Endocrine Functions of Adipose Tissue

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Abstract
Obesity is a risk factor for type 2 diabetes, dyslipidemia, and cardiovascular disease. Dissection of the molecular mechanisms underlying obesity and its relationship to insulin resistance and the metabolic syndrome are essential for developing new strategies for prevention and treatment of these disorders. Both excess adipose tissue and lack of adipose tissue cause insulin resistance and dyslipidemia, suggesting that normal fat is required for the maintenance of systemic glucose and lipid homeostasis. Recent advances in obesity research have led to the recognition that adipose tissue is an active endocrine organ that secretes multiple bioactive factors termed adipokines. Secretion of adipokines provides a link between adipose tissue lipid accumulation and the metabolic function of other tissues such as liver and muscle. Dysregulation of adipokines is emerging as an important mechanism by which adipose tissue contributes to systemic insulin resistance and metabolic disease.
INTRODUCTION

The prevalence of obesity is rising to epidemic proportions worldwide. In the United States, the incidence of obesity (body mass index, or BMI, greater than 30) in adults over 15 years of age is predicted to increase from 32% to 44.2% in males and from 37.8% to 48.3% in females between 2002 and 2010 (1). The financial cost of obesity and related diseases is becoming a heavy burden on healthcare systems. New approaches to the prevention and treatment for obesity are therefore urgently needed.

Obesity, insulin resistance, dyslipidemia, and hypertension are often coincident in an individual, a status referred to as the metabolic syndrome (2). Subjects with the metabolic syndrome are at a greater risk of cardiovascular morbidity and mortality. Obesity is considered to play a principal and causative role in each component of the syndrome (2). However, it is not yet clear how excess fat in adipose tissue causes insulin resistance, dyslipidemia, and atherosclerosis. At the opposite end of the spectrum, patients and mice that lack normal adipose tissue, a pathological condition termed lipoatrophy, also exhibit severe insulin resistance and dyslipidemia. These two extremes make clear that although excess fat contributes to pathology, normal adipose tissue mass is required for the maintenance of systemic glucose and lipid homeostasis.

The identification of leptin in 1994 led to the recognition that adipose tissue functions as an endocrine organ, in addition to a storage depot for excess calories (3). In the 10 years subsequent, researchers have identified a variety of biologically active factors secreted from adipose tissue, termed adipokines. Today, dysregulation of adipokines is recognized as an important factor in the pathogenesis of insulin resistance. Another significant advance in our understanding of the pathogenesis of metabolic disease came with the recognition that inflammatory changes in adipose tissue are associated with obesity and insulin resistance. In 1993, researchers first observed the elevated expression of tumor necrosis factor-alpha (TNF-α) in adipose tissue in obesity (4). Since then, an increasing number of inflammatory mediators have been added to the list of potential factors contributing to insulin resistance. Furthermore, recent studies have focused on macrophages as key players in adipose tissue inflammation and obesity-associated insulin resistance. This review presents an overview of the recent progress in our understanding of the endocrine functions of adipose tissue and highlights the implications of these studies for the pathogenesis and treatment of metabolic disease.

DISCOVERY OF LEPTIN

In 1950, Ingalls et al. (5) first described the obese mutation (ob), an autosomal recessive mutation that causes increased food intake, profound obesity, and type 2 diabetes. Para-biosis experiments predicted that ob/ob mice were deficient for a circulating factor that regulates food intake and metabolism (6, 7). It was more than 40 years, however, before Friedman’s group identified the ob gene by positional cloning (3). This gene, leptin, named after the Greek leptos, meaning thin, is expressed predominantly in adipocytes and encodes a 16 kDa polypeptide secreted into the bloodstream. In ob/ob mice, a nonsense mutation in leptin disrupts the synthesis of functional polypeptide. Supplementation of leptin to ob/ob mice confirmed the significance of leptin for the phenotype of ob/ob mice (8–10); leptin administration dramatically reduced food intake and body weight and reversed the diabetic phenotype. A few years later, leptin receptors were identified through analysis of db/db mice and fa/fa rats (11–13). Leptin receptors belong to the cytokine receptor class I superfamily and are expressed in both brain and peripheral tissues (14).

The discovery of leptin revolutionized the concept of adipose tissue as a passive depot for...
Figure 1
The effect of leptin on feeding behavior, energy expenditure, and adiposity. (a) Leptin secreted from adipocytes directly activates or inhibits neurons expressing the long form of the leptin receptor (Ob-Rb) in the hypothalamic arcuate nucleus, ventromedial hypothalamic nucleus, and dorsal medial hypothalamic nucleus. Leptin also stimulates fatty acid oxidation in muscle and liver by activating 5'-AMP-activated protein kinase (AMPK) both directly and indirectly through the central nervous system. The net effects of leptin action are decreased food intake and increased energy expenditure, resulting in less lipid storage in adipocytes. (b) ob/ob mice are deficient in leptin. Loss of negative feedback from leptin results in increased adiposity. SCD-1, steroyl CoA desaturase-1.

