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Adipocyte-Derived Hormones, Cytokines, and Mediators

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Adipose tissue is responsive to both central and peripheral metabolic signals and is itself capable of secreting a number of proteins. These adipocyte-specific or enriched proteins, termed adipokines, have been shown to have a variety of local, peripheral, and central effects. These secreted proteins, which include tumor necrosis factor (TNF)-α, resistin, IL-6, IL-8, acylation-stimulating protein (ASP), angiotensinogen, plasminogen activator inhibitor-1 (PAI-1) ("bad" adipokines) and leptin, adiponectin ("good" adipokines) seem to play important regulatory roles in a variety of complex processes, including fat metabolism, feeding behavior, hemostasis, vascular tone, energy balance, and insulin sensitivity, but none is without controversy regarding its respective mechanism and scope of action. The present review is focused on the effects of free fatty acids and a restricted number of adipokines, which have been implicated in vascular (angiotensinogen, PAI-1) and energy and glucose homeostasis (ASP, TNFα, IL-6, resistin, leptin, adiponectin).

Key Words: Adipokines; adiponectin; fatty acids; leptin; resistin.

Introduction: Adipose Tissue— An Active Endocrine Organ

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). Lately it has become clear that adipose tissue is an important organ for the development of many diseases related to obesity and metabolic syndrome *(1–3)*. It increases in obesity or conversely shrinks in lipoatrophic syndromes. Both obesity and lipoatrophy are pathologic conditions highly associated with metabolic disorders, including hyperlipidemia, insulin resistance, and type 2 diabetes (T2D) *(4)*. Thus, the idea has emerged that adipose tissue might be instrumental in the pathogenesis of these disorders.

Adipose tissue is no longer viewed as a passive repository for triacylglycerol storage and a source of free FA but as an active endocrine and paracrine organ secreting an ever-increasing number of mediators that participate in diverse metabolic processes including food intake, regulation of energy balance, insulin action, lipid and glucose metabolism, angiogenesis and vascular remodeling, regulation of blood pressure, and coagulation *(2,5–7)*. Several secretory proteins are synthesized in adipose tissue including leptin, resistin, adiponectin, tumor necrosis factor (TNF α), angiotensinogen, adipsin, acylation-stimulating protein (ASP), retinol-binding protein (RBP), interleukin (IL)-1β, IL-6, IL-8, IL-10, plasminogen activator inhibitor-1 (PAI-1), fasting-induced adipose factor, a fibrinogen–angiopoietin-related protein, metallothionein, tissue factor (TF), complement C3, fibronectin, haptoglobin, entactin/nidogen, collagen VIα3, pigment epithelium–derived factor (PEDF), hippocampal cholinergic neurostimulating peptide (HCNP), neutrophil gelatinase-associated lipocalin (NGAL), and adiponutrin *(8)*. Moreover, lipoprotein lipase (LPL), apolipoprotein E (apoE), cholesteryl ester transfer protein (CETP), angiotensinogen, transforming growth factor-beta (TGF-beta), nitric oxide synthase (NOS), acylation stimulating protein (ASP), adipophilin, monobutyrin, agouti protein, and factors related to pro-inflammatory and immune processes *(9)* apelin and zinc-alpha2-glycoprotein [also named lipid mobilizing factor (LMF)] have also been shown to be released by white adipocytes *(10)*. These secretory proteins from the adipose organ *(11)* are named adipokines and have many physiological effects on different organs including the brain, bone, reproductive organs, liver, skeletal muscles, immune cells, and blood vessels *(4)*. It is likely that many more undiscovered fat cell–derived mediators will be causally linked to cardiovascular health, insulin resistance, and diabetes.

Virtually all known adipokines are markedly dysregulated in response to alteration of fat mass, although the molecular link between the size of adipose stores and the rate of production of a specific adipokine remains largely speculative. Weight loss is associated with a decrease in the serum levels of most of these adipokines, with the exception of adiponectin, which is increased *(3,12)*. A large number of adipokines also affect insulin action, glucose, and fat metabolism and, consequently, insulin resistance, which ultimately leads to type 2 diabetes. Hence, they exert direct as well as indirect

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influences on the process of atherosclerosis. The present review is focused on a number of adipokines, which have been implicated in vascular, energy, and glucose homeostasis.

Leptin

Leptin is a 16-kDa protein encoded by the *ob* gene and is synthesized mainly in adipose tissue *(13)*. The potent effect of recombinant leptin to reduce food intake, body weight, and fat mass in leptin-deficient mice brought the ultimate proof that the absence of functional leptin is responsible for the obese phenotype of *ob/ob* mice. Numerous reviews have been published describing its regulation and its role in eating and energy homeostasis *(14,15)*.

Adipocytes are the most important source of leptin, and circulating leptin levels directly correlate with adipose tissue mass *(16)*. Control of appetite is the primary role of leptin. In fact, mice with a mutation in the leptin (*ob*/*ob* mice) or leptin receptor (*db*/*db* mice) gene, as well as human subjects with mutations in the same genes, are massively obese *(15,17)*. The fact that adipose leptin production is increased in obese individuals led to the concept of leptin resistance. The molecular basis of leptin resistance, apart from mutations in the receptor gene, is yet to be determined. Adenoviral or transgenic overexpression of the leptin gene reduced food intake and body weight in rodents *(18–20)*. Attempts to obtain the same effect in humans through daily administration of recombinant leptin were frustrating, because only very high doses of leptin induced a reduction of body weight in a subset of individuals *(21)*. Interestingly, leptin administration has been proposed as a new treatment to ameliorate insulin sensitivity in lipoatrophic diabetes, where low leptin levels prevail *(22)*.

Both leptin-deficient and leptin-resistant obese rodents exhibit severe insulin resistance. This condition is rapidly ameliorated by leptin administration in deficient mice, even before reduction of body weight *(18–20)*. Moreover, the insulin-sensitizing effect of leptin exceeds that seen in pair-fed animals. Accumulating evidence suggests that leptin promotes fatty acid oxidation and reduces ectopic fat accumulation in non-adipose tissues, thereby increasing insulin sensitivity *(23,24)*. This effect is mediated by activation of the AMP-activated kinase (AMPK) by leptin, through a direct effect on certain skeletal muscles and indirectly through the hypothalamic–sympathetic nervous system axis *(25)*. As a result of AMPK activation, the enzyme acetyl coenzyme A carboxylase is inhibited, leading to reduced intracellular levels of the metabolite malonyl CoA. This alleviates inhibition of fatty acid entry into the mitochondria by malonyl CoA and favors fatty acid oxidation.

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. 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 *(26)*. 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 *(27)*. Overexpression of leptin in adipocytes also reduced gene expression of acetyl CoA carboxylase (ACC) *(28)*. Recent data suggest that leptin exerts a paracrine effect on fat cells and that its expression and secretion by fat cells can be induced by IL-6 and inhibited by TNF-α, underscoring the potential role of interaction among adipokines on their release by fat cells *(29)*. 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 *(30)*.

Emerging data also link leptin to cardiovascular disease. Leptin, like CRP, upregulates ET-1 and endothelial NO synthase production in endothelial cells and promotes accumulation of reactive oxygen species *(31,32)*. It stimulates the proliferation and migration of endothelial cells *(33)* and vascular smooth muscle cells *(34)*. MCP-1 expression in aortic endothelial cells is stimulated by leptin *(35)*. Furthermore, leptin increases platelet aggregation and arterial thrombosis via a leptin receptor–dependent pathway *(31,32)*, has a direct action on macrophages by increasing the release of monocyte colony-stimulating factor *(36)*, promotes cholesterol accumulation in macrophages under high glucose conditions *(37)*, and stimulates angiogenesis *(38)*. Leptin also increases peripheral sympathetic tone, with observations of lower arterial pressures in leptin-deficient mice suggesting a possible role for leptin in hypertension *(39,40)*.

