molecules, and inhibition of endothelial function (19). In parallel, TNF-α induces endothelial cell insulin resistance through a MAPK pathway (142). Recent interesting evidence is that insulin sensitivity is improved by treatment through neutralizing TNF-α with the monoclonal antibody infliximab in patients with rheumatoid arthritis or ankylosing spondylitis (86, 127), indicating that TNF-α is indeed an important adipocytokine that may be at least partially responsible for an insulin-resistant state. Other studies, however, showed no effect on improving insulin sensitivity after treatment with a TNF-α monoclonal antibody or receptor (99, 188, 194). These conflicting results could be due to differences in duration of treatment (shorter in the latter experiments) or selection of patient groups, insufficient power of the studies, or the potential paracrine action of the molecule. Although TNF-α may not be a good candidate as a systemic fat-derived signal, due to its low circulating concentration other than in conditions such as septic shock (100), it is still possible that it has important paracrine or autocrine effects within adipose tissue or that its portal delivery is more important than its systemic effect. In fact, in vitro studies demonstrated that the macrophage activation state is influenced by contact with adipocytes and that factors derived from the macrophage, in turn, induce insulin resistance in adipocytes, an action which is partially reversed by a TNF-α-neutralizing antibody (151).

**Interleukin-6** (IL-6) is perhaps the cytokine that most clearly plays a systemic role as the main driver of hepatic production of the acute-phase reactant **C-reactive protein** (CRP) (283). It is generated in sufficient quantities by adipose tissue to represent up to 30% of circulating levels in healthy subjects (169). The role of IL-6 in insulin resistance has been the topic of substantial debate (172, 195). This cytokine may mediate hepatic insulin resistance (115). In fact, in mice with constitutive overexpression of inflammatory pathways in hepatocytes, circulating levels of IL-6 were increased two- to threefold, whereas neutralization of IL-6 using monoclonal antibodies improved insulin resistance (32). A recent study related portal vein concentrations of cytokines to systemic levels of inflammation and showed a substantial secretion of IL-6 from visceral fat as well as a close correlation between portal levels of this cytokine and systemic levels of CRP (76). In contrast, IL-6 generation by exercising muscle has been postulated as a mechanism whereby glucose uptake is increased during physical activity (70); and infusion of IL-6 in doses insufficient to produce systemic symptoms enhances muscle insulin action through activation of AMPK (37). It is interesting to note that CRP itself may have a direct effect on IRS-1 serine phosphorylation in L6 myocytes (54). These effects occur at physiological concentrations of CRP (54) and are consequent upon the activation of extracellular signal-regulated protein kinase-1/2 (ERK1/2) and JNK. This may represent the indirect effect of IL-6 on muscle insulin resistance via increased levels of CRP.

Finally, there is growing evidence linking **plasminogen activator inhibitor-1** (PAI-1) with obesity and insulin resistance; PAI-1 is a key regulatory protein of tissue fibrinolysis, cell migration, angiogenesis, and tissue remodeling (144). It has been shown that adipose tissue is a significant source of PAI-1, with the bulk of production derived specifically from the stromal vascular fraction in visceral fat (92, 162, 163). Fascinatingly, PAI-1 deficiency reduces adiposity, improves the metabolic profile in genetically obese mice (224), and attenuates diet-induced obesity and insulin resistance in C57BL/6 mice (39, 155). Furthermore, in mouse models, the absence or inhibition of PAI-1 through genetic alteration in adipocytes protects against insulin resistance by promoting glucose uptake and adipocyte differentiation via increased PPARγ expression (141).

There are, however, differences between liver and skeletal muscle in the responses to activation of inflammatory signaling pathways. In mice, the liver NF-κB pathway is activated by high-fat feeding and is integral to producing hepatic insulin...
resistance (8, 32). Activation of the NF-κB pathway through muscle-specific transgenic expression of activated IKKβ, however, results in muscle wasting but without increasing expression of cytokines or effects on skeletal muscle insulin action (31, 202). In parallel, inactivation of the NF-κB pathway in mouse skeletal muscle is without effect on glucose homeostasis, even after induction of obesity (213). Interestingly, although skeletal muscle NF-κB appears to be unconnected to obesity-related insulin resistance, the peripheral insulin resistance induced by high-fat feeding in mice is almost completely prevented in animals lacking a myeloid cell line NF-κB pathway (8). Taken together, these data suggest that the peripheral insulin resistance that follows hepatic insulin resistance in high-fat feeding, as described in Obesity and insulin resistance: The portal hypothesis, may depend more significantly on activation of inflammatory pathways in circulating monocellular cells than on such signaling mechanisms in local target tissue.