the storage of excess energy. Instead, adipose tissue is now recognized as an active organ that communicates with other tissues by receiving signals and secreting a variety of hormones and metabolites. The current view is that leptin is an adipose-derived signal to the brain and other tissues, and acts as a negative feedback loop for the maintenance of energy homeostasis (Figure 1). The major effects of leptin are mediated by direct activation or inhibition of neurons expressing the long form of the leptin receptor (Ob-Rb) in the hypothalamic arcuate nucleus, ventromedial hypothalamic nucleus, and dorsal medial hypothalamic nucleus, which are known to regulate food intake (Figure 1) (15, 16). The janus-kinase/signal transducer and activator of transcription-3 pathway is a key mediator of intracellular signaling from the leptin receptor (11).
The effect of leptin is not limited to the reduction of food intake, as leptin treatment of ob/ob mice is more effective than simple food restriction (17). Other documented effects of leptin administration include the promotion of energy expenditure (18), suppression of steroyl CoA desaturase-1 (SCD-1) expression in liver (19, 20), and direct effects on peripheral tissues such as pancreatic β cells to prevent ectopic accumulation of lipid (21). Friedman and colleagues identified SCD-1 as a gene overexpressed in livers of ob/ob mice and repressed by leptin treatment but not by pair feeding. ob/ob mice crossed with mice with a mutation in SCD-1 (ab1/ab1) exhibited an attenuated obese phenotype and markedly increased energy expenditure despite persistent hyperphagia. These data suggested that downregulation of SCD-1 expression mediated leptin’s metabolic effect (19). Recent studies have also demonstrated that leptin stimulates fatty acid oxidation in muscle and liver by activating 5′-AMP-activated protein kinase (AMPK) both directly and indirectly through the central nervous system (22).

Not surprisingly, many researchers expected leptin to be a magic cure for obesity and accompanying diseases such as diabetes and dyslipidemia. In fact, as in ob/ob mice, leptin supplementation therapy is quite effective in human subjects with a mutation in the leptin gene (23–25). Similarly, leptin replacement therapy improves hyperglycemia and hyperlipidemia in patients with lipodystrophy who are also leptin deficient (26). However, patients with leptin deficiency are extremely rare. The majority of obese patients and of obese mouse models are characterized by elevated leptin levels in the circulation, a state termed leptin resistance (27–29). Leptin
therapy in this context is ineffective. The precise mechanism of leptin resistance is still unclear, but transport across the blood-brain barrier and intracellular signaling are likely to be altered in leptin resistance (30–32).

LESSONS FROM LIPOATROPHIC MOUSE MODELS

Lipoatrophic diabetes is a syndrome characterized by generalized or localized deficiency in adipose tissue, insulin resistance, hepatic steatosis, hyperphagia, and hypertriglycemia (33). Genetic and acquired (spontaneous or HIV drug-induced) forms are known (34). In the 1990s, three mouse models of lipoatrophic diabetes were described (35–38). Ross et al. (38) overexpressed diphtheria toxin A (DTA) under the control of the aP2 promoter. Mice highly expressing the transgene died soon after birth, whereas aP2-DTA mice with lower levels of transgene expression survived but developed decreased fat mass and insulin resistance after six months. Moitra et al. (35) created A-ZIP/F-1 transgenic mice overexpressing a dominant-negative protein that inactivates CAAT/enhancer binding protein and Jun in adipose tissue. These mice were viable but developed no visible white adipose tissue (WAT), and their livers were enlarged and engorged with lipid. Furthermore, the mice were hyperphagic, diabetic, and showed severely reduced leptin levels and elevated serum glucose, insulin, free fatty acids (FFAs), and triglycerides. Shimomura et al. (36) overexpressed a constitutively active form of sterol response element binding protein-1c (SREBP-1c) in adipose tissue under the control of the aP2 promoter. aP2-SREBP-1c mice showed ∼70% reduction in WAT mass, a milder phenotype than the A-ZIP/F-1 mice. aP2-SREBP-1c mice also exhibited fatty liver, low leptin levels, and elevated glucose, insulin, and triglycerides. Together, analysis of these three mouse lines not only provided models for human lipoatrophic diabetes, but also clearly established the physiological importance of adipose tissue in maintaining glucose and lipid homeostasis. Regardless of the strategy employed, loss of WAT led to the same pathologies.

Transplantation of Adipose Tissue into A-ZIP/F-1 Mice

Reitman and colleagues (39) took a unique approach to verifying the important role of adipose tissue in whole-body lipid and glucose homeostasis. They surgically transplanted adipose tissue from wild-type mice back into lipoatrophic A-ZIP/F-1 mice (Figure 2a). Reconstitution of adipose tissue almost completely reversed the phenotypes of A-ZIP/F-1 mice, including hyperphagia, insulin resistance, hepatic steatosis, and hypoleptinemia. This experiment established that lack of adipose tissue caused the abnormal feeding behavior and the dysregulated glucose and lipid metabolism in A-ZIP/F-1 mice. What is the mechanism by which the ablation of WAT causes these effects? One possible cause is leptin deficiency. To determine the contribution of leptin to the pathogenesis of A-ZIP/F-1 mice, Reitman and colleagues transplanted WAT from ob/ob mice, which lack leptin, into A-ZIP/F-1 mice (Figure 2b) (40). Strikingly, WAT from ob/ob mice was unable to reverse the abnormalities in blood glucose, insulin, triglycerides, food intake, and hepatic lipid accumulation (40). However, the combination of exogenous leptin administration and transplantation of ob/ob WAT improved these symptoms to the same extent as WAT from wild-type mice (Figure 2c).

The above results established that leptin deficiency plays a principal role in the development of metabolic defects in A-ZIP/F-1 mice. But is leptin the only factor involved? In aP2-SREBP-1c transgenic mice, treatment with physiological concentrations of leptin normalized glucose and insulin levels (41). By contrast, in A-ZIP/F-1 mice, leptin treatment at higher doses led to more modest reductions in these parameters (Figure 2d) (42). One possible explanation for the difference in leptin efficacy in the two models is the severity of...
Figure 2
Reconstitution of adipose tissue in lipoatrophic mice establishes the importance of adipokines in metabolic homeostasis. (a) Transplantation of adipose tissue from wild-type mice into lipoatrophic A-ZIP/F-1 mice. (b) Transplantation of adipose tissue from ob/ob mice, which lack leptin, into lipoatrophic A-ZIP/F-1 mice. (c) Combination of leptin supplementation and transplantation of adipose tissue from ob/ob mice into lipoatrophic A-ZIP/F-1 mice. (d) Treatment of lipoatrophic A-ZIP/F-1 mice with leptin alone. (e) Treatment of ob/ob mice with leptin alone.