Leptin's role in regulating immunity has been fueled by early observations of thymus atrophy in *db*/*db* mice. Leptin protects T lymphocytes from apoptosis and regulates T-cell proliferation and activation. Leptin also influences cytokine production from T lymphocytes, generally switching the phenotype toward a TH1 response *(41–43)*. Leptin also influences monocyte activation, phagocytosis, and cytokine production. Signal transduction pathways activated by leptin in immune cells include the Janus kinase–signal transducer and activator of transcription system (particularly signal transducer and activator of transcription 3), as well as phosphatidylinositol 3-kinase and mitogen-activated protein kinase. In endothelial cells leptin induces oxidative stress and upregulation of adhesion molecules *(42)*. Thus, leptin has also an important role of in modulating immunity and inflammation.

Adiponectin

Adiponectin (ACRP30, adipoQ, apM1, or GBP28) was identified as the product of a gene induced during adipocyte differentiation *(44)*. 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 glo-

bular domain that can undergo homotrimerization *(45)*. In plasma, adiponectin circulates as either a trimer, a hexamer (called the low-molecular-weight or LMW form), or as multimeric forms of 12–18 subunits (called the high-molecular-weight or HMW form) *(46)*.

A series of recent studies reveal that administration of recombinant adiponectin, either full length or in the form of its isolated globular head, exerts glucose lowering effects and ameliorates insulin resistance in mice models of obesity or diabetes *(47–50)*. In addition, adiponectin has antiatherogenic properties, as shown by its capacity to inhibit monocyte adhesion to endothelial cells and the macrophage-to-foam-cell transformation in vitro *(51,52)*. The phenotype of adiponectin-null mice confirmed the protective role of the protein against atherosclerosis and diet-induced insulin resistance *(53,54)*. Adiponectin-deficient mice develop insulin resistance on a high-fat diet *(53,54)*, although in one study, adiponectin-null mice did not show aggravated insulin resistance on high fat diet as compared to wild-type mice *(55)*. Interestingly, insulin resistance in lipoatrophic mice was fully reversed by a combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone *(48)*. This suggests that adiponectin and leptin may work hand in hand to sensitize peripheral tissues to insulin. In addition, transgenic expression of adiponectin in adipose tissue resulted in increased plasma levels and inhibition of hepatic glucose production with improved glucose tolerance *(56,57)*. Administration of recombinant adiponectin to lipodystrophic or obese mice results in reductions in plasma FA and TG levels *(48)*, whereas transgenic mice with increased secretion of full-length adiponectin had improved clearance of an exogenous fat load *(56)*.

The insulin-sensitizing effect of adiponectin is mediated, at least in part, by an increase in fatty acid oxidation through activation of AMPK in skeletal muscles *(58,59)*, similar to the action of leptin. Moreover, adiponectin also activates AMPK in the liver, resulting in reduced rate of hepatic glucose production *(60)* and in isolated rat adipose cells, thereby increasing glucose uptake *(61)*. Although the signaling pathways evoked by adiponectin are not fully deciphered, two receptors have recently been cloned, Adipo R1 and Adipo R2, that are expressed predominantly in muscles and liver, respectively *(62)*.

Adiponectin levels in plasma, which are very high compared with other adipokines, are reduced in obese, insulin-resistant, diabetic, or dyslipidemic subjects as compared to healthy controls *(63–65)*. These findings indicate that adiponectin may represent a biological link between obesity and obesity-related disorders such as atherosclerosis and diabetes. Moreover, adiponectin has been found to increase with weight loss, and be negatively correlated with changes in body mass index (BMI), waist and hip circumference, and plasma glucose levels *(66)*. The mechanism by which the insulin-resistant state is associated with low levels of adiponectin is not clear. However, TNF-α, which is increased in the adipose tissue of obese subjects, might downregulate adiponectin production *(67)*. On the other hand, adiponectin reduces the production and activity of TNF-α *(68)*. The anti-inflammatory activities of adiponectin extend to inhibition of IL-6 production accompanied by induction of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist *(69–71)*. Inhibition of nuclear factor κB (NF-κB) by adiponectin might explain at least part of these effects *(71)*.

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 *(72)*. In addition, increased adipose tissue LPL activity was present in a transgenic mouse that secretes increased quantities of full-length adiponectin from adipose tissue *(56)*. 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 *(73)*.

The current literature suggests that replenishment of adiponectin in patients with type 2 diabetes having decreased adiponectin levels could represent a novel therapeutic for the treatment of insulin resistance and type 2 diabetes. Increased adiponectin levels might have the added benefit of reducing weight and, as an anti-inflammatory agent, inhibit atherogenesis and the risk of cardiovascular disease.

Resistin

Resistin is an adipokine that belongs to a family of cysteine-rich secreted proteins named FIZZ *(74)*. 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 *(75)*. Resistin levels are elevated in both diet-induced obesity and genetic mouse models of obesity/diabetes *(75)*. 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 *(76)*. Neutralization of resistin by specific antibodies resulted in decreased blood glucose levels and improved insulin sensitivity *(75)* and antisense oligodeoxynucleotides that normalized resistin levels also reversed hepatic insulin resistance *(77)*. 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 high-fat diet, the resistin-deficient mice also showed significantly better glucose tolerance than the wild-type mice *(78)*. Deletion of the resistin gene was associated with increased AMPK activity in hepatocytes, decreases in gluconeogenic enzymes, and decreased hepatic glucose pro-

duction *(78)*. On the other hand, transgenic overexpression of resistin was associated with increased hepatic glucose production and glucose intolerance *(78)*.

Resistin also exerts direct vasoactive effects in cultured endothelial cells *(79)*. Resistin treatment activated endothelial cells by promoting ET-1 release, in part by inducing ET-1 mRNA expression, suggesting it participates in the endothelial dysfunction observed in patients with insulin resistance. Resistin also significantly augmented the expression of the cell adhesion molecule VCAM-1 and the chemoattractant chemokine MCP-1, key processes in early atherosclerotic lesion formation. Resistin has also been demonstrated recently to have a proinflammatory effect on smooth muscle cells. Resistin, in a dose-dependent manner, induced human aortic smooth muscle cell proliferation, suggesting it may play a role in the increased incidence of restenosis observed in diabetic patients *(80)*.

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 or Wistar rats, plasma TG levels increased, and this was associated with increased secretion of TG from the liver *(81,82)*. 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 *(81)*.

The role of resistin in humans is less certain. Clinical studies in humans do not show a consistent link between resistin levels and either obesity or insulin resistance *(83, 84)*. There is also controversy regarding the importance of adipocytes as a source of resistin in humans *(85,86)*. However, recent data indicate that stimulation of macrophages in vitro with endotoxin or proinflammatory cytokines leads to a marked increase in resistin production *(87)*. Furthermore, administration of endotoxin to human volunteers is associated with dramatically increased circulating resistin levels *(87)*. Thus, in human subjects resistin seems to act as a critical mediator of the insulin resistance associated with sepsis and possibly other inflammatory conditions. Human resistin is only 64% homologous (53% identical) 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 *(86)*. 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.

Tumor Necrosis Factor-α and IL-6: Bad Adipocytokines?

