Adipocyte Signaling and Lipid Homeostasis
Sequelae of Insulin-Resistant Adipose Tissue

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Abstract—For many years adipose tissue was viewed as the site where excess energy was stored, in the form of triglycerides (TGs), and where that energy, when needed elsewhere in the body, was released in the form of fatty acids (FAs). Recently, it has become clear that when the regulation of the storage and release of energy by adipose tissue is impaired, plasma FA levels become elevated and excessive metabolism of FA, including storage of TGs, occurs in nonadipose tissues. Most recently, work by several laboratories has made it clear that in addition to FA, adipose tissue communicates with the rest of the body by synthesizing and releasing a host of secreted molecules, collectively designated as adipokines. Several recent reviews have described how these molecules, along with FA, significantly affect total body glucose metabolism and insulin sensitivity. Relatively little attention has been paid to the effects of adipokines on lipid metabolism. In this review, we will describe, in detail, the effects of molecules secreted by adipose tissue, including FA, leptin, adiponectin, resistin, TNF-α, IL-6, and apolipoproteins, on lipid homeostasis in several nonadipose tissues, including liver, skeletal muscle, and pancreatic β cells. (Circ Res. 2005;96:1042-1052.)

Key Words: lipids ■ fatty acids ■ adipose tissue ■ insulin resistance ■ cytokines

For many years adipose tissue was viewed as playing a passive role in total body lipid and energy homeostasis. Adipose tissue was the site where excess energy was stored, in the form of triglycerides (TGs), and where that energy, when needed elsewhere in the body, was released in the form of fatty acid (FA). Work by numerous laboratories during the second half of the 20th century made it clear that under normal conditions, the storage and release of TG and FA, respectively, are both coordinated and tightly regulated so that lipid fuels are stored during the immediate postprandial periods and released during periods of fasting. The biochemistry of key molecules critical to coordinating storage and release of energy, such as lipoprotein lipase (LPL) and hormone sensitive lipase (HSL), was characterized in detail during that period. More recently, it has become clear that when the regulation of storage and release of energy by adipose tissue is impaired, particularly when release of FA becomes dissociated from energy requirements in other organs and tissues, plasma FA levels become elevated and excessive metabolism of FA, including storage of TG, occurs in nonadipose tissues.

Our initial understanding of how fat tissue communicates with other tissues and organs to integrate total body lipid homeostasis was limited to the effects of circulating FA on hepatic lipid and glucose metabolism. During the last 15 years, however, it has become clear that adipose tissue—
derived FA can affect lipid metabolism in several tissues, including muscle and pancreatic β cells. The expanded view of ectopic metabolism or accumulation of FA or TG is that it causes dysfunction, or lipotoxicity, in these organs and tissues. Most recently, work by several laboratories has further expanded our view of the way adipose tissue communicates with, and affects, total body energy homeostasis. We now know that in addition to FA, adipose tissue communicates with the rest of the body by synthesizing and releasing a host of secreted molecules, collectively designated as adipokines. These molecules, along with FA, have significant effects on total body glucose and lipid metabolism, and insulin sensitivity. Several recent articles have described the effects of the adipokines on glucose metabolism, insulin sensitivity, and inflammation;1-5 little has been written about their effects on lipid metabolism. In this review, we will combine a detailed description of the effects of FA on total body lipid metabolism with an update on the rapidly evolving understanding of the role of adipokines in lipid homeostasis.

**Normal Adipose Tissue Regulates Partitioning and Utilization of Lipids by the Body**

After a meal, dietary TG is digested by pancreatic lipase in the small intestines and absorbed by intestinal enterocytes as FA and sn-2-monocacylglycerols.6 These lipid molecules reassemble as TG and are then exported into the circulation as chylomicrons. In the fed state, adipose tissue is the major site for uptake of dietary TG FA, after LPL-mediated hydrolysis of chylomicron TG. LPL is produced mainly in adipose tissue and muscle, secreted by those tissues, and transported to the surfaces of capillary endothelial cells in these tissues. LPL binds to proteoglycans at the luminal side of the capillary blood vessels, where it interacts with TG-rich lipoprotein particles7,8 and, in the presence apolipoprotein (apo) CII,9 hydrolyzes TG. ApoCII inhibits LPL-mediated TG hydrolysis, and the ratio of apoCII to apoCIII may be critical in regulating the delivery of dietary TG to adipose tissue.10 Insulin is a major regulator of LPL synthesis and activity in adipose tissue. In the fed state, whereas LPL is downregulated in muscle, it is upregulated in adipose tissue.7,8 The opposite regulation characterizes the fasted state.