Additionally, subsequent studies revealed that differences in the treatment regimen (12 days for aP2-SREBP-1c mice and six days for A-ZIP/F-1 mice) may explain the difference. Although one week of therapy was not sufficient to reverse hyperglycemia in A-ZIP/F-1 mice, two weeks of treatment almost normalized the glucose level (40). However, compared to ob/ob mice treated with leptin in a similar regimen (Figure 2d,
and e), the effects of leptin treatment on A-ZIP/F-1 mice were more modest (42). One possible mechanism to account for the response of the A-ZIP/F-1 mice is the effect of lipid repartitioning in the complete absence of adipose tissue, which causes lipid accumulation in tissues such as liver and skeletal muscle and in β cells (43). Alternatively, there may be additional insulin-sensitizing factors produced by WAT that are absent in lipoatrophy but present in ob/ob mice. As outlined below, a number of possible candidates for such a factor have been identified in recent years.

**ADIPOKINES**

The discovery of leptin led to the recognition that adipose tissue is an endocrine organ. In fact, fat is a major source of secreted proteins. A random sequencing survey of complementary DNA (cDNA) expressed in adipose tissue revealed that transcripts for secretory proteins accounted for 19.6% of the total transcripts (44). The past decade witnessed the characterization of a range of adipocyte-secreted factors involved in diverse biological functions such as energy homeostasis, insulin sensitivity, lipid metabolism, inflammation, and immunity (Table 1). These factors are collectively referred to as adipokines (45, 46). Dysregulation of adipokine expression is now thought to be one of the key events in the pathogenesis of metabolic diseases. Selected adipokines are reviewed here.

**Adiponectin**

Adiponectin is a 30 kDa secreted protein produced exclusively by adipocytes. Adiponectin was identified initially in the mid-1990s by four independent groups using different approaches. Scherer et al. (47) and Hu et al. (48) cloned adiponectin, termed Acrp30 and AdipoQ, respectively, through differential displays of genes expressed during adipocyte differentiation. Maeda et al. (49) cloned adiponectin, termed apM1, as one of the most abundantly expressed genes in adipocytes.

Table 1 Examples of secretory factors from adipose tissue

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<th>Energy homeostasis, glucose, and lipid metabolism</th>
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<td>Leptin</td>
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<td>Adiponectin</td>
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<td>Resistin</td>
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<td>Retinol-binding protein 4</td>
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<td>Visfatin</td>
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<td>Angiopoietin-like protein 4 (ANGPTL4)</td>
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<td>Lipoprotein lipase (LPL)</td>
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<th>Complement-related factor</th>
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<td>Adipsin</td>
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<td>Complement factor B</td>
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<td>Acylation-stimulating protein (ASP)</td>
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<th>Fibrinolytic system</th>
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<td>Plasminogen activator inhibitor-1 (PAI-1)</td>
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<th>Inflammatory mediator</th>
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<td>Interleukin-6 (IL-6)</td>
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<td>Monocyte chemoattractant protein-1 (MCP-1)</td>
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<td>Macrophage migration inhibitory factor (MIF)</td>
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<td>Interleukin-1 receptor antagonist (IL-1Ra)</td>
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<th>Renin-angiotensin system</th>
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Nakano et al. (50) purified adiponectin from human plasma as a gelatin-binding protein, GBP28. On the basis of its structure, adiponectin belongs to the complement 1q family of proteins that consists of a carboxyl-terminal globular domain and an amino-terminal collagenous domain. Adiponectin forms multimeric structures ranging from trimers to higher-order multimer species up to several hundred kilodaltons (51–53). The formation of high molecular weight (HMW) multimers depends on the disulfide bond between Cys residues at the amino terminus (51, 53, 54). Investigators have reported that a truncated globular domain form of adiponectin is generated by proteolytic cleavage (53, 55, 56). The globular domain of adiponectin (gAd) appears more potent than the full-length protein in at least some actions (55, 57). Although the existence of gAd in vivo has not been determined conclusively,
Hypoadiponectinemia: present in both obesity and lipodystrophy; may cause insulin resistance and atherosclerosis.

TZD: thiazolidinedione

PPARγ: peroxisome proliferator-activated receptor gamma

The concentration of adiponectin in human serum is $\sim 10 \mu g/ml$ (58), and levels are higher in females than in males (51, 58, 59). Most importantly, there is a strong negative correlation between plasma adiponectin concentration in humans and BMI (58). Adiponectin levels are decreased in obese humans despite increased fat mass (48, 57). A high-fat diet induces hypoadiponectinemia (57), whereas weight reduction in obese patients leads to an increase in adiponectin levels (60). Interestingly, the thiazolidinedione (TZD) class of insulin sensitizers, which acts as agonists of peroxisome proliferator-activated receptor gamma (PPARγ), increases both adiponectin gene expression and plasma protein levels (57, 61, 62). Hypoadiponectinemia is also associated with cardiovascular disease (63), hypertension (64), and the metabolic syndrome (65). Hypoadiponectinemia is also present in lipodystrophic diabetes in both humans and mice (40, 57, 66, 67). Thus, hypoadiponectinemia correlates with insulin resistance in most circumstances. This distinctive feature of adiponectin contrasts with leptin, the level of which is actually elevated in obesity. Furthermore, prospective longitudinal studies using rhesus monkeys revealed that plasma adiponectin levels begin to drop before the onset of diabetes, suggesting that hypoadiponectinemia contributes to the pathogenesis of diabetes (68).