Tumor Necrosis Factor

Tumor 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 metallo-roteinase *(88)*. TNF-α was the first adipose-secreted product proposed to represent a molecular link between obesity and insulin resistance *(89,90)*.

A number of studies have demonstrated that TNF-α alters insulin signaling in cultured cells and in vivo *(91)*. Chronic exposure of cells or whole animals to TNF-α induces insulin resistance, and treatment with soluble forms of TNF-α receptors can neutralize this effect. Furthermore, TNF-α or TNF-α receptor knockout mice showed improved insulin sensitivity in both diet-induced obesity and in the *ob*/*ob* model of obesity *(89)*. Obese TNF-α-deficient mice had lower levels of circulating FFAs and were partially protected from the obesity-related reduction in the insulin receptor signaling in muscle and fat tissues *(89)*. Locally, TNF-α increases PAI-1 and C3 gene expression and decreases adiponectin in fat *(92)* and this hormone seems to be a crucial mediator of insulin sensitivity, potentially explaining how paracrine effects of TNF-α within fat could cause systemic insulin resistance. Thus, TNF-α–regulated pathways in fat may mediate, at least in part, the obesity-induced alteration in circulating levels of certain adipokines *(93)*.

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 *(94)* leading to elevation of plasma FA levels through activation of p44/42 and JNK. These kinases can phosphorylate perilipin, thereby displacing it from lipid droplets and making TG accessible to HSL *(95)*. Additionally, TNF-α causes reductions in the expression of genes involved in adipogenesis and lipogenesis in adipocytes, likely through NF-κB–mediated transcription *(93)*. 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 also acutely stimulates VLDL production from liver *(96)*. Increased VLDL secretion, together with TNF-α–mediated inhibition of LPL in adipose tissue *(97)*, results in significant hypertriglyceridemia. In addition, TNF-α impairs insulin signaling through serine phosphorylation (inactivation) of both the insulin receptor (IR) and insulin receptor substrate 1 (IRS-1), both of which result in diminished activation of phosphoinositol-3-kinase, the essential second messenger signal that governs most of insulin's metabolic effects *(98,99)*.

The role of TNF-α in the systemic inflammatory response triggered by obesity has been studied extensively *(100)*. Circulating TNF-α concentrations rise with increasing obesity and correlate with insulin resistance *(101,102)*. Within adipose tissue, macrophages account for nearly all TNF-α production *(103)*, and both TNF-α mRNA content and TNF-α production increase in adipose tissue of obese individuals *(101)*. However, net secretion of TNF-α from visceral fat into the circulation has not yet been documented. Therefore, it remains unclear whether TNF-α secretion from adipose tissue directly accounts for the elevated serum TNF-α con-

centration seen in obesity. In vivo studies have complicated the interpretation of the role of circulating TNF-α, as two rodent studies using chimeric TNF-α receptor *(90)* or overexpression of a soluble TNF-α receptor fragment *(104)* both resulted in significant improvement of insulin resistance in obese rats. However, despite initial promising results in animal studies, neutralization of TNF-α activity has proved ineffective in ameliorating insulin sensitivity in diabetic patients *(105)*. Therefore, although TNF-α is a macrophage-derived inflammatory factor that contributes to insulin resistance in adipose tissue and muscle via paracrine and potentially endocrine mechanisms, other inflammatory molecules may be able to compensate for the absence of TNF-α signaling; thus, the viability of antagonists of TNF-α signaling in the treatment of the metabolic syndrome remains unclear.

Interleukin-6 (IL-6)

IL-6 is a protein of 22–27 kDa, with various degrees of glycosylation *(106)*. Human fat tissue produces substantial amounts of IL-6 and this secretion might represent 10–30% of circulating levels *(107)*. Plasma IL-6 is highly correlated with body mass and inversely related to insulin sensitivity *(108,109)*. Recent data suggest that IL-6 plays a direct role in insulin resistance by altering insulin signaling in hepatocytes *(110)*. This effect is mediated by the induction of SOCS-3 (suppressor of cytokine signaling-3), which inhibits insulin-dependent insulin receptor autophosphorylation *(111)*. Moreover, IL-6 suppresses LPL activity and inhibits adiponectin production in adipocytes *(112)*, an action that may contribute to IL-6–induced hepatic insulin resistance.

IL-6 signaling is complex because of its apparently opposite role in the central nervous system. Central administration of IL-6 in rodents causes increased energy expenditure, resulting in decreased body fat *(113)*. The existence of this powerful central action of IL-6 may be used to explain the phenotype of the IL-6 knockout mice. IL-6–deficient mice developed mature-onset obesity associated with glucose intolerance *(114)*. Central replacement of IL-6 in these animals partially reversed obesity, whereas peripheral administration of IL-6 had no effect *(114)*. It should be noted that these results were not confirmed in a recent report *(115)*, and the reason for the different findings was not clear. However, these observations suggest that IL-6 might act at multiple levels, both centrally and on peripheral tissues, to influence body weight, energy homeostasis, and insulin sensitivity. Therefore, if IL-6 secretion by adipose tissue contributes to energy homeostasis through an endocrine action on the CNS, then one could invoke a state of obesity-induced IL-6 resistance, much as described for the effects of obesity on leptin and insulin signaling. This supposition also suggests the possibility that increased adipose-tissue IL-6 secretion associated with obesity may be a regulatory mechanism attempting to correct excess body weight and achieve negative energy balance, as hypothesized for obesity-related increases in leptin. The systemic inflammation resulting from IL-6 effects on liver and endothelium therefore could be an unintended consequence of appropriately elevated IL-6 levels in the face of obesity and central IL-6 resistance.

Administration of IL-6 to humans results in elevations of plasma FA consistent with increased adipocyte lipolysis of TG and increase in fatty acid oxidation *(116,117)*. IL-6 infusions also produce dose-dependent increases in plasma glucose secondary to the development of insulin resistance or increased levels of glucagon *(118)*.

In addition, circulating IL-6 is the single most important factor controlling the hepatic acute-phase response, the rapid, coordinated physiologic reaction to tissue damage or infection designed to recruit host defense mechanisms, eliminate damaged cells, contain pathogens, and begin tissue repair *(119)*. Of the many positive and negative acute-phase reactants, the most recognized is CRP, a member of the pentraxin family that attaches to the plasma membrane of damaged cells causing cell death through activation of the complement cascade *(120)*.

The endocrine cytokine IL-6, therefore, is a likely mediator of proinflammatory signaling from adipose tissue; however, strategies designed to block IL-6 action remain to be evaluated as treatments of the metabolic syndrome. Importantly, limiting IL-6 effects on liver and endothelium may well impair the normal host response to acute infection, whereas preventing IL-6 secretion from adipose tissue could potentially worsen obesity if peripheral IL-6 secretion provides negative feedback to hypothalamic areas that govern energy balance.

Other Adipocyte-Secreted Proteins

Angiotensinogen (AGT)

Angiotensinogen (AGT), a precursor to the major proatherogenic vasoconstrictor ANG II, is expressed and produced in adipocytes *(1)*. ANG II directly stimulates ICAM-1, VCAM-1, MCP-1, and macrophage colony stimulating factor (M-CSF) expression in vascular cells by activating NF-κB–regulated genes *(121)*. ANG II also promotes the formation of free oxygen radicals from NO, thereby decreasing the availability of NO and incurring damage to the vascular tissue *(122)*. Augmented angiotensinogen production by adipose tissue in obesity has been linked to angiogenesis *(123)* and the development of hypertension *(1)*, both of which are known to be associated with endothelial dysfunction.