The FAs that are released in high concentrations by lipolysis of TG-enriched lipoproteins have to be efficiently trapped in the local adipose tissue to prevent high FA flux through the plasma to tissues that do not, at that moment, require FA as an energy source.8 Both inhibition of adipocyte HSL-mediated TG lipolysis during the hyperinsulinemic postprandial period and efficient acylation of newly arrived FA inside adipocytes help create a gradient for FA uptake in adjacent adipose tissue. Physical channeling of FA through the endothelial cells to the adipocyte also occurs during lipolysis.11 Then, FA can be rapidly taken up and trapped, most likely, by a combination of passive diffusion12 and the action of fatty acid transport proteins.13 Acylation-stimulating protein (ASP) is an adipocyte-secreted protein that may also play an important role in the efficient adipocyte trapping of FA released after lipolysis of lipoprotein TG.14 ASP is generated by the interaction of complement C3 with factors B and D (the latter is also called adipins). The result is cleavage of the N-terminal of C3, producing C3a, which is then cleaved by plasma carboxypeptidase to produce C3desarg or ASP. Studies in adipocytes indicate that ASP increases glucose uptake (a substrate for glycogen formation) and the activity of diacylglycerol acyltransferase (DGAT), thereby facilitating use of FA for TG synthesis. Additionally, ASP has been shown to increase reesterification of FA released from TG by HSL, as well as reduce HSL-mediated lipolysis. In humans, fasting ASP levels correlate with postprandial TG clearance.15 ASP-deficient mice have delayed postprandial TG clearance (males on a mixed C57BL/6x129Sv background)16 and reduced body weight and fat mass (females on the same mixed background),17 even on the ob/ob background.18 Additionally, intraperitoneal injection of ASP facilitated postprandial TG clearance in several mouse models of obesity and insulin resistance.16 However, other investigators, using the same mouse (they had originally generated a complement C3 knockout mouse that would, by definition, also be deficient in ASP), did not find alterations in energy metabolism or lipid metabolism.19 This controversy remains unresolved at present, although the weight of evidence favors some role for ASP in postprandial TG and FA partitioning into fat.

As a result of these various processes, 90% to 100% of LPL-released FA is trapped by adipocytes after feeding.20 The efficiency of TGFA uptake by adipose tissue in normal humans is evidenced by the fact that postprandial plasma FFA levels are actually lower than fasting levels.20 This reduction in plasma FA results both from inhibition of adipose tissue HSL-mediated TG lipolysis and the facilitated uptake of chylomicron or very low density lipoprotein (VLDL)-generated FA.

By contrast, during the fasting state, insulin levels are low, HSL is activated, and catecholamine-stimulated adipose tissue lipolysis becomes unopposed. Additionally, adipose tissue LPL activity is downregulated and muscle LPL is upregulated. Thus, during fasting, FA is released from fat, plasma FA levels rise, and muscle uptake of FA increases. FA flux to liver also increases during fasting, contributing energy for hepatic gluconeogenesis and ketogenesis. During longer periods of fasting, FA becomes the primary fuel in organs where glucose is not an obligatory fuel, including skeletal muscle, heart, and the renal cortex. In this manner, glucose is spared for its required utilization by the central nervous system.

**Insulin-Resistant Adipose Tissue and Dysregulation of Lipid Partitioning and Utilization**

Insulin resistance is traditionally assessed by insulin’s ability to promote normal glucose metabolism. The physiological role of insulin is, however, much broader, and includes the metabolism of all 3 macronutrients (carbohydrates, lipids, and proteins) as well as cellular growth. Insulin’s action on lipid metabolism is analogous to its role in glucose metabolism, ie, promoting anabolism and inhibiting catabolism. Specifically, insulin upregulates LPL and stimulates gene expression of intracellular lipogenic enzymes, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS).21 In
addition, insulin inhibits adipocyte HSL through inhibition of its phosphorylation.22

In the insulin-resistant state, the responses of both LPL and HSL to insulin are blunted. Thus, with insulin resistance, inefficient trapping of dietary energy occurs both because of decreased LPL-mediated lipolysis of chylomicron-TG and ineffective inhibition of HSL-mediated lipolysis in adipose tissue.23 Postprandial lipemia and elevated plasma FA levels are well-recognized abnormalities in obesity and insulin resistance.24 Reduced adipose tissue uptake and storage of TG FA during the postprandial period results in greater partitioning of dietary lipids to nonadipose tissues, including muscle and liver.25,26 Thus, the metabolic consequence of inadequate insulin action at the adipocyte during the fed state is a condition similar to the normal fasting state, when insulin levels are appropriately low. However, in the fed state, this maladaptive response creates a situation in which postprandial FA are directed to various nonadipose tissues and organs at a time when lipid energy is not needed. The specific consequences of increased FA flux to nonadipose tissues are discussed in more detail in the following sections.