Human genetic studies also support a causal role for adiponectin in the pathogenesis of type 2 diabetes. Genome-wide scans in multiple ethnic groups have identified several chromosomal loci linked to type 2 diabetes (69). One locus, 3q27, contains the adiponectin gene. Screening of the adiponectin gene for single nucleotide polymorphisms (SNPs) revealed that subjects carrying the G/G genotype at SNP 276 in intron 2 have hypoadiponectinemia and increased insulin resistance, compared with those with the T/T genotype (70). To date, SNP 276, SNP 45, SNP 11,377, and SNP 11,391 have all been significantly associated with hypoadiponectinemia and type 2 diabetes in various ethnic groups (71–73). Eight rare missense mutations have also been identified in the adiponectin gene, and some of these have been associated with hypoadiponectinemia and type 2 diabetes (71, 74, 75). In vitro experiments, the R112C and I164T mutations, which are associated with hypoadiponectinemia, disrupted adiponectin trimer formation, resulting in impaired secretion from the cell. The G84R and G90S mutations, which are associated with diabetes and hypoadiponectinemia, impair HMW multimer formation (51). These observations suggest that impaired multimerization may be a principal cause of the diabetic phenotype in subjects carrying these mutations.

Direct evidence for the ability of adiponectin to ameliorate insulin resistance came from experiments in which recombinant adiponectin was administered to diabetic mouse models. Both KKAy and db/db mice develop obesity and diabetes in an age-dependent manner. Feeding them a high-fat diet further exacerbates obesity, diabetes, and hypoadiponectinemia in these models. Yamauchi et al. (57) demonstrated that adiponectin treatment of KKAy and db/db mice on a high-fat diet ameliorated insulin resistance and lowered serum triglycerides. Replenishment of a physiological dose of both adiponectin and leptin completely reversed insulin resistance in PPARγ heterozygous knockout mice made lipoatrophic by treatment with the PPARγ antagonist HX531 (57). These data strongly support the hypothesis that the hypoadiponectinemia in both obesity-induced diabetes and lipodystrophic diabetes contributes to the development of insulin resistance.

Adiponectin appears to exert its beneficial effects through actions in both skeletal muscle and liver. Yamauchi et al. (57) found that reduced triglyceride content and increased expression of genes involved in fatty acid β oxidation and in energy dissipation in skeletal muscle appeared to contribute to the effects...
of adiponectin. Berg et al. (76) demonstrated that adiponectin treatment acutely lowered plasma glucose levels by inhibiting hepatic glucose production. Suppression of glucose production by adiponectin also occurred in cultured hepatocytes in vitro (76). Fruebis et al. (55) reported that the gAd increased fatty acid oxidation in muscle and lowered plasma FFA and glucose levels. Similar to leptin, adiponectin increased phosphorylation of AMPK. This pathway stimulates glucose uptake and fatty acid oxidation in muscle, and reduces the expression of enzymes involved in gluconeogenesis such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase in liver (77, 78). Adenoviral expression of a dominant-negative mutant of AMPK in liver significantly blunted the ability of adiponectin to reduce PEPCK expression and plasma glucose, suggesting that adiponectin exerts such effects through activation of AMPK (77).

Adiponectin transgenic and knockout mice have proven to be useful tools in investigating the effect of chronic overexpression or deficiency of adiponectin on insulin sensitivity. Globular adiponectin transgenic mice crossed with ob/ob mice showed improved insulin resistance with no change in body weight (79). Amelioration of insulin resistance was associated with increased expression of fatty acid oxidation genes, such as acyl-CoA oxidase, and of genes involved in energy dissipation, such as uncoupling proteins 2 and 3 in skeletal muscle of gAd Tg ob/ob mice (79). Combs et al. (80) generated transgenic mice for adiponectin lacking the collagenous domain. Elevated lipid clearance, elevated lipoprotein lipase activity, and suppressed insulin-mediated endogenous glucose production were observed in these transgenic mice (80). Adiponectin knockout mice showed impaired insulin signaling, elevated TNF-α expression, reduced fatty acid transport protein-1 mRNA expression in muscle, and delayed FFA clearance (81, 82). Nawrocki et al. (83) also generated adiponectin knockout mice that showed severe hepatic, but not peripheral, insulin resistance in euglycemic/insulin clamp studies.

As mentioned above, ligand activation of PPARγ induces adiponectin mRNA expression in adipocytes through direct activation of the promoter (57, 61, 62). Recent studies using adiponectin knockout mice have examined the contribution of adiponectin to the insulin-sensitizing effect of PPARγ agonists (83, 84). Kubota et al. (84) showed that the insulin-sensitizing effect of pioglitazone was abolished in ob/ob mice crossed with adiponectin knockout mice. This lack of response was associated with a failure to improve hepatic insulin resistance and to activate AMPK. Nawrocki et al. (83) also crossed adiponectin knockout mice with ob/ob mice and reported that the ability of rosiglitazone to improve glucose tolerance was diminished in ob/ob mice that also lacked adiponectin. Together, these studies suggest that the antidiabetic actions of PPARγ agonists are dependent, at least in part, on expression of adiponectin.

In recent years, cell surface receptors for adiponectin have been cloned by cDNA expression library screening (85). AdipoR1 and AdipoR2 encode proteins with seven transmembrane domains, although the topology of the receptors is opposite from G protein–coupled receptors: The N terminus is internal and the C terminus is external. These receptors are well conserved from yeast to humans, and the yeast homolog (YOL002c) regulates lipid metabolism (86). AdipoR1 is expressed abundantly in skeletal muscle, whereas AdipoR2 is expressed mainly in liver. AdipoR1 shows a higher affinity for globular adiponectin, and AdipoR2 has a higher affinity for full-length adiponectin. Overexpression and siRNA loss-of-function experiments suggest that AdipoR1 and AdipoR2 mediate adiponectin activation of AMPK and stimulation of fatty acid oxidation and glucose uptake. Expression of AdipoR1 and AdipoR2 is regulated negatively by insulin via activation of phosphatidylinositol 3-kinase and inactivation of Foxo1 (87).
adipose tissue of ob/ob mice, which are hyperinsulinemic, expression of both AdipoR1 and AdipoR2 is significantly reduced (87). Thus, reduction of adiponectin receptor expression may contribute to the diminished action of adiponectin in obesity.