Hypertension is a frequent complication of obesity and a major risk for the development of cardiovascular diseases. Epidemiological studies have reported a significant positive correlation between blood pressure and circulating levels of AGT. Although AGT is mainly produced by the liver, adipose tissue is considered a major extrahepatic source of AGT, which could contribute to increased circulating levels in obese individuals *(1)*. The pathophysiological importance of adipose tissue production was clarified by genetic manip-

ulations in mice. In wild-type mice, overexpression of AGT mRNA in fat resulted in elevated plasma AGT, hypertension, and increased fat mass *(124)*. In addition, AGT-deficient mice are partially protected from diet-induced obesity *(125)* supporting the idea that increased AGT production could also contribute to enhancement of fat mass, an effect that has been attributed to angiotensin II acting locally as a trophic factor for new adipose cell formation.

Plasminogen Activator Inhibitor 1 (PAI-1)

Plasma plasminogen activator inhibitor 1 (PAI-1) is a factor which is the most important endogenous inhibitor of tissue plasminogen activator and uro-plasminogen activator, and is a main determinant of fibrinolytic activity *(126)*. PAI-1 is a glycoprotein that is composed of 379 amino acids and has an apparent molecular weight of 48 kDa. PAI-1 is a member of the superfamily of serine-protease inhibitors (serpins) and serves as a pseudosubstrate for PA *(126)*. The main production sites for PAI-1 known so far are liver, endothelial cells, adipose tissue, and thrombocytes. After release into the blood stream, PAI-1 is present either in an active form or, to a greater extent, complexed with either t-PA, one of the two main activators of fibrinolysis, or vitronectin, which converts PAI-1 into an inactive, latent form *(127, 128)*. In addition, PAI-1 has been shown to influence cell migration and angiogenesis by competing with integrin binding on extracellular matrix vitronectin. Visceral PAI-1 can also impair pre-adipocyte migration and attachment to vitronectin *(129)*.

PAI-1 is overexpressed in adipose tissue of obese mice and humans *(130,131)*. Obese mice, humans with type 2 diabetes *(132–134)*, and offspring of patients with type 2 diabetes *(135)* have elevated plasma PAI-1 levels. Elevated PAI-1 has been considered to be a consequence of obesity and to be a marker of risk of type 2 diabetes *(136)*. Effects of modification of PAI-1, through genetic knockout or overexpression of PAI-1, on the development of obesity have been reported, but with controversial results *(137–139)*. Accordingly, disruption of PAI-1 gene reduced adiposity in genetically obese mice *(138)* or had significant effect on fat mass in diet-induced obesity *(140)*. In contrast, other reports hypothesized that PAI-1 is not merely a product of obesity, but reduces obesity and insulin resistance. Their findings show that PAI-$1^{-/-}$ mice gain more weight and have elevated adipose tissue cellularity *(137)*. Thus, at this time it is not clear whether inhibition of PAI-1 might provide a novel anti-obesity and anti-diabetes treatment and might have marked beneficial effects on both obesity and type 2 diabetes.

Acylation-Stimulating Protein (ASP)

Acylation-stimulating protein (ASP) is an adipocyte-secreted protein that is generated by the interaction of complement C3 with factors B and D (adipsin). The result is cleavage of the N-terminal of C3, producing C3a, which is then cleaved by plasma carboxypeptidase to produce ASP *(141)*. ASP stimulates triglyceride storage in adipose cells through different processes: stimulation of glucose transport, enhancement of fatty-acid re-esterification, and inhibition of lipolysis *(141)*. However, the receptor and signaling pathways mediating ASP effects are as yet uncharacterized.

Studies in adipocytes indicate that ASP increases glucose uptake (a substrate for glycerol formation) and the activity of diacylglycerol acyltransferase (DGAT), thereby facilitating use of FA for TG synthesis *(141)*. Additionally, ASP has been shown to increase reesterification of FA released from TG by HSL, as well as reduce HSL-mediated lipolysis *(142)*. ASP-deficient mice have delayed postprandial TG clearance and reduced body weight and fat mass *(143–145)*. Additionally, intraperitoneal injection of ASP facilitated postprandial TG clearance in several mouse models of obesity and insulin resistance *(143)*. However, other investigators, using the same mouse did not find alterations in energy metabolism or lipid metabolism *(146)*. Whatever the strain, the absence of ASP resulted in a moderate reduction of WAT mass, on both standard and high fat diets, indicating decreased triglyceride strorage. In addition, ASP-deficient mice appear to be more sensitive to insulin, although this could be the consequence of their relative leanness *(145)*.

In humans, fasting ASP levels correlate with postprandial TG clearance *(147)*. Most, but not all studies in humans report substantial increases in plasma ASP in obese subjects, along with moderate overexpression of C3 mRNA in fat *(148)*. It is not known whether increased circulating levels of the protein reflect increased activity or resistance to ASP. This controversy remains unresolved at present, although the weight of evidence favors some role for ASP in postprandial TG and FA partitioning into fat.

Fatty Acids (FA)

Fatty acids themselves and as part of complex lipids play a number of key roles in metabolism as a major metabolic fuel (storage and transport of energy), as essential components of all membranes and as ligands for transcription factors *(149)*. Fatty acids or their derivatives (acyl–CoA or eicosanoids) may interact with nuclear receptor proteins that bind to certain regulatory regions of DNA and thereby alter transcription of the target genes.

Plasma FAs are primarily an important energy substrate for a number of organs. FAs are also precursors for the formation of triacylglycerol (TG) stores in adipose tissue, liver, and muscle through esterification. In addition, FA may be involved in the regulation of a number of metabolic processes in the body. If they are present in excess, they are involved directly in the pathogenesis of metabolic disturbances leading to insulin resistance and metabolic syndrome, and they may also exert adverse effects on heart chronotropic

function. The availability of FA for all these processes is governed mainly by their release from the the adipose tissue.

The concentration of free fatty acid (FFA) in plasma is the result of a balance between lipolytic production, the uptake by the liver and the oxidation by muscle, heart, liver, and other tissues. Thus, a poor regulation of lipolysis can significantly affect the plasma FFA levels. A reduced lipolytic activity may lead to the accumulation of adipose tissue stores and this increase together with an impaired insulin-mediated inhibition of lipolysis may increase circulating FFA concentrations. This imbalance in FFA and glucose plasma levels is closely related to obesity, insulin resistance, dyslipidaemia, and type 2 diabetes mellitus.

Lipolysis is important for the maintenance of body energy homeostasis as well as for the prevention of systemic metabolic disorders. The key rate-limiting enzyme of the reaction is hormone-sensitive lipase (HSL) *(150)*. The main hormones involved in the regulation of HSL activity are catecholamines and insulin. Catecholamines stimulate lipolysis through the activation of β1 and β2 adrenoreceptors and inhibit lipolysis through activation of the α2 adrenoreceptor. HSL activation is mediated by a cascade beginning with coupling to Gs or Gi protein and subsequent formation of cAMP through the adenylylcyclase system and subsequent activation of protein kinase A and phosphorylation of HSL *(150,151)*. Insulin is a potent inhibitor of lipolysis and acts primarily through its effect on phosphodiesterase and subsequent suppression of the formation of cAMP *(152)*. Work in recent years has revealed that both hormone-sensitive lipase (HSL), generally thought to be the rate-limiting enzyme, and perilipin, a lipid droplet surface protein, are required for optimal lipid storage and fatty acid release *(153)*. The perilipin proteins are polyphosphorylated by protein kinase A and phosphorylation is necessary for translocation of HSL to the lipid droplet and enhanced lipolysis *(153,154)*.