Again, the multitude and complexity of inflammatory cytokines produced by adipose tissue would seem to confirm the concerted effect of these products at points upstream in the insulin-signaling pathway on subsequent insulin resistance. The questions then become: why does adipose tissue from obese subjects secrete more inflammatory cytokines than that from lean subjects; what happens in the adipose tissue bed to make it more inflammatory?

INFLAMMATORY SIGNALING: THE ROLE OF ADIPOSE TISSUE MACROPHAGES. Not all adipose tissue beds are alike in the amount of adipocytokines they produce. Please refer to Table 1, which outlines the cellular origins and depot specificity of the various adipocytokines discussed here. Surgical fat removal in animals, an established method for studying the influence of fat accumulation on systemic metabolism, could provide valuable information regarding the role of visceral fat, as subcutaneous lipoectomy results in a compensatory increase in the amount of visceral fat (160). A study in which mice underwent partial subcutaneous lipoectomy showed the development of insulin resistance in association with a significantly greater fat accumulation and higher TNF-α expression in visceral adipose tissue (VAT) (109). The transplantation of VAT back into the subcutaneous compartment after lipoectomy reversed the systemic and tissue features of insulin resistance. This indicates that the functional difference between SAT and VAT is, at least in part, consequent upon the interaction of adipocytes with their milieu, a hypothesis that is supported by in vitro studies (237). The expression and secretion of IL-6 are substantially greater in visceral and truncal fat than in subcutaneous fat (9), and its production is also increased by lipid accumulation in the adipocyte (67, 220).

One major difference between different adipose tissue beds and between states of over- and undernutrition is the degree of infiltration by macrophages. Observations demonstrating the existence of adipose tissue macrophages, their increase in number with obesity, a high-fat diet, and increasing adipocyte size (52, 264, 272), and reduction in number with weight loss (35) or increased intake of n-3 fatty acids (252), have raised the possibility that these cells, rather than the adipocytes themselves, are the main source of adipocytokine production. Cell separation studies have shown that adipose tissue macrophages are responsible for the expression of most of the TNF-α and substantial amounts of other cytokines (264). Moreover, if macrophage inflammatory pathways are inactivated, such as by creating a mouse lacking the IKKβ enzyme in myeloid cells, the animal is protected from the effect of a high-fat diet on both hepatic and peripheral insulin resistance (8). Recent elegant studies have shown that the phenotypic shift of adipose tissue macrophages to a proinflammatory state in obesity results from recruitment of inflammatory macrophages from the circulation rather than from a switch of resident macrophage phenotype (150, 178). This suggests that the consequences of adipose tissue excess are mediated through recruitment of macrophages and their secretion of inflammatory mediators, which may also play a part in further stimulating cytokine release from adjacent adipocytes. The systemic effects arising from such a process will thereby depend on endocrine signals generated in fat by both adipocytes and macrophages, with other inflammatory molecules, such as TNF-α, exerting effects in a paracrine, rather than an endocrine, fashion. It is also possible that the circulating inflammatory signal in obesity is not an individual cytokine but a systemic state of blood monocyte activation (83), which may respond to local chemoattractant molecules and trigger inflammatory cascades in remote tissues. In support of this paradigm, a recent study showed that in obesity the circulating mononuclear cell population is indeed characterized by increased levels of inflammatory mediators such as IKKβ and suppressor of cytokine signaling-3 (SOCS3), suggesting that the circulating mononuclear cell in obesity is inflamed (82).