The ultimate example of the loss of physiological lipid partitioning is total lipodystrophy, a disorder in which there is no adipose tissue.27 Several genes have been identified that can cause total lipodystrophy, including 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2), which converts 1-acyl glycerol phosphate to 1,2 diacylglycerol phosphate (phosphaditic acid); without AGPAT2, TG synthesis and adipogenesis are disrupted.28,29 A second gene, called seipin, has no known function, and the way in which it causes lipodystrophy is unknown.29 There are partial lipodystrophies, some of which have been associated with mutations in the gene for lamins A and C (LMNA), and in the PPARγ gene.30 In total lipodystrophy, plasma FA levels are very high, and TG accumulates in muscle, liver, islet cells, and plasma, resulting in insulin resistance, hypertriglyceridemia, and defective insulin secretion with diabetes.31 It is noteworthy that patients with total lipodystrophy have no intradominal fat, including no visceral fat, and yet have severe insulin resistance. In partial lipodystrophy, ectopic fat deposition occurs in several organs, and there is also visceral fat, hypertriglyceridemia, and insulin resistance.

Rodent models of lipodystrophy32–34 have provided important insights regarding the effects of a failure to partition FA to adipose tissue. These models all have ectopic fat, particularly fatty livers, and both insulin resistance and increased secretion of VLDL TG.

Role of Increased Plasma FA Flux to the Liver and Increased VLDL Secretion

As noted, insulin resistance in adipose tissue will disrupt normal lipid partitioning in both the fed and fasting states, and nonadipose tissues will be faced with increased FA delivery. Based on blood flow, and a very high capacity to take up plasma FA, the liver will be a major recipient of those FA. It is likely that both “flip-flop” diffusion processes12 and one or more fatty acid “receptors” or “transport proteins”13 are involved in the high capacity of the liver to take up FA. In addition, intracellular proteins that bind or chemically modify FA, such as long-chain acyl-CoA synthetase (ACLS).35,36 are very likely to facilitate the net transport of FA from the plasma into hepatocytes.

Increased plasma FA flux to the liver as a cause of increased VLDL apoB and TG secretion has been observed both in vitro and in vivo.37–39 Studies of cultured liver cells have often, but not always, shown FA to stimulate apoB secretion.39 Some of the inconsistencies seem to derive from the fact that when studying primary rodent hepatocytes, the composition and caloric content of the prior diet may be important determinants of the response to FA; fast rats, or rats fed high carbohydrate diets, respond to increased FA with a rise in VLDL assembly and secretion. Several in vivo rodent models also support a close link between increased plasma FA flux to the liver and increased apoB secretion. In the sucrose-fed hamster,40 which is a model of insulin resistance and hypertriglyceridemia, increased plasma FA levels are correlated with increased assembly and secretion of apoB lipoproteins (LPs). Studies with mice having genetic alterations in the fatty acid transporter, CD36 (also called FA translocase or FAT), indicate that plasma FA flux to the liver is a critical stimulus for VLDL secretion. Thus, when CD36 was deleted in all tissues (CD expression is normally low in the liver), plasma FA and TG levels were increased despite an apparent increase in overall insulin sensitivity.41 By contrast, in mice overexpressing CD36 in muscle, lower levels of plasma FA and TG were observed despite concomitant overall insulin resistance. Importantly, mice lacking HSL had significantly reduced levels of plasma FA and TG, and also reduced rates of hepatic TG secretion.42 We recently demonstrated, in vivo, that intravenous delivery of albumin-bound FA could stimulate secretion of apoB from livers of normal mice.43

Increased apoB secretion was first demonstrated in insulin-resistant and diabetic subjects,44 in whom increased FA flux was linked to both VLDL TG and apoB secretion.45 Obese patients were also shown to incorporate plasma FA efficiently into VLDL TG.46 Increased VLDL apoB and TG secretion has been documented in a patient with lipodystrophy, severe insulin resistance, and high levels of plasma FA.47 Impaired insulin-mediated inhibition of FA release by adipose tissue has been related to increased VLDL secretion in subjects with familial combined hyperlipidemia.48 The most direct evidence was provided by studies in which intravenous infusions of the TG-phospholipid emulsion, intralipid, resulted in both acute elevations in plasma FA levels and increased secretion of both apoB and TG in healthy young men.38

FA delivery to the liver could also lead to increased secretion of VLDL by inhibiting insulin-stimulated apoB degradation.49 Increased availability of FA, either from endogenous TG stores or as dietary TG, not only causes insulin resistance in skeletal muscle, but also in the liver.50,51 This would affect insulin’s ability to inhibit VLDL secretion. Indeed, although hyperinsulinemia has been shown to reduce apoB secretion in normal humans,52,53 insulin is ineffective in obese52 or diabetic54 subjects.