Magnetic resonance spectroscopy studies in humans suggest that a defect in insulin-stimulated glucose transport in skeletal muscle is the primary metabolic abnormality in insulin-resistant type 2 diabetics *(155)*. Fatty acids appear to cause this defect in glucose transport by inhibiting insulin signaling *(156–158)*. A number of different metabolic abnormalities may increase intramyocellular/intrahepatic fatty acid metabolites; these include increased fat delivery to muscle/liver as a consequence of either excess energy intake or defects in adipocyte fat metabolism and acquired or inherited defects in mitochondrial fatty acid oxidation *(159)*. Increases in intramyocellular fatty acid–derived metabolites (e.g., fatty acyl–CoA, diacylglycerol, etc.), due to increased delivery and/or decreased mitochondrial oxidation of fatty acids, triggers the activation of a serine/threonine kinase cascade results in increased serine phosphorylation of insulin receptor substrate-1 (IRS-1) on critical sites, which in turn reduces the ability of IRS-1 to bind and activate phosphoinositol 3-kinase (PI3K), resulting in reduced insulin-stimulated glucose transport acitivty and other events downstream of PI3K *(156–158)*.

Increased plasma FFA concentrations are typically associated with many insulin-resistant states, including obesity and type 2 diabetes mellitus *(159)*. In a cross-sectional study of young, normal-weight offspring of type 2 diabetic patients, inverse relationship between fasting plasma fatty acid concentrations and insulin sensitivity, consistent with the hypothesis that altered fatty acid metabolism might contribute to insulin resistance in patients with type 2 diabetes *(160)*. Furthermore, studies measuring intramyocellular triglyceride content have shown an even stronger relationship between accumulation of intramyocellular triglyceride and insulin resistance *(160)*. Thus, targeting pathways involved in the regulation of fatty acid oxidation, fatty acid delivery and storage might provide novel anti-obesity and anti-diabetes treatments and might have marked beneficial effects on both obesity and type 2 diabetes.

Conclusions

Adipose tissue secretes different factors capable of influencing several physiological processes (Fig. 1). Some adipokines like leptin and adiponectin exert a beneficial effect on energy balance, insulin action, and vasculature. Conversely, excessive production of FA and adipokines like TNF-α, IL-6, and resistin is deleterious. For example, TNF-α, IL-6, or resistin might deteriorate insulin action, while angiotensinogen and PAI-1 are likely to participate in the vascular complications linked to obesity. Dysregulation of adipokines production with alteration of fat mass has been implicated in metabolic and cardiovascular complications of obesity. In obese individuals, excessive production of ASP, TNF-α, IL-6, or resistin deteriorates insulin action in muscles and/or in liver, while increased angiotensinogen and PAI-1 secretion favors hypertension and impaired fibrinolysis. Thus, the possibility now exists to develop drugs targeting adipose secreted factors or their cognate receptors, representing a new therapeutic approach to sensitize peripheral tissues to insulin and protect patients from atherosclerosis. This could be of particular therapeutic benefit in pathology associated with fat mass dysregulation, such as lipodystrophy and obesity.

References

1. Ahima, R. and Flier, J. S. (2000). *Trends Endocrinol. Metab.* **11,** 327–332.
2. Mohamed-Ali, V., Pinkney, J. H., and Coppack, S. W. (1998). *Int. J. Obes. Relat. Metab. Disord.* **22,** 1145–1158.
3. Wajchenberg, B. L. (2000). *Endocr. Rev.* **21,** 697–738.
4. Rajala, M. W. and Scherer, P. E. (2003). *Endocrinology* **144,** 3765–3773.
5. Ferroni, P., Basili, S., Falco, A., and Davi, G. (2004). *Curr. Atheroscler. Rep.* **6,** 424–431.
6. Lau, D. C., Schillabeer, G., Li, Z. H., Wong, K. L., Varzaneh, F. E., and Tough, S. C. (1996). *Int. J. Obes. Relat. Metab. Disord.* **20,** S16–S25.

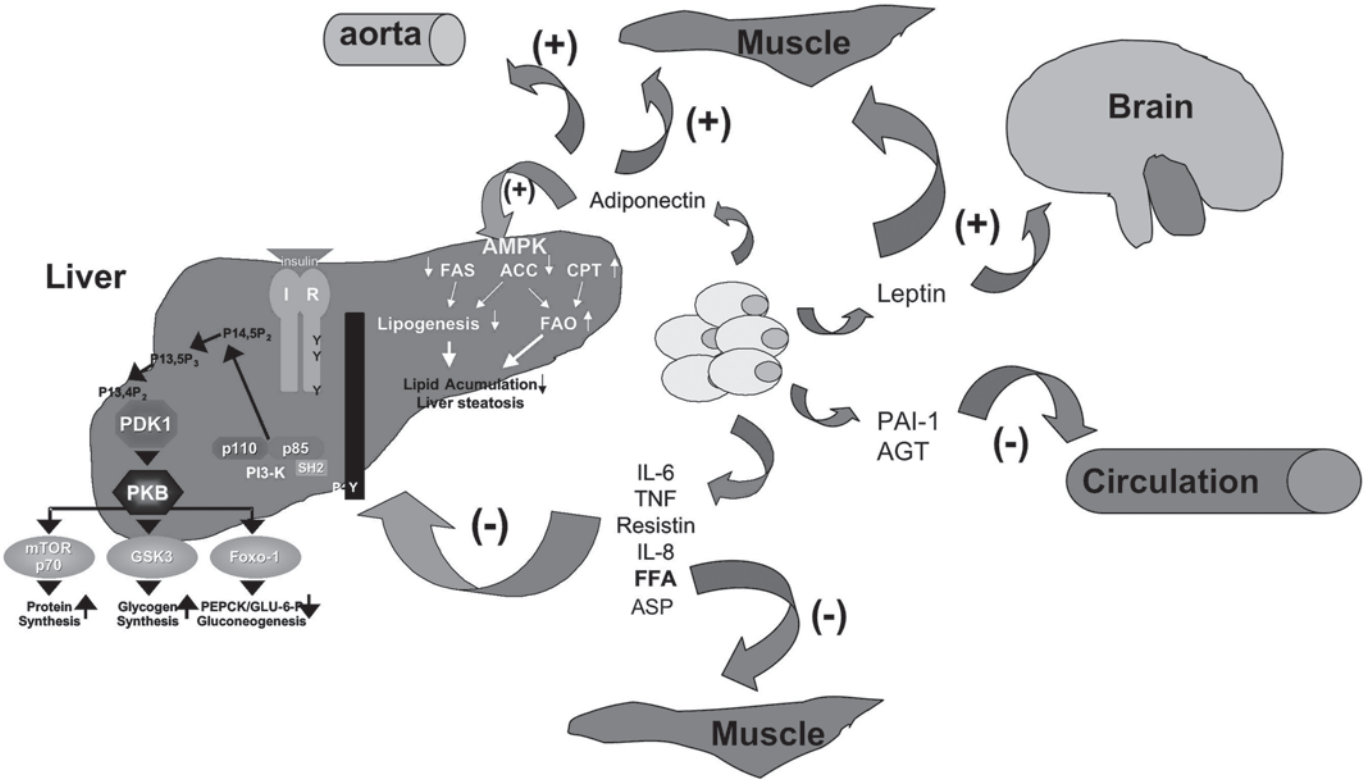

Fig. 1. Adipose tissue: an active endocrine organ. Adipose tissue is not only a source of free FA (FFA) but an active endocrine and paracrine organ secreting a number of adipokines that participate in diverse metabolic processes. These secreted proteins, which include tumor necrosis factor (TNF)-α, resistin, IL-6, acylation-stimulating protein (ASP), angiotensinogen (AGT), plasminogen activator inhibitor-1 (PAI-1) ("bad" adipokines) and leptin, adiponectin ("good" adipokines) seem to play important regulatory roles in a variety of complex processes, including fat metabolism, feeding behavior, hemostasis, vascular tone, energy balance, and insulin sensitivity.