Thus, if obesity is a low-grade inflammatory state, the question arises how macrophages are recruited into the adipose tissue. As mentioned before, it has been shown that macrophage content increases with adipocyte size (272). Interestingly, adipose tissue macrophages in obesity form crown-like structures around necrotic adipocytes, indicating the possibility that adipocytes may achieve a certain maximum size before cell death which then attracts macrophages to clear the necrotic debris (46). This finding is consistent with the previously mentioned study (263), which showed higher numbers of macrophages in adipose tissue consisting mainly of larger adipocytes.

It may be hypothesized, then, that hypertrophied adipocytes, as a result of obesity, have abnormal secretory properties, such as increased expression of monocyte chemoattractant protein-1 (MCP-1), colony-stimulating factor-1 (CSF-1), and transforming growth factor β (TGF-β) (24, 139, 153, 223), thereby recruiting more monocytes into adipose tissue than normal adipocytes (114, 176, 263) (Fig. 1). Previous studies have further supported this concept of an interaction between adipocytes and macrophages (151, 193). It is notable that the inflammatory properties of macrophages in fat seem to require the presence of adipocytes. FAT-ATTAC mice, whose adipocytes undergo apoptosis, showed significantly lower concentrations of circulating proinflammatory cytokines compared with wild-type mice despite the significant number of macrophages in the adipocyte-free adipose tissue pads (193). Furthermore, as referred to above, a series of studies by Lumeng et al. (149, 151, 152) suggests two-way cellular cross talk between adipocytes and macrophages, mediated in part by TNF-α.

Among the chemoattractants responsible for monocyte recruitment, MCP-1, also known as CC-motif chemokine ligand-2 (CCL-2), is considered one of the most important candidates. Mice deficient in the receptor for this ligand are
resistant to the adverse effects of high-fat feeding on insulin action and show decreased adipose tissue macrophage infiltration, reduced production of adipocytokines, and less hepatic steatosis (263). Another recent study has shown that overexpressing or knocking out MCP-1 in mouse adipocytes produces increased or decreased hepatic steatosis and insulin resistance, respectively (114). Interestingly, Saltiel’s group (149, 152) has recently shown that macrophages in mice recruited during a high-fat diet have a different inflammatory profile from resident macrophages or those found in lean mice that such macrophages are recruited from the circulation and that such recruitment is in part dependent on the CCL-2 receptor (CCR) (150). This suggests that macrophages recruited during high-fat feeding and dependent on MCP-1 signaling may be responsible for the inflammation and consequent insulin resistance. MCP-1 exerts its effects primarily on the more proinflammatory classically activated subtype of macrophages, recruiting them to sites of tissue damage (187). MCP-1 also circulates in plasma and may itself play an endocrine role in inhibiting insulin action in skeletal muscle (230). Surprisingly, in a recent study by Inouye et al. (107), MCP-1-deficient mice on a high-fat diet did not show any reductions in adipose tissue macrophages yet demonstrated worsened hyperinsulinemia and hyperglycemia. This study therefore suggests an independent metabolic role of MCP-1.

MCP-1 may also have an auxiliary function in establishing greater adipose tissue accumulation in obesity (133, 154, 208, 221, 222, 261). WAT has the capability to expand, and histological evaluation reveals that fat is highly vascularized, suggesting that the presence of blood vessels may be necessary to maintain its mass. Indeed, studies have demonstrated that either general or adipose-specific inhibition of angiogenesis can prevent the development of obesity in mice in association with an improved metabolic profile (133, 221). MCP-1, in addition to acting as an important adipocyte-generated chemotactic factor for monocytes and macrophages, as described above, is also substantially produced by several tumors and contributes directly to tumor angiogenesis by mechanisms involving TGF-β-mediated vascular smooth muscle cell migration (154), independent of monocyte recruitment (222). It is possible, then, that, in situations of obesity or positive energy balance, recruited macrophages may significantly contribute to the expansion of adipose tissue vasculature, thereby activating a vicious cycle (261).