Diminished LPL activity could reduce partitioning of TGFA to adipose tissue from chylomicron TG (in the fed state) and VLDL TG (in the fasted state). This would leave
TG-enriched remnant lipoproteins in the circulation, and the liver would be the major repository for these TGFA. Hepatic uptake of apoB-lipoprotein remnants occurs by a multioption process that involves LDL receptors (the primary mediator of uptake), LDL-receptor–related protein (LRP), hepatic lipase (HL), possibly LpL associated with remnants, proteoglycans, and scavenger receptor-B1 (SR-B1).55,56 It is clear from both in vitro57 and in vivo58 studies that uptake by the liver of TG-enriched LPs can stimulate subsequent secretion of hepatic apoB-LPs. It is not clear, however, if LpL/HL-liberated lipoprotein FA or lysosomal-liberated lipoprotein FA have similar effects on apoB-LP assembly and secretion. Indeed, our recent studies53 demonstrate qualitative differences between FA delivered by albumin and FA derived from the internalization of remnants on the assembly and secretion of VLDL.

Despite the ability of the liver to assemble and secrete VLDL TG, and thereby “unload” excess FA, fatty liver is a common consequence of inappropriate flux of plasma FA and lipoprotein remnant TGFA to that organ.59,60 Why the liver is unable to efficiently unload the excess FA is unclear, but several possibilities can be considered. First, delivery of albumin-bound FA may have different quantitative and qualitative effects compared with delivery of remnant lipoprotein TGFA, with the latter less potent at stimulating secretion of VLDL.43 Second, the state of lipogenesis in the liver may be important; excessive de novo fatty acid synthesis, driven by hyperinsulinemia61 or hyperglycemia,62 in the face of increased exogenous FA influx, may overwhelm the secretory capacity of the liver. Third, insulin-mediated degradation of apoB will clearly be a major determinant of the liver’s ability to assemble and secrete VLDL. Thus both the degree of hyperinsulinemia, and the degree of hepatic insulin resistance, will be critical determinants of VLDL secretion and, therefore, TG accumulation in the liver. For example, if insulin-mediated degradation of apoB is preserved in the setting of increased FA flux and hepatic lipogenesis, fatty liver would be a likely outcome.

**Role of Increased FA Flux to Muscle and Insulin Resistance**

Beginning with Randle’s publication of the “glucose-fatty acid cycle” in skeletal muscle in 1963,63 it has become increasingly clear that disordered FA metabolism is critical in the pathophysiology of muscle insulin resistance.50 Although this is not a review of insulin action or glucose metabolism, it is important to review the effects of increased FA flux to muscle on FA metabolism, and therefore, insulin/glucose metabolism in that tissue. Recent studies have provided strong evidence that the ability of FA to interfere with insulin signaling and glucose transport into muscle correlates with the generation of FA metabolites, such as fatty acyl CoA molecules or diacylglycerol. Increased generation of these metabolites as a response to inappropriate increases in FA uptake can stimulate serine-phosphorylation of IRS-1, and possibly IRS-2, via the actions of certain protein kinase C isoforms.64,65 Targeted disruption of PKC-θ in mice protected them from FA-induced insulin resistance.66 Recent evidence also suggests that PKC-mediated serine phosphorylation of inhibitor of κβ kinase (IKKβ), leading to its degradation and the unregulated translocation of nuclear factor-κβ (NF-κβ) into the nucleus, may also be important to FA-induced insulin resistance.67 We realize that there are many other potential reasons for muscle to be insulin resistant, but it is clear that simply providing excess FA can induce insulin resistance.50

Why are potentially harmful intermediates of FA metabolism accumulating in muscle of insulin-resistant individuals? Is it just increased FA flux into the muscle, especially when those FA are not needed for energy, or is there a defect in FA utilization? Of interest in this regard are the findings that insulin-resistant and diabetic individuals have decreased fractional and, in some cases, absolute uptake of FA by muscle.68,69 However, the combination of normal or only slightly reduced plasma FA levels during the immediate postprandial period in insulin-resistant individuals, a time when plasma FA levels should be dramatically suppressed,20 together with modestly reduced extraction of FA by muscle, would still create a situation where FA supply is out of proportion to the need for FA to supply energy to that tissue. Additionally, there is considerable evidence that mitochondrial oxidation of FA is defective in insulin-resistant individuals,70,71 and that this can be reversed by weight loss and exercise.72 Excess FA trapping in muscle, together with reduced FA oxidation, would result in increased TG synthesis. Indeed, increased intramyocellular TG is highly correlated with insulin resistance.73 Although some investigators have suggested that intramyocellular TG is the lipotoxice substance in muscle, it is more likely only a marker of reduced oxidation of FA relative to FA availability.74