7. Lau, D. C. W. (2000). *Int. J. Obes. Relat. Metab. Disord.* (2000). **24,** S20–S22.
8. Kratchmarova, D. E., Kalume, B., Blagoev, B., et al. (2002). *Mol. Cell Proteomics* **1,** 213–222.
9. Frunbeck, G. (2004). *Curr. Med. Chem. Cardiovasc. Hematol. Agents* **2,** 197–208.
10. Bing, C., Bao, Y., Jenkins, J., et al. (2004). *Proc. Natl. Acad. Sci. USA* **101,** 2500–2505.
11. Cinti, S. (2002). *J. Endocrinol. Invest.* **25,** 823–835.
12. Yamamoto, Y., Hirose, H., Saito, I., et al. (2002). *Clin. Sci. (Lond.)* **103,** 137–142.
13. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). *Nature* **372,** 425–432.
14. Leibel, R. L. (2002). *Nutr. Rev.* **60,** S15–S19.
15. Friedman, J. M. (2002). *Nutr. Rev.* **60,** S1–S14.
16. Maffei, M., Fei, H., Lee, G. H., et al. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 6957–6960.
17. Elmquist, J. K. (2001). *Physiol. Behav.* **74,** 703–708.
18. Halaas, J. L., Gajiwala, K. S., Maffei, M., et al. (1995). *Science* **269,** 543 546.
19. Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., and Burn, P. (1995). *Science* **269,** 546–549.
20. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., et al. (1995). *Science* **269,** 540–543.
21. Hemysfield, S. B., Greenberg, A. S., Fujioka, K., et al. (1999). *JAMA* **282,** 1568–1575.
22. Oral, E. A., Simha, V., Ruiz, E., et al. (2002). *N. Engl. J. Med.* **346,** 570–578.
23. Toyoshima, Y., Gavrilova, O., Yakar, S., et al. (2005). *Endocrinology* **46,** 4024–4035.
24. Minokoshi, Y., Kim, Y. B., Peroni, O. D., et al. (2002). *Nature* **415,** 339–343.
25. Kahn, B. B., Alquier, T., Carling, D., and Hardie, D. G. (2005). *Cell. Metab.* **1,** 15–25.
26. Dobbins, R. L., Szczepaniak, L. S., Zhang, W., and McGarry, J. D. (2003). *Am. J. Physiol. Endocrinol. Metab.* **284,** E778–E787.
27. Fruhbeck, G., Aguado, M., and Martinez, J. A. (1997). *Biochem. Biophys. Res. Commun.* **240,** 590–594.
28. Bai, Y., Zhang, S., Kim, K. S., Lee, J. K., and Kim, K. H. (1996). *J. Biol. Chem.* **271,** 13939–13942.
29. Lau, D., Yan, H., Abdel-Hafez, M., and Kermouni, A. (2002). *Int. J. Obes. Relat. Metab. Disord.* **26,** S111.
30. Nogaska, A., Sucajtys-Szulc, E., and Swierczynski, J. (2005). *Metabolism* **54,** 1041–1047.
31. Cooke, J. P. and Oka, R. K. (2002). *Circulation* **106,** 1904–1905.
32. Konstantinides, S., Schafer, K., Koschnick, S., and Loskutoff, D. J. (2001). *J. Clin. Invest.* **108,** 1533–1540.
33. Park, H. Y., Kwon, H. M., Lim, H. J., et al. (2002). *Exp. Mol. Med.* **33,** 95–102.
34. Artwohl, M., Roden, M., Holzenbein, T., et al. (2002). *Int. J. Obes. Relat. Metab. Disord.* **26,** 577–580.
35. Yamagishi, S. I., Edelstein, D., Du, X. L., Kaneda, Y., Guzman, M., and Brownlee, M. (2001). *J. Biol. Chem.* **276,** 25096–25100.