In light of the importance of the adipose macrophage in insulin resistance, a series of somewhat counterintuitive observations relate to the role of adipose tissue cortisol metabolism. Many previous animal studies have shown that the administration of an inhibitor of 11β-hydroxysteroid dehydrogenase (11β-HSD) type I, or genetic deficiency of 11β-HSD type I, ameliorated insulin resistance (3, 158, 173), whereas adipose specific overexpression of 11β-HSD type I produced a phenotype remarkably similar to that of human metabolic syndrome (158, 159), including visceral obesity, insulin-resistant diabetes, hyperlipidemia, hyperphagia, and hypertension. 11β-HSD type I is an enzyme that regenerates active cortisol from inactive cortisone in a tissue-specific manner, leading to local cortisol excess within key metabolic tissues as a consequence of heightened enzyme activity in obesity (164). Although the anti-inflammatory properties of cortisol might suggest that adipose macrophages may be further activated by lowering local cortisol levels through inhibition of 11β-HSD type I, a recent study opened up the possibility that inhibiting 11β-HSD type I renders the macrophage less rather than more inflammatory (108), suggesting discordant effects of local endogenous cortisol production vs. high-dose exogenous glucocorticoid therapy.

The proposed paradigm, then, is that, in response to any change in milieu which induces insulin resistance in the adipocyte, whether it be positive energy balance, high glucose concentrations, high NEFA levels, or locally or systemically produced cytokines, the adipocyte or stromal vascular fraction in adipose tissue will generate MCP-1. This would result in macrophage recruitment and/or activation with subsequent hepatic and systemic insulin resistance, potentially in association with further fat mass growth. Any of the mechanisms that have been proposed as responsible for insulin resistance within target tissues, such as reactive oxygen species (ROS) (64, 101, 146), endoplasmatic reticulum (ER) stress (191), and AMP kinase/malonyl-CoA (220, 230), or mitochondrial dysfunction (196), may act as the mechanism within the adipocyte whereby MCP-1 is induced and macrophages are recruited (50). The reader is referred to a recent review for a detailed discussion of the potential mechanisms whereby insulin-sensitive tissues sense nutrient excess, thereby generating insulin resistance in adipose tissue and skeletal muscle (226).

To hypothesize which mechanisms are operative in adipose tissue, it is necessary to delineate changes that occur in response to metabolic challenges in other target tissues. Elegant work from Hotamisligil’s group has shown that a high-fat diet and obesity cause ER stress in liver cells, perhaps in consequence of ROS generation (191). The chronic metabolic milieu of hyperglycemia, increased levels of NEFA, and proinflammatory cytokines also increases the generation of ROS in endothelial cells and adipocytes (64, 101, 146). Systemic markers of oxidative stress increase with obesity, consistent with a role for ROS in the development of obesity-induced insulin resistance (117). These alterations could be the downstream effect of activation of NADPH oxidase by lipid accumulation in the adipocyte, with subsequent increases in ROS production (77). Ruderman et al. (220) have defined an AMP-kinase/malonyl-CoA nutrient-sensing mechanism in muscle, liver, and endothelial cells that plays an important role in activating or inhibiting insulin signaling. Some data suggest that these mechanisms may also occur in adipocytes, where they may regulate inflammatory cascades (230). Indeed, this may explain how positive or negative energy balance rapidly influences insulin resistance and several associated characteristics much more dramatically than might be anticipated with small changes in fat mass. Additionally, mitochondrial dysfunction is found in skeletal muscle of elderly insulin-resistant subjects (196), although whether this is a consequence of the AMP-kinase/malonyl-CoA mechanism, oxidant stress, and/or adiponectin action is unclear (226).

Recently, a family of proteins collectively known as sirtuins is gaining increasing attention as possible regulators of insulin action, mitochondrial function, and systemic and adipose inflammation (91). Sirt1 reproduces some of the beneficial effects of caloric restriction, promoting fat mobilization through suppression of peroxisome proliferator-activated receptor-γ (PPARγ), resulting in decreased triglyceride storage and increased secretion of free fatty acids (FFA) (197). Sirt1 may
also modulate inflammation, by inhibiting the transcription of nuclear factor-κB (NF-κB), one of the key regulators of inflammation (165). Finally, a role has been proposed for adipose tissue hypoxia, consequent upon tissue hypoperfusion in obesity, causing dysregulated adipocytokine production in obesity (97). In particular, local adipose tissue hypoxia in obese mice has been shown to decrease adiponectin mRNA expression and increase gene expression of PAI-1, leptin, and C/EBP homologous protein (CHOP), an ER stress-mediated protein, thus contributing to the metabolic syndrome in obesity (97).