**Role of Increased FA Flux to β Cells and Insulin Secretory Defects**

Plasma FA can be taken up by pancreatic β cells where it can stimulate insulin secretion.73 The latter appears to occur as FAs are used to generate long-chain fatty acyl CoA, which can contribute to the synthesis of signaling molecules such as DAG and phosphatidic acid (PA) or directly stimulate PKC isoforms involved in secretion.76 The ability of FA to stimulate insulin secretion requires the presence of stimulatory glucose; the potential reasons for this interaction include the generation from glucose of (1) α glycerol phosphate, which is needed as the backbone molecule for DAG and PA, and (2) malonyl CoA, which inhibits FA oxidation and stimulates lipid formation. Of particular relevance to insulin resistance is the finding that circulating FA are critical for the response of the pancreas to glucose in the fasting state.77 Thus, the high basal insulin response characteristic of insulin resistance might derive, at least in part, from increased levels of FA before the administration of glucose. High-plasma FA uptake by β cells will also lead to increased storage of FA as TG. The release of TGFA from cytosolic TG droplets by HSL and associated lipases is another source of FA that could stimulate insulin release. Indeed, HSL-deficient mice have reduced insulin response to glucose.78

There is also clear evidence, however, that continued high-cytosolic FA concentrations lead ultimately to decreased insulin secretion.76 Several mechanisms have been reported, including inhibition of insulin synthesis,79 inhibition of the β
Role of Adipokines in Lipid Metabolism

Leptin

Leptin is a 16-kDa protein synthesized mainly in adipose tissue. The leptin gene was identified as the causative mutation in ob/ob mice. Numerous reviews have been published describing its regulation and its role in eating and energy homeostasis. However, studies from a number of laboratories, using in vitro and in vivo rodent models, indicate a direct role for leptin in lipid metabolism. The effect on lipid metabolism may be mediated both through central and peripheral actions of leptin. For example, central administration of leptin increased resting metabolic rates, resulting in reduced TG content in both adipose and nonadipose tissues, as well as reduced plasma-free FA and TG levels in pair-fed Sprague-Dawley rats. Leptin may also have autocrine or paracrine effects on adipocyte fat metabolism: incubation of mouse adipocytes with leptin stimulates lipolysis of intracellular TG, and this effect was not seen in db/db mice lacking leptin receptors. Overexpression of leptin in adipocytes also reduced gene expression of acetyl CoA carboxylase (ACC). In other tissues, leptin also appears to inhibit lipogenesis and stimulate FA oxidation. The mechanism underlying these actions of leptin may be via binding to its receptor with succeeding activation of the Jak/Stat pathway or by direct stimulation of AMP-activated protein kinase (AMPK), which will phosphorylate and thereby inhibit ACC activity and lipogenesis. Leptin also can inhibit the expression of sterol response element binding protein-1c (SREBP-1c) in liver, pancreatic islets, and adipose tissue, thereby inhibiting lipogenesis in those tissues.

The importance of leptin in regulating lipid metabolism in several tissues is best exemplified by the lipotoxicity that is associated with the complete absence of leptin or leptin action. For example, as described earlier in this review, individuals without adipose tissue, who also lack leptin, have ectopic fat deposition and lipotoxicity. Furthermore, rodent models of either naturally occurring leptin deficiency or unresponsiveness, or transgenically created lipodystrophy, have similar lipotoxic phenotypes. Importantly, administration of leptin to people with lipodystrophy or to rodent models of leptin deficiency, reverses many of the lipotoxic sequelae. This is due partly to direct effects of leptin on metabolism, although some of the effects of administered leptin may be through suppression of hyperphagia. The importance of leptin for FA metabolism in nondiaposite tissue of obese people who have, in general, increased levels of leptin, remains to be determined.

Adiponectin

Adiponectin (ACRP30, adipQ, apM1, or GBP28) was identified as the product of a gene induced during adipocyte differentiation. It is a 30-kDa protein that is synthesized and secreted from adipocytes. Adiponectin has an N-terminal collagenase domain followed by a C-terminal globular domain that can undergo homotrimerization. In plasma, adiponectin circulates as either a trimer, a hexamer (called the low molecular weight or LMW form), or as multimeric forms of 12 to 18 subunits (called the high molecular weight or HMW form). Of note, recent studies suggest that the HMW form may undergo cleavage to smaller units, including the trimer, and that the latter may be the active form or be further cleaved to an even smaller fragment that transduces adiponectin’s signal to cells, particularly to hepatocytes. On the other hand, the globular domain itself (which has not yet been observed in an in vivo setting) can increase fatty acid oxidation in mouse muscle, probably via activation of AMPK. Indeed, transgenic overexpression of the globular domain of adiponectin in the liver of ob/ob mice improved total body insulin sensitivity and increased fatty acid oxidation in skeletal muscle. To make matters more complex, studies have indicated that different forms of circulating adiponectin have effects in specific tissues. Three putative receptors for adiponectin have been cloned. One is mainly in liver, one in skeletal muscle, and one endothelial cells and smooth muscle. PPARγ agonists increase, whereas both tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 inhibit, adiponectin gene expression.

Adiponectin-deficient mice develop insulin resistance on a high-fat diet. Transgenic expression of adiponectin in adipose tissue resulted in increased plasma levels and inhibition of hepatic glucose production with improved glucose tolerance. Administration of recombinant adiponectin to lipodystrophic or obese mice results in reductions in plasma FA and TG levels, whereas transgenic mice with increased secretion of full-length adiponectin had improved clearance of an exogenous fat load.