36. Loffreda, S., Yang, S. Q., and Lin, H. Z. (1998). *FASEB J.* **12,** 57–65.
37. O'Rourke, L., Gronning, L. M., Yeaman, S. J., and Shepherd, P. R. (2002). *J. Biol. Chem.* **277,** 42557–42562.
38. Sierra-Honigmann, M. R., Nath, A. K., Murakami, C., et al. (1998). *Science* **281,** 1683–1386.
39. Bouloumie, A., Marumo, T., Lafontan, M., and Busse, R. (1999). *FASEB J.* **13,** 1231–1238.
40. Quehenberger, P., Exner, M., Sunder-Plassmann, R., et al. (2002). *Circ. Res.* **90,** 711–718.
41. Fantuzzi, G. and Faggioni, R. (2000). *J. Leukoc. Biol.* **68,** 437–446.
42. Sanchez-Margalet, V., Martin-Romero, C., Santos-Alvarez, J., Goberna, R., Najib, S., and Gonzalez-Yanes, C. (2003). *Clin. Exp. Immunol.* **133,** 11–19.
43. La Cava, A., Alviggi, C., and Matarese, G. (2004). *J. Mol. Med.* **82,** 4–11.
44. Pajvani, U. B. and Scherer, P. E. (2003). *Curr. Diab. Rep.* **3,** 207–213.
45. Pajvani, U. B., Du, X., Combs, T. P., et al. (2003). *J. Biol. Chem.* **278,** 9073–9085.
46. Pajvani, U. B., Hawkins, M., Combs, T. P., et al. (2004). *J. Biol. Chem.* **279,** 12152–12162.
47. Fruebis, J., Tsao, T. S., Javorschi, S., et al. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 2005–2010.
48. Yamauchi, T., Kamon, J., Waki, H., et al. (2001). *Nat. Med.* **7,** 941–946.
49. Berg, A. H., Combs, T. P., Du, X., Brownlee, M., and Scherer, P. E. (2001). *Nat. Med.* **7,** 947–953.
50. Shimomura, I., Hammer, R. E., Ikemoto, S., Brown, M. S., and Goldstein, J. L. (1999). *Nature* **401,** 73–76.
51. Funahashi, T., Nakamura, T., Shimomura, I., et al. (1999). *Intern. Med.* **38,** 202–206.
52. Ouchi, N., Kihara, S., Arita, Y., et al. (1999). *Circulation* **100,** 2473–2476.
53. Kubota, N., Terauchi, Y., Yamauchi, T., et al. (2002). *J. Biol. Chem.* **277,** 25863–25866.
54. Maeda, N., Shimomura, I., Kishida, K., et al. (2002). *Nat. Med.* **8,** 731–737.
55. Ma, K., Cabrero, A., Saha, P. K., et al. (2002). *J. Biol. Chem.* **277,** 34658–34661.
56. Combs, T. P., Pajvani, U. B., Berg, A. H., et al. (2004). *Endocrinology* **145,** 367–383.
57. Yamauchi, T., Kamon, J., Waki, H., et al. (2003). *J. Biol. Chem.* **278,** 2461–2468.
58. Fruebis, J., Tsao, T. S., Javorschi, S., et al. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 2005–2010.
59. Tomas, E., Tsao, T. S., Saha, A. K., et al. (2002). *Proc. Natl. Acad. Sci. USA* **99,** 16309–16313.
60. Yamauchi, T., Kamon, J., Minokoshi, Y., et al. (2002). *Nat. Med.* **8,** 1288–1295.
61. Wu, X., Motoshima, H., Mahadev, K., Stalker, T. J., Scalia, R., and Goldstein, B. J. (2003). *Diabetes* **52,** 1355–1363.
62. Yamauchi, T., Kamon, J., Ito, Y., et al. (2003). *Nature* **423,** 762–769.
63. Kadowaki, T., Hara, K., Yamauchi, T., Terauchi, Y., Tobe, K., and Nagai, R. (2003). *Exp. Biol. Med.* **228,** 1111–1117.
64. Hotta, K., Funahashi, T., Arita, Y., et al. (2000). *Arterioscler. Thromb. Vasc. Biol.* **20,** 1595–1599.
65. Weyer, C., Funahashi, T., Tanaka, S., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1930–1935.
66. Yang, W. S., Lee, W. J., Funahashi, T., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 3815–3819.
67. Kappes, K. and Loffler, G. (2000). *Hormone Metab. Res.* **32,** 548–554.
68. Masaki, T., Chiba, S., Tatsukawa, H., et al. (2004). *Hepatology* **40,** 177–184.
69. Kumada, M., Kihara, S., Ouchi, N., et al. (2004). *Circulation* **109,** 2046–2049.
70. Wolf, A. M., Wolf, D., Rumpold, H., Enrich, B., and Tilg, H. (2004). *Biochem. Biophys. Res. Commun.* **323,** 630–635.
71. Wulster-Radcliffe, M. C., Ajuwon, K. M., Wang, J., Christian, J. A., and Spurlock, M. R. (2004). *Biochem. Biophys. Res. Commun.* **316,** 924–929.
72. Von Eynatten, M., Schneider, J. G., Humpert, P. M., et al. (2004). *Diabetes Care* **27,** 2925–2929.
73. Bajaj, M., Suraamornkul, S., Piper, P., et al. (2004). *J. Clin. Endocrinol. Metab.* **89,** 200–206.
74. Rea, S. and Donnelly, R. (2004). *Diabetes Obes. Metab.* **6,** 163–170.
75. Steppan, C. M., Bailey, S. T., Bhat, S., et al. (2001). *Nature* **409,** 307–312.
76. Rajala, M. W., Obici, S., Scherer, P. E., and Rossetti, L. (2003). *J. Clin. Invest.* **111,** 225–230.
77. Muse, E. D., Obici, S., Bhanot, S., et al. (2004). *J. Clin. Invest.* **114,** 232–239.
78. Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., et al. (2004). *Science* **303,** 1195–1198.
79. Verma, S., Li, S. H., Wang, C. H., et al. (2003). *Circulation* **108,** 736–740.
80. Calabro, P., Samudio, I., Willerson, J. T., and Yeh, E. T. (2004). *Circulation* **110,** 3335–3340.
81. Sato, N., Kobayashi, K., Inoguchi, T., et al. (2005). *Endocrinology* **146,** 273–279.
82. Satoh, H., Nguyen, M. T., Miles, P. D., Imamura, T., Usui, I., and Olefsky, J. M. (2004). *J. Clin. Invest.* **114,** 224–231.
83. Banerjee, R. R. and Lazar, M. A. (2003). *J. Mol. Med.* **81,** 218–226.
84. Kielstein, J. T., Becker, B., Graf, S., Brabant, G., Haller, H., and Fliser, D. (2003). *Am. J. Kidney Dis.* **42,** 62–66.
85. Fain, J. N., Cheema, P. S., Bahouth, S. W., and Lloyd Hiler, M. (2003). *Biochem. Biophys. Res. Commun.* **300,** 674–678.
86. Steppan, C. M., Brown, E. J., Wright, C. M., et al. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 502–506.
87. Lehrke, M., Reilly, M. P., Millington, S. C., Iqbal, N., Rader, D. J., and Lazar, M. A. (2004). *PLoS* **1,** 45.
88. Gearing, A. J., Beckett, P., Christodoulou, M., et al. (1994). *Nature* **370,** 555–557.
89. Uysal, K. T., Wiesbrock, S. M., Marino, M. W., and Hotamisligil, G. S. (1997). *Nature* **389,** 610–614.
90. Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993). *Science* **259,** 87–91.
91. Hotamisligil, G. S. (1999). *J. Intern. Med.* **245,** 621–625.
92. Wang, B., Jenkins, J. R., and Trayhurn, P. (2005). *Am. J. Physiol. Endocrinol. Metab.* **288,** E731.
93. Ruan, H., Hacohen, N., Golub, T. R., Van Parijs, L., and Lodish, H. F. (2002). *Diabetes* **51,** 1319–1336.
94. Green, A., Dobias, S. B., Walters, D. J., and Brasier, A. R. (1994). *Endocrinology* **134,** 2581–2588.
95. Zhang, H. H., Halbleib, M., Ahmad, F., Manganiello, V. C., and Greenberg, A. S. *Diabetes* 2002, **51,** 2929–2935.
96. Grunfeld, C. and Feingold, K. R. (1992). *Proc. Soc. Exp. Bio. Med.* **200,** 224–227.
97. Beutler, B., Greenwald, D., Hulmes, J. D., et al. (1985). *Nature* **316,** 552–554.
98. Peraldi, P., Hotamisligil, G. S., Buurman, W. A., White, M. F., and Spiegelman, B. M. (1996). *J. Biol. Chem.* **271,** 13018–13022.
99. Hotamisligil, G. S., Peraldi, P., Budavari, A., Ellis, R., White, M. F., and Spiegelman, B. M. (1996). *Science* **271,** 665–668.
100. Moller, D. E. (2000). *Trends Endocrinol. Metab.* **11,** 212–217.
101. Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., and Spiegelman, B. M. (1995). *J. Clin. Invest.* **95,** 2409–2415.
102. Tsigos, C., Kyrou, I., Chala, E., et al. (1999). *Metabolism* **48,** 1332–1335.

103. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. Jr. (2003). *J. Clin. Invest.* **112,** 1796–1808.
104. Cheung, A. T., Ree, D., Kolls, J. K., Fuselier, J., Coy, D. H., and Bryer-Ash, M. (1998). *Endocrinology* **139,** 4928–4935.
105. Ofei, F., Hurel, S., Newkirk, J., Sopwith, M., and Taylor, R. (1996). *Diabetes* **45,** 881–885.
106. Gross, V., Andus, T., Castell, J., Vom Berg, D., Heinrich, P. C., and Gerok, W. (1989). *FEBS Lett.* **247,** 323–326.
107. Mohamed-Ali, V., Goodrick, S., Rawesh, A., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 4196–4200.
108. Kern, P. A., Ranganathan, S., Li, C., and Ranganathan, G. (2001). *Am. J. Physiol. Endocrinol. Metab.* **280,** E745–E751.
109. Vozarova, B., Weyer, C., Hanson, K., Tataranni, P. A., Borgardus, C., and Pratley, R. E. (2001). *Obes. Res.* **9,** 414–417.
110. Senn, J. J., Klover, P. J., Nowak, I. A., et al. (2003). *J. Biol. Chem.* **278,** 13740–13746.
111. Senn, J. J., Klover, P. J., Nowak, I. A., and Mooney, R. A. (2002). *Diabetes* **51,** 3391–3399.
112. Fasshauer, M., Kralisch, S., Klier, M., et al. (2003). *Biochem. Biophy. Res. Commun.* **301,** 1045–1050.
113. Wallenius, K., Wallenius, V., Sunter, D., Dickson, S. L., and Jansson, J.-O. (2002). *Biochem. Biophys. Res. Commun.* **293,** 560–565.
114. Wallenius, V., Wallenius, K., Ahren, B., et al. (2002). *Nat. Med.* **8,** 75–79.
115. Di Gregorio, G. B., Hensley, L., Lu, T., Ranganathan, G., and Kern, P. A. (2004). *Am. J. Physiol. Endocrinol. Metab.* **287,** E182–E187.
116. Van Hall, G., Steensberg, A., Sacchetti, M., et al. (2003). *J. Clin. Endocrinol. Metab.* **88,** 3005–3010.
117. Petersen, E. W., Carey, A. L., Sacchetti, M., et al. (2005). *Am. J. Physiol. Endocrinol. Metab.* **288,** E155–E162.
118. Tsigos, C., Papanicolaou, D. A., Kyrou, I., Defensor, R., Mitsiadis, C. S., and Chrousos, G. P. (2001). *J. Clin. Endocrinol. Metab.* **82,** 4167–4170.
119. Heinrich, P. C., Castell, J. V., and Andus, T. (1990). *Biochem. J.* **265,** 621–636.
120. Pepys, M. B. and Hirschfield, G. M. (2003). *J. Clin. Invest.* **111,** 1805–1812.
121. Tham, D. M., Martin-McNulty, B., Wang, Y. X., et al. (2002). *Physiol. Genomics* **11,** 21–30.
122. Verma, S. and Anderson, T. J. (2002). *Circulation* **105,** 546–549.
123. Emanueli, C. and Madeddu, P. (2002). *Hypertension* **39,** 29.
124. Massiera, F., Bloch-Faure, M., Ceiler, D., et al. (2001). *FASEB J.* **15,** 2727–2729.
125. Massiera, F., Seydoux, J., Geloen, A., et al. (2001). *Endocrinology* **142,** 5220–5225.
126. Dellas, C. and Loskutoff, D. J. (2005). *Thromb. Haemost.* **93,** 631–640.
127. Loskutoff, D. J. and Samad, F. (1998). *Arterioscler. Thromb. Vasc. Biol.* **18,** 16.
128. Kohler, H. P. and Grant, P. J. (2000). *N. Engl. J. Med.* **342,** 1792–1801.
129. Crandall, D. L., Busler, D. E., McHendry-Rinde, B., Groeling, T. M., and Kral, J. G. (2000). *J. Clin. Endocrinol. Metab.* **85,** 2609–2614.
130. Loskutoff, D. J., Fujisawa, K., and Samad, F. (2000). *Ann. NY Acad. Sci.* **902,** 272–281.
131. Alessi, M. C., Peiretti, F., Morange, P., Henry, M., Nalbone, G., and Juhan-Vague, I. (1997). *Diabetes* **46,** 860–867.
132. Festa, A., D'Agostino, R. Jr., Mykkanen, L., et al. (1999). *Arterioscler. Thromb. Vasc. Biol.* **19,** 562–568.
133. Meigs, J. B., Mittleman, M. A., Nathan, D. M., et al. (2000). *JAMA* **283,** 221–228.
134. Aso, Y., Matsumoto, S., Fujiwara, Y., Tayama, K., Inukai, T., and Takemura, Y. (2002). *Metabolism* **51,** 471–476.
135. Gurlek, A., Bayraktar, M., and Kirazli, S. (2000). *Diabetes Care* **23,** 88–92.
136. Festa, A., D'Agostino, R. Jr., Tracy, R. P., and Haffner, S. M. (2002). *Diabetes* **51,** 1131–1137.
137. Morange, P. E., Lijnen, H. R., Alessi, M. C., Kopp, F., Collen, D., and Juhan-Vague, I. (2000). *Arterioscler. Thromb. Vasc. Biol.* **20,** 1150–1154.
138. Schafer, K., Fujisawa, K., Konstantinides, S., and Loskutoff, D. J. (2001). *FASEB J.* **15,** 1840–1842.
139. Lijnen, H. R., Maquoi, E., Morange, P., et al. (2003). *Arterioscler. Thromb. Vasc. Biol.* **23,** 78–84.
140. Ma, L.-J., Mao, S.-L., Taylor, K. L., et al. (2004). *Diabetes* **53,** 336–346.
141. Cianflone, K. (1997). *J. Endocrinol.* **155,** 203–206.
142. Koisiten, H. A., Vidal, H., Karonen, S. L., et al. (2001). *Arterioscler. Thromb. Vasc. Biol.* **21,** 1034–1039.
143. Murray, I., Sniderman, A. D., and Cianflone, K. (1999). *J. Lipid. Res.* **40,** 1671–1676.
144. Murray, I., Havel, P. J., Sniderman, A. D., and Cianflone, K. (2000). *Endocrinology* **141,** 1041–1049.
145. Xia, Z., Sniderman, A. D., and Cianflone, K. (2002). *J. Biol. Chem.* **277,** 45874–45879.
146. Wetsel, R. A., Kildsgaard, J., Zsigmond, E., Liazo, W., and Chan, L. (1999). *J. Biol. Chem.* **274,** 19429–19433.
147. Cianflone, K., Zakarian, R., Couillard, C., Delplanque, B., Despres, J. P., and Sniderman, A. (2004). *J. Lipid Res.* **45,** 124–131.
148. Xia, Z. and Cianflone, K. (2003). *Metabolism* **52,** 1360–1366.
149. Rustan, A. C. and Drevon, C. A. (2000). In: *Encyclopedia of life sciences.* Nature Publishing: London.
150. Holm, C., Osterlund, T., Laurell, H., and Contreras, J. A. (2000). *Annu. Rev. Nutr.* **20,** 365–393.
151. Londos, C., Brasaemle, D. L., Schultz, C. J., Segrest, J. P., and Kimmel, A. R. (1999). *Semin. Cell Dev. Biol.* **10,** 51–58.
152. Shakur, Y., Holst, L. S., Landstrom, T. R., Movsesian, M., Degerman, E., and Manganiello, V. (2001). *Prog. Nucleic Acid Res. Mol. Biol.* **66,** 241–277.
153. Greenberg, A. S., Egan, J. J., Wek, S. A., Moos, M. C. Jr., Londos, C., and Kimmel, A. R. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 12035–12039.
154. Londos, C., Brasaemle, D. L., Schultz, C. J., et al. (1999). *Ann. NY Acad. Sci.* **892,** 155–168.
155. Shulman, G. I. (2004). *Physiology* **19,** 183–190.
156. Itani, S. I., Ruderman, N. B., Schmieder, F., and Boden, G. (2002). *Diabetes* **51,** 2005–2011.
157. Dresner, A., Laurent, D., Marcucci, M., et al. (1999). *J. Clin. Invest.* **103,** 253–259.
158. Griffin, M. E., Marcucci, M. J., Cline, G. W., et al. (1999). *Diabetes* **48,** 1270–1274.
159. Boden, G. and Shulman, G. I. (2002). *Eur. J. Clin. Invest.* **32,** 14–23.
160. Szczepaniak, L. S., Babcock, E. E., Schick, F., et al. (1999). *Am. J. Physiol. Endocrinol. Metab.* **276,** E977–E989.