**Obesity and insulin resistance: The overflow and vasocrine hypotheses.** When adipocytes exceed their storage capacity and the process of adipocyte proliferation fails, fat begins to accumulate in tissues not suited for lipid storage, such as liver, muscle, or blood vessel, which then may form specific metabolites that inhibit insulin signal transduction in these tissues (Fig. 2). High-fat feeding results in triglyceride accumulation in hepatocytes (176, 262), termed hepatosteatosis (18, 120), and consequent hepatic insulin resistance. In a similar fashion, the accumulation of intramyocellular lipids may contribute to the muscle insulin resistance of physical inactivity, obesity, or a high-fat diet (118). The deposition of intracellular triglycerides in these insulin target organs may contribute to insulin resistance by the intracellular presence of a constant NEFA source, acting either as a substrate or a signal modulator for these cells (23) or by activation of inflammatory pathways (71, 247). The triggers for ectopic fat deposition are unclear, with possible roles for elevated concentrations of NEFA (either fasting or throughout the day) or for circulating inflammatory cytokines (43). A recent patient study has shown a close correlation between the degree of macrophage infiltration in omental fat and the degree of hepatic inflammation and fibrosis (36). In mice, modulation of macrophage infiltration in adipose tissue by manipulating the expression of MCP-1 (114) or its receptor (263) produced corresponding changes in hepatic steatosis and hepatic/muscle insulin sensitivity. It is possible, then, that the overflow and inflammatory hypotheses of insulin resistance are complementary, with the deposition of ectopic fat both consequent upon and contributing to (32, 43, 71) circulating inflammatory signals.

Ectopic fat deposition also occurs around blood vessels. While there may be parallel roles for this ectopic fat in propagating signals to remote organs and tissues, this fat differs in microanatomy and physiology in that it is intrace-
lular in its deposition and, hence, signals to insulin-sensitive cells in a paracrine fashion. We have demonstrated that there is a cuff of adipose tissue around the origin of nutrient arterioles, isolated from cremaster muscles from obese Zucker rats (282). Using a variety of insulin-signaling pathway inhibitors, we have shown that, in these animals, the PI 3-kinase insulin-signaling pathway is impaired, and NO production is suppressed (161). This has led us to propose that in states of obesity perivascular fat may signal to the vessel wall, both locally (paracrine) and downstream (vasocrine), through outside-to-inside signaling, with TNF-α being a likely mediator (282) (Fig. 2). Perivascular fat around nutrient arterioles may inhibit the effects of systemic insulin on local vasodilatation, with consequent inhibition of postprandial increases in nutritive blood flow and insulin action. Crucially, it is possible that such downstream delivery of inflammatory mediators through the nutritive capillary bed may impact on the insulin-signaling pathways in the skeletal myocytes, producing a state of muscle insulin resistance independent of constraints on nutritive blood flow and substrate delivery. Such a hypothetical mechanism might explain the observations that inducing overactivity of the NF-κB pathway in skeletal muscle itself is without effect on muscle insulin sensitivity (31, 202). It is also postulated that the increased intravascular concentration of TNF-α will be limited to the muscle nutritive capillary bed, with TNF-α binding proteins likely to inhibit any activity of the molecule in the systemic circulation. The predominant source of these adipocytokines in perivascular fat, again, is likely to be macrophages recruited from the circulation or through activation of local resident macrophages (264), although this hypothesis needs further experimental testing.