Adiponectin levels in plasma, which are very high compared with other adipokines, are reduced in obese, insulin-resistant, diabetic, or dyslipidemic subjects. In humans, adiponectin levels are inversely correlated with plasma TG levels and positively correlated with HDL cholesterol concentration, although it is not clear if there are direct links between adiponectin and plasma lipid levels or these findings are related to adiponectin’s effects on insulin sensitivity. However, a recent study suggested a strong relationship between adiponectin levels and LPL activity in humans, and increased adipose tissue LPL activity was present in a transgenic mouse that secretes increased quantities of full-length adiponectin from adipose tissue. In humans, adiponectin levels are predictive of hepatic steatosis and are inversely related to hepatic fat content and hepatic insulin resistance before and after treatment with a PPARγ agonist.

Resistin

Resistin is a 12-kDa protein that is synthesized and secreted from adipose tissue. It was discovered in mice by searching
for genes that were suppressed by a PPARγ agonist.113 Resistin levels are elevated in both diet-induced obesity and genetic (leptin or leptin receptor deficient) mouse models of obesity/diabetes.113 Recombinant resistin decreases insulin sensitivity in mice, and antibodies against resistin block this effect. Resistin infusions in rodents during euglycemic hyperinsulinemic clamp conditions, caused increased hepatic glucose production.114 Mice treated with Western-type high-fat diets had concomitant increases in resistin levels, and antisense oligodeoxynucleotides that normalized resistin levels also reversed hepatic insulin resistance.115 These results are consistent with the data obtained from resistin knockout mice, which despite having no changes in body weight or fat mass, showed significantly lower fasting plasma glucose levels when fed with normal chow diet. When fed with Western-type high-fat diet, the resistin-deficient mice also showed significantly better glucose tolerance than the wild-type mice.116 Deletion of the resistin gene was associated with increased AMPK activity in hepatocytes, decreases in gluconeogenic enzymes, and decreased hepatic glucose production.116 On the other hand, transgenic overexpression of resistin was associated with increased hepatic glucose production and glucose intolerance.117 Of interest, like adiponectin,95 resistin appears to circulate in mice in both trimeric and hexameric forms, with the smaller form (or possibly the monomer) being the most biologically active molecule.118

Resistin has more recently been shown to have effects on lipid metabolism in mice. When adenovirus-mediated overexpression of resistin was achieved in normal chow-fed mice, plasma TG levels increased, and this was associated with increased secretion of TG from the liver.119 LDL cholesterol also increased whereas HDL cholesterol fell in the mice, and these changes were associated with reduced expression of the LDL receptor and apoAI genes, respectively.119 Wistar rats that had been made “hyper-resistinemic” by adenoviral administration also had increased plasma TG levels.120 No mechanistic studies of lipid metabolism were performed in the latter study.

The role of resistin in humans is less certain, however. Clinical studies in humans do not show a consistent link between resistin levels and either obesity or insulin resistance.4,121,122 There is also controversy regarding the importance of adipocytes as a source of resistin in human.123,124 Human resistin is only 64% homologous (53% identical with) with the murine counterpart, and both are members of the family of resistin like molecules, RELM (also called FIZZ), which are C-terminal cysteine-rich proteins.124 Given the diverse roles and tissue specificities of this protein family, and the low homology between the rodent and human forms, it is unclear at present whether human resistin plays a similar role as murine resistin, and if it does, how important human resistin is in the pathogenesis of the human insulin resistance.

Role of Adipocytokines in Lipid Metabolism

During acute injury or infection, a host of defense responses are elicited, including changes in the partitioning of lipid-derived energy that are associated with insulin resistance and increases in plasma TG and FFA levels. It is believed that these acute metabolic responses are initiated, at least in part, by proinflammatory cytokines. Further, dyslipidemia, insulin resistance, atherosclerosis, and obesity125 have been linked with chronic inflammation. Cytokines are produced not only by immune cells, but also by adipocytes (hence the term adipocytokine).125,126 In fact, adipose tissue is able to secrete a range of “acute-phase reactants,” which have been linked, both epidemiologically and biochemically, to the chronic disorder, insulin resistance. The demonstration of cytokine production in adipose tissue suggests that obesity is either seen by the whole organism as an inflammatory state, or that adipocytokines are playing a physiological role in attempting to maintain normal fuel homeostasis.