In separate studies, Hug et al. (88) reported that T-cadherin is a receptor for adiponectin. They also employed a strategy of cDNA library expression cloning using cDNA from C2C12 muscle cells. Interestingly, T-cadherin binds specifically to hexametric or HMW species of adiponectin, but not to trimeric adiponectin. Furthermore, only adiponectin produced in eukaryote cells bound to T-cadherin, whereas bacterially expressed adiponectin did not. This implies that eukaryotic posttranslational modifications and multimer specificity are involved in receptor binding. To date, the role of T-cadherin in mediating the metabolic effects of adiponectin has not been tested.

Adiponectin also has direct anti-inflammatory and antiatherosclerotic effects. Adiponectin inhibits TNF-α-induced inhibitor of nuclear factor-kappa B (NF-κB)-α phosphorylation and subsequent NF-κB activation, as well as the expression of monocyte adhesion molecules in human aortic endothelial cells (89). Researchers have proposed that this effect inhibits the ability of monocytes to adhere to endothelial cells, a key step in the formation of the atherosclerotic lesion (90). Adiponectin also suppresses the expression of the class A macrophage scavenger receptor (SR-A) and lipid accumulation in human monocyte–derived macrophages (91), and inhibits smooth muscle cell proliferation (92). HMW multimers of adiponectin reportedly suppressed apoptosis and caspase-3 activity in human umbilical vein endothelial cells through the AMPK pathway (93). In apoE-deficient mice, adenooviral overexpression of adiponectin suppressed mRNA levels of vascular cell adhesion molecule-1 and SR-A and reduced atherosclerotic lesion formation (94). Similarly, globular adiponectin transgenic mice crossed with apoE-deficient mice showed reduced atherosclerosis without significant changes in serum cholesterol, triglyceride, or glucose levels, suggesting a direct antiatherosclerotic effect of adiponectin (79).

In summary, recent studies suggest that hypoadiponectinemia caused by genetic factors or by environmental factors such as a high-fat diet is a key contributor to the development of type 2 diabetes and the metabolic syndrome (Figure 3). Targeting the adiponectin pathway by increasing adiponectin concentrations or through alteration of postreceptor signaling represents a promising new approach to the treatment of metabolic disease.

**Resistin**

Resistin is a 10 kDa polypeptide secreted from adipocytes, which was identified in a screen for genes induced during adipocyte differentiation but downregulated in mature adipocytes exposed to TZD (95). In mice, resistin is expressed primarily in adipose tissue. Serum resistin levels are elevated in rodent models of obesity and diabetes such as ob/ob or db/db mice. Administration of recombinant resistin to mice worsens glucose tolerance, whereas injection of neutralizing antibody into obese mice improved insulin sensitivity, suggesting that resistin contributes to the pathogenesis of insulin resistance in obesity (95). Infusion of resistin rapidly induces hepatic insulin resistance, resulting in increased hepatic glucose production (96). Transgenic mice expressing resistin also showed higher fasting glucose levels due to increased hepatic glucose production in a glucose clamp study (97). Similarly, adenoviral expression of resistin in mice caused insulin resistance in liver, skeletal muscle, and adipose tissue (98). Resistin knockout mice exhibit low blood glucose levels after fasting owing to reduced hepatic glucose production (99). This effect was due partially to activation of AMPK and partially to the reduced expression of gluconeogenic genes. Consistent with these findings, transgenic
Hypoadiponectinemia induced by obesity contributes to the development of insulin resistance and atherosclerosis. Hypoadiponectinemia is associated with obesity and is caused by either genetic factors or environmental factors such as a high-fat diet. Adiponectin deficiency causes insulin resistance and dysregulation of lipid homeostasis and may contribute directly or indirectly to the development of atherosclerosis through impaired glucose tolerance and dyslipidemia.

Some controversy exists regarding the regulation of resistin by both obesity and TZDs, and there have been reports of differences between rodent and humans in resistin expression (102). Resistin was initially reported to increase in obese mouse models such as ob/ob and db/db mice and to decrease in response to TZD treatment in adipocytes in vitro (95). However, subsequent studies reported finding decreases or no change in resistin levels in obese mice (103, 104). In addition, the effect of TZD on resistin expression in vivo...
is controversial, with studies reporting both upregulation and downregulation (102). Although murine resistin is expressed almost exclusively in adipose tissue, human resistin is poorly expressed in adipose tissue (105), and nonadipocyte cells within the tissue express more resistin than adipocytes do (106). Because of these species differences, the clinical relevance of murine studies for human subjects needs to be addressed in future studies.

Plasminogen Activator Inhibitor
Plasminogen activator inhibitor-1 (PAI-1) is a member of the serine protease inhibitor family that inactivates urokinase-type and tissue-type plasminogen activator and thereby inhibits fibrinolysis. PAI-1 is expressed in adipose tissue as well as in endothelial cells, liver, and platelets. Elevated PAI-1 levels increase the risk of atherothrombotic events and may promote the progression of vascular disease (107). Elevated expression of PAI-1 is observed in individuals who exhibit myocardial infarction (108, 109), deep vein thrombosis (110), type 2 diabetes (111), and obesity (112). Interestingly, high PAI-1 levels are strongly associated with visceral fat mass (113). Expression of PAI-1 is induced by interleukin-1 (IL-1), TNF-α, transforming growth factor-beta (TGF-β), estrogen, thrombin, and insulin (107). Transgenic mice expressing PAI-1 developed spontaneous coronary arterial thrombosis (114). Knockout studies have shown conflicting results, with both protective and detrimental effects of PAI-1 deficiency on the development of atherosclerosis reported (115–117). PAI-1 knockout mice also exhibited reduced adiposity and improved insulin resistance (118, 119), suggesting a role for PAI-1 in obesity and insulin resistance.