We have proposed that the relationship between insulin resistance and vascular disease could represent the coexistent downstream consequences of inflammatory cytokines inducing endothelial dysfunction in parallel with its effects on other insulin responsive organs (284). The production of these cytokines from perivascular fat may imply that the effects on both nutritive blood flow and insulin action are mediated via local rather than systemic signaling, although as indicated above there is a possible systemic contribution to the process through recruitment of activated circulating mononuclear cells to perivascular fat depots (Fig. 2). Perivascular adipocytes show reduced adipocytic differentiation compared with those from subcutaneous and visceral depots and secrete less adiponectin and more proinflammatory cytokines, particularly after high-fat feeding (41). Intriguingly, the role of perivascular fat may also be important in arterial disease, as has been proposed by Mazurek and colleagues (105, 161). This group described substantial macrophage infiltration in epicardial fat corresponding anatomically to the location of atheromatous plaques in underlying coronary vessels. The observation that epicardial fat volume closely correlates with that of visceral fat (105) suggests that there may be a homology between perivascular fat around large arteries (with resultant outside-to-inside signaling and atherosclerosis) and visceral fat around the heart and in the peritoneum (282). If there is a corresponding homology between perivascular fat and visceral fat, the relationships of visceral obesity, insulin resistance, and atherothrombotic vascular disease become clear.

Considering the aforementioned organ cross talk, the question then follows how fat mass might signal to endothelium, initiating endothelial inflammation and damage. Potential signals as circulating molecules have been considered earlier. NEFA do not seem to be a likely candidate, because fasting concentrations are generally not substantially elevated in insulin-resistant states (11). Of the cytokines, TNF-α can certainly produce endothelial activation and inhibition of NO production (168) but, as pointed out earlier, is not present in the systemic circulation of healthy obese subjects in sufficient concentrations for this effect to be likely (100). IL-6 on its own does not have a major impact on endothelial function (19). Adiponectin may function as an endothelial protector by increasing NO synthesis and reducing inflammation (56, 189, 227). It is also possible that endothelial cells themselves, like adipocytes, respond to overnutrition or a high-fat diet by changes in cell redox state or through activation of AMP-kinase/malonyl-CoA pathways (218). A recent study investigating the time course of high-fat diet on generating insulin resistance in adult mice showed effects on aortic inflammation, endothelial dysfunction, and insulin resistance within 1 wk, whereas skeletal muscle and liver insulin resistance was measurable only after 4–14 wk (124). These observations support a major contributing role of vascular insulin resistance to the systemic effects of high-fat feeding, although the role of peri-aortic fat was not explored in that study.

The above hypothesis now leaves one further question: if perivascular and truncal fat act as an integrated organ responsible for generating local and systemic inflammatory signals, what is it that determines this pattern of fat distribution? Central adiposity is a feature of low birth weight (212, 274), aging (55), and physical inactivity (116). The Whitehall study showed the association of low social class and work-related stress with central obesity (29). Possible mechanisms for this include the activation of the hypothalamo-pituitary-adrenal axis (21), local production of glucocorticoids (30), or effects of a dysfunctional autonomic nervous system (94). Moreover, in a rat hindlimb model, increases in nonnutritive blood flow (as would occur with inactivity) produce deposition of septal fat rather than uptake of NEFA by muscle as a substrate (49). Therefore, it is likely that the individual pattern of fat distribution is of multifactorial origin, and future studies are likely to uncover additional contributing factors.

Obesity and insulin resistance: A neural hypothesis. As elaborated earlier in this article, it has become clear that the brain is an insulin-sensitive organ and glucose metabolism in insulin-resistant individuals is disrupted in the brain as in other target organs. What, then, could be the cause of insulin insensitivity of the brain in the state of obesity? First of all, insulin transport may become rate limiting under certain circumstances, as has been outlined above regarding insulin’s access to the interstitial space and its action in muscle and fat (18, 77, 230). It is notable that a capillary endothelium that comprises the blood-brain barrier is surrounded not only by pericytes but by macrophages, which are stimulated by cytokines and chemokines in inflammatory brain diseases (134) (Fig. 3). It could then be proposed that, in the low-grade inflammatory state found in obesity, these macrophages are also involved in the generalized activation of the NF-κB pathway (31, 213, 214). While the insulin receptor-mediated transporter in brain capillary endothelium has not been characterized at the cellular level, it is possible that serine phosphorylation of IRS-1 might adversely affect
insulin transport as it does in other insulin-sensitive cells (8). In such a way, the etiology of insulin resistance may be similar in the brain, blood vessels, and, through vasocrine effects, muscle.