Tissue Necrosis Factor-α

TNF-α is a 26-kDa transmembrane protein, which is released into the circulation as a 17-kDa soluble protein after extracellular cleavage by a metalloproteinase.127 TNF-α has 2 main receptors, type 1 and 2, which are expressed on many cells, including adipocytes.128 Although circulating TNF-α levels are relatively low and have no clear correlation with obesity or insulin resistance, tissue expression levels of TNF-α correlate positively with both conditions. In mice, chronic exposure of cells or whole animals to TNF-α induces insulin resistance, and treatment with soluble forms of TNF-α receptors neutralize this effect. Furthermore, mice with targeted gene deletion of TNF-α or its receptors showed increased insulin sensitivity and improved plasma FFA levels.129

TNF-α has multiple effects on lipid metabolism, via both paracrine effects on adipocytes and effects in the liver. In adipose tissue, TNF-α promotes lipolysis,130 leading to elevation of plasma FA levels. Although there are differences between murine and human adipocytes regarding mechanistic details, TNF-α–induced lipolysis is, in general, mediated by p44/42 and JNK. These kinases can phosphorylate perilipin, thereby displacing it from lipid droplets and making TG accessible to HSL.131 Additionally, TNF-α causes reductions in the expression of genes involved in adipogenesis and lipogenesis in adipocytes, likely through NF-κB–mediated transcription.132 By contrast, in liver, TNF-α increases the expression of genes involved in de novo fatty acid synthesis while decreasing expression of those involved in fatty acid oxidation. It is not surprising, therefore, that TNF-α acutely stimulates VLDL production from liver.133 Increased VLDL secretion, together with TNF-α–mediated inhibition of LPL in adipose tissue,134 results in significant hypertriglyceridemia. TNF-α impairs insulin signaling as well, probably by activating Ser/Thr kinases (PKCε, PKCζ, Raf1, and IKK-β) that act on insulin receptor and IRS molecules, making them poor substrates for insulin-mediated tyrosine phosphorylation and signal propagation.135 Hepatic insulin resistance increases apolipoprotein B secretion by reducing intracellular degradation of this protein.136 Finally, TNF-α can affect lipid metabolism by altering the secretion of other adipokines. For example, TNF-α potently reduces expression of adiponectin (Acrep30) and increases expression of IL-1 and IL-6.132

Interleukin-6

IL-6 is a protein of 22 to 27 kDa, with various degrees of glycosylation.136 In contrast to TNF-α, IL-6 circulates at
relatively high levels, and adipocytes contribute \( \approx 1/3 \) of the plasma IL-6.\(^{137} \) Plasma levels of IL-6 correlate positively with fat mass, insulin-resistant syndrome, and plasma FFA levels.\(^{138,139} \) Administration of IL-6 to humans results in elevations of plasma FA and increased FA appearance in plasma, consistent with increased adipocyte lipolysis of TG.\(^{140,141} \) Fatty acid oxidation also appears to increase during IL-6 administration to humans.\(^{140} \) These results are consistent with the demonstration that an IL-6 receptor, homologous to the leptin receptor, is present in adipose tissue.\(^4 \) IL-6 influsions also produce dose-dependent increases in plasma glucose secondary to the development of insulin resistance or increased levels of glucagon.\(^{142} \) Studies in hepatocytes showed that IL-6 is able to impair insulin signaling by downregulation of IRS and upregulation of SOCS-3 (suppressor of cytokine signaling 3), a negative regulator of insulin signaling. Recently, upregulation of SOCS-3 by IL-6 was affirmed in human skeletal muscle and adipose tissue.\(^{143} \) Moreover, IL-6 inhibits adiponectin production in adipocytes,\(^{144} \) an action that may contribute to IL-6–induced hepatic insulin resistance. Finally, IL-6 suppresses LPL activity in adipose tissue.\(^{145} \)

IL-6 signaling is complex, however, because of its apparently opposite role in the central nervous system.\(^4 \) Central administration of IL-6 in rodents causes increased energy expenditure, resulting in decreased body fat. This is consistent with the negative correlation between IL-6 levels in the central nervous system and body fat mass observed in overweight human subjects.\(^{146} \) The existence of a powerful central action of IL-6 may be used to explain the phenotype of the IL-6 knockout mice. Thus, it was reported that these mice were actually obese, leptin resistant, glucose intolerant, and in the females, hypertriglyceridemic and hypercholesterolemic\(^{147} \): phenotypes opposite to what would be expected from the lack of peripheral action of IL-6. Central replacement of IL-6 in these animals partially reversed obesity, whereas peripheral administration of IL-6 had no effect.\(^{147} \) It should be noted, however, that the obese and insulin-resistant phenotypes in IL-6–deficient mice were not confirmed in a recent report,\(^{148} \) and the reason for the different findings was not clear.