Retinol-Binding Protein 4
Yang et al. (120) recently identified retinol-binding protein 4 (RBP4) as an adipokine. Their previous work established that although adipose-Glut4-/- mice exhibited insulin resistance in liver and muscle (121), adipose-Glut4-trangenic mice showed systemically enhanced glucose tolerance and insulin sensitivity (122). Because Kahn and colleagues did not observe any changes in previously identified adipocyte-secreted molecules in their model, they hypothesized the existence of an additional factor that regulates insulin sensitivity in liver and muscle (121). Through microarray analysis of adipose tissue from adipose-Glut4-/- and adipose-Glut4-trangenic mice, RBP4 mRNA was found to be elevated in adipose-Glut4-/- and reduced in adipose-Glut4-trangenic mice (120). Although RBP4 is a retinol-binding protein highly expressed in liver, hepatic RBP4 expression was not altered in these mice. The authors also showed that serum RBP4 levels were elevated in mouse models of obesity and diabetes, as well as in diabetic human subjects, suggesting that high RBP4 levels were universally associated with insulin resistance. To establish a direct link between RBP4 and insulin resistance, they studied insulin signaling in RBP4 transgenic mice, mice treated with recombinant RBP4, and RBP4-/- mice. As expected, elevation of RBP4 caused systemic insulin resistance, whereas reduced RBP4 levels improved insulin resistance. The deleterious effects of RBP4 on insulin action were explained by impaired insulin signaling in muscle and increased expression of PEPCK and glucose production in liver. Together, these data identify RBP4 as a new adipokine that may contribute to the pathogenesis of insulin resistance. Although the mechanism by which RBP4 causes insulin resistance in muscle and increases hepatic glucose production is not yet known (including whether this effect is retinol dependent or independent), lowering RBP4 levels could be a new strategy for the treatment of insulin resistance in obesity.

Visfatin
Visfatin (also known as pre-B cell colony-enhancing factor) is a newly identified 52 kDa
adipokine. This protein is more highly expressed in visceral fat than in subcutaneous fat, although it is also expressed in other tissues such as bone marrow, liver, and muscle (123). Despite its lack of a signal sequence, visfatin is present in the circulation. Plasma concentrations of visfatin reportedly correlate strongly with the amount of visceral fat. Unexpectedly, visfatin was found to bind to the insulin receptor with high affinity (3 nM). This affinity is comparable to insulin itself, but visfatin does not compete with insulin, which suggests that the two proteins bind to different sites. Consistent with binding to the insulin receptor, visfatin induced the phosphorylation of insulin receptor, insulin receptor substrate (IRS)-1, and IRS-2; binding of phosphatidylinositol 3-kinase to IRS-1 and IRS-2; and phosphorylation of Akt and mitogen-activated protein kinase (MAPK) in adipocytes, myocytes, and hepatocytes. An insulin-like effect of visfatin was also reported in vivo. Administration of visfatin to diabetic mice caused a rapid reduction of plasma glucose levels without changing insulin levels. Furthermore, adenoviral expression of visfatin improved insulin sensitivity in KKAy mice. In these studies, the plasma concentration of visfatin was 10% that of fasting insulin levels and only 3% that of fed insulin levels. Unlike insulin, plasma visfatin levels did not change upon fasting or feeding in mice. Although the physiological role of visfatin in the regulation of glucose homeostasis remains to be established, the mechanism whereby this protein exerts its insulin-mimetic action is of obvious interest from a drug development standpoint.

ADIPOSE TISSUE, INFLAMMATION, AND INSULIN RESISTANCE

In recent years, an increasing number of studies have focused on the relationship between chronic inflammation and obesity-induced diabetes. Researchers suspected a causal role for inflammation in insulin resistance because many inflammatory factors directly affect insulin signaling (124). Inflammatory factors secreted from adipocytes are thought to work mainly in an autocrine or paracrine fashion, but can also be categorized as adipokines. Adipocyte-derived TNF-α was the first such inflammatory factor to be identified (4). TNF-α expression is elevated in adipose tissue in obesity and may contribute to the development of insulin resistance. c-jun N-terminal kinase (JNK), inhibitor of NF-κB kinase beta (IKKβ), protein kinase C theta (PKCθ), IL-6, and SOCS proteins are additional examples of mediators proposed to link inflammation with insulin signaling. Adipose tissue appears to play an essential role in inflammatory-metabolic cross talk because adipocytes secrete a number of inflammatory factors such as TNF-α, IL-6, monocyte chemoattractant protein-1 (MCP-1), and IL-1β. However, recent findings have also implied an important role for resident macrophages in the production of inflammatory mediators and in the development of systemic insulin resistance. Although it remains to be determined what the initial trigger of inflammation in obesity is and which cell types or organs emit and receive the inflammatory signals, further studies promise to expand our understanding of the pathology of obesity-induced insulin resistance.