It is likely that the brain’s involvement in the regulation of glucose homeostasis serves more as a master regulator rather than as a mere target organ (Fig. 3). Phrased differently, the brain’s insensitivity to insulin and/or other nutrients such as glucose or NEFA seems to influence endogenous glucose production, adipose tissue lipolysis, and adipocyte proliferation. In insulin-sensitive subjects, the brain processes information from adiposity signals such as insulin and leptin and integrates this input with nutrient signals such as NEFA and glucose (183, 229), thereby favoring the return of food intake and glucose production by the liver to their original levels. On the contrary, the brain in insulin-resistant individuals will result in elevated levels of both body fat content and hepatic glucose production due to defects in brain nutrient sensing.

Rossetti et al. (185) provided supporting evidence in a study where restoring hypothalamic levels of long-chain fatty acyl-CoAs in overfed rats normalized food intake and glucose production by the liver to their original levels. On the contrary, the brain in insulin-resistant individuals will result in elevated levels of both body fat content and hepatic glucose production due to defects in brain nutrient sensing. Rossetti et al. (185) provided supporting evidence in a study where restoring hypothalamic levels of long-chain fatty acyl-CoAs in overfed rats normalized food intake and glucose production by the liver to their original levels. On the contrary, the brain in insulin-resistant individuals will result in elevated levels of both body fat content and hepatic glucose production due to defects in brain nutrient sensing. Rossetti et al. (185) provided supporting evidence in a study where restoring hypothalamic levels of long-chain fatty acyl-CoAs in overfed rats normalized food intake and glucose production by the liver to their original levels. On the contrary, the brain in insulin-resistant individuals will result in elevated levels of both body fat content and hepatic glucose production due to defects in brain nutrient sensing.
sets of autonomic neurons in the central nervous system. Body fat mass is tightly regulated, as partial surgical lipectomy in several species triggers compensatory increases in nonexercised WAT mass, independent of changes in food intake (160). In Siberian hamsters, surgical denervation of inguinal WAT produces pronounced increases in fat cell number with little change in fat cell size in vivo (75, 280), suggesting that SNS innervation of WAT plays a role in fat pad mass. It is notable that the magnitude of the denervation-induced increase in fat cell number varies between fat pads. This finding is in accord with the observation that obesity typically is associated with decreases in SNS activity and WAT hypercellularity (63). However, it remains to be studied whether the regulation of body fat mass could be the result of a fine balance between the sympathetic and parasympathetic nervous systems.

Conclusions

In conclusion, the associations between obesity and insulin resistance, most importantly, may represent the consequences of various metabolically active substances produced by excess adipose tissue mass. First, recent studies suggest that the cellular origin of adipose-generated inflammatory signals is likely, to a considerable degree, to be macrophages. These macrophages are recruited to inflamed fat, mostly by MCP-1 production, and are responsible for both the generation of inflammatory signals themselves and modulating production of cytokines from adipocytes. Second, the effect of these fat-generated inflammatory molecules on liver, muscle, and adipose tissue itself is local insulin resistance. Organ cross talk is possibly mediated by elevated NEFA levels, one or more circulating cytokines, or even the circulating monocytes themselves. Additionally, these inflammatory signals, as well as the over-supply of NEFA, may contribute to ectopic fat production. The downstream effect of ectopic fat deposition is local insulin resistance, perhaps partially mediated by local accretion of triglycerides in hepatocytes or myocytes and the metabolic effects of adipocytokines. Moreover, at least in the liver and in blood vessels, ectopic fat is associated with the activation of inflammatory cascades and overproduction of cytokines, including TNF-α. Third, the association of insulin resistance and central obesity with structural vascular disease and abnormal vascular function contributes to insulin insensitivity by limiting nutritive blood flow and, thus, insulin and substrate delivery to target tissues. This vascular insulin resistance may represent the consequences of outside-to-inside signaling from perivascular fat. Finally, the brain seems to be a key mediator in this multiple organ cross talk mechanism, responding to signals from adipose tissue and to the general nutritional state, with its output determining fat mass and distribution, hepatic glucose production, and, through the effects of NEFA on TLR4, the inflammatory milieu.

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Mechanisms of Cross Talk by Adipose Tissue

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Review