**Secretion of Apolipoproteins by Adipocytes**

It is not totally surprising that adipose tissue, which not only contain the large majority of the body’s TG stores, but also the largest store of cholesterol in the body, synthesizes and secretes several apolipoproteins. ApoE is a 34-kDa protein that is well known as a key apolipoprotein involved in both secretion and uptake of lipoproteins by tissues and in cholesterol efflux from cells.\(^{149} \) Expression of apoE by adipocytes, and regulation of that expression by cellular cholesterol, were demonstrated over a decade ago.\(^{150} \) Consistent with the latter finding, and with prior studies in macrophages indicating a role for apoE in cholesterol efflux, recent studies have shown that the apoE gene is a target of adipocyte liver X receptors (LXR).\(^{151} \) Potentially relevant to this review are recent findings indicating that PPAR\(\gamma\) agonists increased apoE mRNA in human adipose tissue and 3T3-L1 cells.\(^{152} \) Additionally, although incubation of cultured 3T3-L1\(^{153} \) or SW872 liposarcoma cells\(^{153} \) with insulin did not affect apoE mRNA, apoE secretion fell in the liposarcoma cells exposed to increasing insulin concentrations.\(^{153} \) If adipose apoE secretion is responsive to insulin in otherwise insulin-resistant adipocytes, then adipocyte apoE secretion might be reduced in states of whole-body hyperinsulinemia. This could lead to defective cholesterol efflux from fat.

Two other apolipoproteins, apoC-I and apoD, have also been identified as adipocyte products.\(^{153,154} \) ApoC-I is a 6.6-kDa protein that plays a role in regulating receptor-mediated removal of lipoproteins from plasma.\(^ {10} \) In SW872 liposarcoma cells, expression of apoC-I increased with increasing cell lipids, and this was associated with increased apoC-I secretion. Insulin treatment, however, reduced both apoC-I mRNA levels and apoC-I secretion into the media.\(^ {153} \) The relevance of these findings remains unclear, but the fact that transgenic mice overexpressing apoC-I have defects in fatty acid uptake by adipose tissue\(^ {155} \) and resistance to obesity\(^ {156} \) suggests that in hyperinsulinemic states, reduced apoC-I synthesis and secretion could contribute to obesity. ApoD is a 6.6-kDa protein that is a member of the lipocalin family of lipid transporters.\(^ {154} \) ApoD has recently been identified as a target for LXR\(\alpha\) in adipocytes, suggesting a role in cholesterol efflux.\(^ {157} \) What role apoD plays in adipocyte function in vivo and whether apoD might be relevant to adipocyte dysfunction in insulin-resistant states remain to be determined.

**Summary**

In this review, we have focused particularly on how unregulated secretion of FA from insulin-resistant adipocytes can affect lipid metabolism in liver, muscle, and pancreatic \(\beta\) cells. It is clear from published literature that dysregulated partitioning of FA between storage sites (adipocytes) and sites of utilization (muscle, liver, and \(\beta\) cells) upsets lipid homeostasis in the latter tissues, with deleterious outcomes, including TG accumulation and insulin resistance. We did not mean to suggest, however, that adipose tissue insulin resistance is the only cause of the insulin resistance present either in those other sites, or in the whole body, in rodents or people with the metabolic syndrome. Although we believe that adipose tissue insulin resistance plays a key role in the “typical” insulin resistance found in obese humans, it is clear from several mouse models that muscle\(^{158} \) and liver\(^ {41} \) can be the sites of primary insulin resistance, with the possibility for subsequent secondary insulin resistance in the other tissues, including adipose tissue. Further, the basis of adipocyte insulin resistance is very poorly defined. Indeed, even the roles of insulin receptors and PPAR\(\gamma\), 2 key proteins involved in energy storage by adipose tissue, are complex and incompletely understood. For example, adipocyte-specific targeted deletion of the insulin receptor (FIRKO mice) results in smaller mice with less body fat, increased whole-body insulin sensitivity, and resistance to age- and obesity-related insulin resistance, despite a clear lack of insulin stimulated glucose and lipid metabolism in adipocytes.\(^ {159} \) However, the FIRKO mice do not have increased plasma FA or TG levels, have normal basal and isoproterenol-stimulated lipolysis, and have increased plasma levels of both leptin and adiponectin; all
factors that could protect against systemic insulin resistance in this complicated model. Overall, mouse models of tissue-specific loss of insulin receptors or glucose transporters are indicative of this species’ ability to shift metabolic substrates from an insulin-resistant tissue to unaffected tissues.\(^\text{160}\) We propose that when humans are unable to store energy normally, whether because of the quantitative or qualitative absence of adequate adipose tissue, they also seem to transfer that energy, in the form of FA, to alternative sites such as liver, muscle, and B cells. Whether the accumulation of FA at those sites is adequate to produce the detrimental metabolic sequelae we described in detail in this review or whether concomitant dysregulation of the secretion of adipokines and adipocytokines is required is not clear. What is clear, however, is that the inability to store energy in adipocytes leads to abnormal lipid metabolism and potentially insulin resistance, inflammation, and cell death in the alternative storage sites.

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Adipocyte Signaling and Lipid Homeostasis


Adipocyte Signaling and Lipid Homeostasis: Sequelae of Insulin-Resistant Adipose Tissue
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