TNF-α

TNF-α was initially identified as an endotoxin-induced serum factor that caused necrosis of tumors (125) and was later found to be identical to cachectin (126). Researchers also proposed that TNF-α may be a cytokine that links inflammation in adipocytes with obesity-induced insulin resistance. TNF-α is synthesized as a 26 kDa transmembrane protein, and the biologically active trimer is formed after the cleavage of transmembrane precursor by the TNF-α converting enzyme. TNF-α is expressed in adipose tissue and, importantly, overexpressed in obese mice (4). Elevation of TNF-α has also been observed...
in adipose tissue in human subjects (127, 128). Chronic exposure of culture cells or animals to TNF-α was shown to cause insulin resistance (129, 130). By contrast, neutralization of TNF-α in obese fa/fa rats significantly increased the peripheral uptake of glucose in response to insulin (4). Consistent with this result, knockout mice for TNF-α or its receptors are protected from insulin resistance induced by a high-fat diet or ob/ob background and exhibit improved insulin signaling in WAT and skeletal muscle (131). There are several possible mechanisms to explain how TNF-α inhibits insulin signaling. One such mechanism is stimulation of serine-phosphorylation of IRS-1, which reduces tyrosine-phosphorylation of IRS-1 by insulin (132). Other proposed mechanisms include activation of a serine/threonine kinase IKKβ, leading to serine-phosphorylation of IRS-1 (133), increased lipolysis leading to higher FFA levels, and suppression of adiponectin expression by TNF-α (134).

IKKβ and JNK

IKKβ and JNK are serine/threonine kinases activated by inflammatory stimuli shown to inhibit insulin signaling through phosphorylation of serine residues on IRS-1. IKKβ activates the NF-kB pathway by phosphorylating and causing degradation of IκB. Interestingly, salicylates exerted their anti-inflammatory effects in part through the inhibition of IKKβ (135). Both salicylate and targeted disruption of IKKβ also reportedly reversed obesity-induced insulin resistance (133, 136, 137), which allowed investigators to identify IKKβ as a physiological contributor to insulin resistance and as a potential therapeutic target. Cai et al. (138) generated transgenic mice overexpressing constitutive active IKKβ in liver. These mice exhibited insulin resistance in liver as well as in skeletal muscle. TNF-α, IL-1β, and IL-6 expression by liver were also increased in the transgenic mice, a finding that may explain insulin resistance in other tissues. The study of tissue-specific IKKβ knockout mice experiments has further established that the IKKβ pathway in liver and macrophages is important in insulin resistance in obesity (139). By contrast, IKKβ action in muscle does not appear to be a major contributor to this pathology (140).

The JNK subgroup of the MAPK family (JNK-1,2,3) is activated by diverse stimuli, including cytokines, stress, and fatty acids. The biological effects of JNK are mediated mainly through their ability to phosphorylate activator protein-1 complexes, i.e., c-jun and c-fos (141). JNK activity is abnormally elevated in liver, muscle, and adipose tissue in obese animals (142). Moreover, JNK-1 knockout mice exhibit decreased adiposity, improved insulin sensitivity, and enhanced insulin receptor signaling capacity in both diet-induced obesity and on the ob/ob background (142). These observations suggest that the JNK pathway is also an important mediator of insulin resistance in obesity.

Interleukin-6

IL-6 is expressed abundantly in adipose tissue, and production from this tissue is thought to account for as much as one-third of circulating IL-6 (143). Similar to TNF-α, IL-6 levels in plasma are correlated positively with adiposity (144). Peripheral administration of IL-6 increased insulin resistance (145) and inhibited insulin signaling by inducing SOCS-3 (146). Surprisingly, IL-6 knockout mice developed mature-onset obesity and insulin resistance, and these phenotypes were reversed by IL-6 replacement (147). IL-6 is thought to exert its anti-obesity effects through the central nervous system because central administration of IL-6 decreased energy expenditure and decreased adiposity. IL-6 transgenic mice exhibited growth retardation with reduced body weight and adiposity (148). Therefore, the effects of IL-6 on adiposity and insulin resistance appear to be different in the periphery and in the central nervous system.
THE ROLE OF MACROPHAGES IN INFLAMMATION AND INSULIN RESISTANCE

Adipose tissue is composed not only of adipocytes, but also contains other cell types such as preadipocytes, macrophages, and endothelial cells. Collectively, these nonadipose cells comprise the stromal-vascular fraction of adipose tissue. Recent attention has focused on macrophages as potential contributors to the increased inflammation in adipose tissue in obesity. Recently, two papers documented the infiltration and accumulation of macrophages into adipose tissue of obese mice and of human subjects (149–151). Furthermore, these macrophages were a source of inflammatory factors such as TNF-α, inducible nitric oxide synthase, IL-6, and MCP-1 (149–151). Both studies emerged from a microarray analysis comparing transcripts in adipose tissue from obese mice with that from control mice. Interestingly, many transcripts elevated in obese adipose tissue were genes characteristic of macrophages. The authors therefore looked for the presence of macrophages in adipose tissue by immunohistochemistry and found marked accumulation of macrophages in obese adipose tissue, but not in liver or skeletal muscle (Figure 4). Weisberg et al.

Figure 4

Recruitment and accumulation of macrophages in adipose tissue in obesity. Macrophages infiltrate into adipose tissue from the circulation. Increased MCP-1, CCL2, or other chemotactic factor expression in obese adipose tissue may trigger the recruitment of macrophages. MCP-1 acts by binding to its receptor CCR2 on monocytes. Macrophages are a source of inflammatory factors such as TNF-α, IKKβ, iNOS, IL-6, and MCP-1, which may be elevated in obesity. Apoptosis of adipocytes in obese adipose tissue may also play a role in the recruitment of macrophages.
(150) further investigated the origin of adipose tissue macrophages by transplantation of bone marrow from wild-type mice expressing the CD45.1 leukocyte marker into wild-type mice expressing CD45.2. After six weeks on a high-fat diet, 85% of the F4/80+ cells in the adipose tissue of recipient mice were of donor origin, suggesting that adipose tissue macrophages are derived from bone marrow cells that infiltrate into adipose tissue from the circulation.

Although these results are provocative, several questions remain. For example, what triggers the recruitment and accumulation of macrophages in adipose tissue in obesity? Do macrophages in adipose tissue actually cause insulin resistance? One candidate molecule that may trigger macrophage recruitment is MCP-1, also known as chemokine (C-C motif) ligand 2 (CCL2). MCP-1 expression is increased significantly in WAT in obesity (149, 152). MCP-1 is expressed abundantly in the stromal-vascular fraction and, to a lesser extent, in adipocytes (149). The receptor for MCP-1 is chemokine (C-C motif) receptor 2 (CCR2), which is also a receptor for CCL8 (MCP-2) and CCL7 (MCP-3). CCR2 is expressed in both macrophages and adipocytes. Weisberg et al. (153) recently investigated the role of CCR2 in macrophage accumulation in obese adipose tissue and its contribution to metabolism (154). CCR2 knockout mice on a high-fat diet showed reduced food intake and attenuated obesity. In obese mice matched for adiposity, CCR2 deficiency reduced macrophage accumulation and inflammatory gene expression in adipose tissue. The mice also showed increased adiponectin, ameliorated hepatic steatosis, and improved insulin sensitivity. Weisberg et al. (153) further demonstrated that pharmacological treatment of high-fat-diet-induced obese mice with a CCR2 antagonist reduced macrophage accumulation in adipose tissue and improved insulin sensitivity (154). These data suggest that CCR2 signaling is involved in the recruitment of macrophages and in inflammatory changes in obese adipose tissue, as well as in the development of systemic insulin resistance.

Recent work using conditional knockout mice for IKKβ also implied a causal role for macrophages in the development of obesity-linked insulin resistance. Arkan et al. (139) demonstrated that myeloid-specific IKKβ knockout mice on a high-fat diet improved their global insulin sensitivity. This result is consistent with a significant contribution of macrophages to systemic insulin sensitivity in obesity. However, as is the case with the CCL2/CCR2 studies mentioned above, it is not yet clear which tissue macrophages are responsible for these effects. Are only adipose tissue macrophages involved, or do Kupffer cells and skeletal muscle macrophages also contribute? How the deficiency of IKKβ in macrophages results in improved insulin sensitivity also remains to be determined. Recently, Cinti et al. (155) carried out a detailed histological examination of macrophage accumulation in adipose tissue in obese mice using light and electron microscopy, and immunohistochemistry. More than 90% of macrophage accumulation was actually associated with apoptotic adipocytes. Furthermore, the macrophages fused to form syncytia that sequester the residual free lipid droplet and, ultimately, formed multinucleate giant cells. The rate of adipocyte death was 30-fold higher in obese mice in comparison with controls. These data suggest that adipocyte death plays a significant role in recruiting macrophages to adipose tissue in obesity.

Together, recent studies on adipose tissue macrophages have introduced a new paradigm into obesity research. Further investigation will continue to define the biological role of adipose tissue macrophages and their contribution to obesity-induced insulin resistance.

**CONCLUSIONS**

The discovery of leptin in 1994 also brought the recognition of fat as a dynamic organ that communicates with other tissues by secreting endocrine factors termed adipokines.
Characterization of the biological and pathological functions of various adipokines over the past decade has expanded our understanding of the pathogenesis of obesity and related disorders. In addition, human genetics has taught us that obesity is a complex disorder and that multiple genetic factors contribute to its development. Although the challenge to fully understand the etiology of obesity and insulin resistance continues, the research outlined in this review illustrates how a better understanding of adipokines may facilitate the development of rational approaches to the treatment of metabolic disease.

**SUMMARY POINTS**

1. Leptin is a hormone secreted from adipocytes that controls feeding behavior and energy expenditure to maintain energy homeostasis and adiposity.
2. Normal adipose tissue is required for systemic glucose and lipid homeostasis.
3. Adiponectin is an antidiabetic adipokine. Hypoadiponectinemia, present in obesity, appears to contribute to the pathogenesis of insulin resistance in obesity.
4. A number of adipokines involved in glucose and lipid homeostasis have been identified and the search for additional factors continues.
5. Inflammation in adipose tissue may play a causal role in obesity-linked insulin resistance.
6. Macrophages in adipose tissue may participate in adipose tissue inflammation in obese subjects and may also be involved in the development of insulin resistance.

**FUTURE ISSUES**

1. What is the physiological contribution of each adipokine to the function of normal adipose and to the maintenance of systemic glucose and lipid homeostasis? What is their contribution to the dysregulation of glucose and lipid homeostasis in obesity?
2. Which organs or cell types are involved in the inflammatory process leading to insulin resistance in obese subjects?
3. Can we reverse the primary disorder, i.e., obesity, or can we uncouple obesity from disorders such as insulin resistance, type 2 diabetes, dyslipidemia, and atherosclerosis?

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**LITERATURE CITED**

3. Described positional cloning of leptin as a gene responsible for obese phenotype of ob/ob mice.

4. First report that an inflammatory mediator was upregulated in adipose tissue of obese mice.


35–38. Described the ablation of WAT in mice, which resulted in severe insulin resistance, dyslipidemia, hyperphagia, and liver steatosis.

39. Examined the effect of adipose tissue transplantation into lipoatrophic mice and demonstrated the importance of adipose tissue in glucose/lipid metabolism.

57. Demonstrated that replenishment of adiponectin ameliorates insulin resistance in both obesity and lipoatrophy.

67. Documented that adiponectin enhances hepatic insulin action.
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120. Described the identification of RBP4 as an adipokine.


139. Highlighted the role of macrophage inflammatory mediators in systemic glucose metabolism.

149 and 150. Reported increased macrophage accumulation in adipose tissue in obese animals.


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