

Adipose tissue and adipokines: for better or worse

M Guerre-Millo

SUMMARY

It is now recognized that the white adipose tissue (WAT) produces a variety of bioactive peptides, collectively termed "adipokines". Alteration of WAT mass in obesity or lipoatrophy, affects the production of most adipose secreted factors. Since both conditions are associated with multiple metabolic disorders and increased risk of cardiovascular diseases, the idea has emerged that WAT could be instrumental in these complications, by virtue of its secreted factors. Several adipokines are increased in the obese state and have been implicated in hypertension (angiotensinogen), impaired fibrinolysis (PAI-1) and insulin resistance (ASP, TNF α , IL-6, resistin). Conversely, leptin and adiponectin both exert an insulin-sensitizing effect, at least in part, by favoring tissue fatty-acid oxidation through activation of AMP-activated kinase. In obesity, insulin resistance has been linked to leptin resistance and decreased plasma adiponectin. In lipoatrophic mice, where leptin and adiponectin circulating levels are low, administration of the two adipokines synergistically reverses insulin resistance. Leptin and adiponectin also have distinct properties: leptin, as a long-term integrative signal of energy store and adiponectin, as a potent anti-atherogenic agent. The thiazolidinedione anti-diabetic drugs increase endogenous adiponectin production in rodents and humans, supporting the idea that the development of new drugs targeting adipokines might represent a promising therapeutic approach to protect obese patients from insulin resistance and atherosclerosis.

Key-words: Adipose tissue · Obesity · Secretion · Insulin resistance · Thiazolidinediones.

Guerre-Millo M. Adipose tissue and adipokines: for better or worse
Diabetes Metab 2004,30,13-9

RÉSUMÉ

Tissu adipeux et adipokines : pour le meilleur et pour le pire

Il est maintenant reconnu que le tissu adipeux blanc (TAB) produit une variété de peptides bioactifs, dénommés « adipokines ». Les altérations de la masse du TAB au cours de l'obésité ou des lipoatrophies affectent la production de la plupart des facteurs adipeux sécrétés. Dans la mesure où ces deux affections sont associées avec de multiples anomalies métaboliques et un risque cardiovasculaire accru, l'idée a émergé que le TAB participerait à ces complications, par l'intermédiaire de ses facteurs sécrétés. Plusieurs adipokines sont augmentées au cours de l'obésité et ont été impliquées dans l'hypertension (angiotensinogène), l'altération de la fibrinolyse (PAI-1) et l'insulinorésistance (ASP, TNF α , IL-6, résistine). Inversement, la leptine et l'adiponectine exercent un effet insulinosensibilisant, au moins en partie en favorisant l'oxydation des acides gras par activation de l'AMP-activated kinase. Dans l'obésité, l'insulinorésistance a été reliée à une résistance à la leptine et une diminution de l'adiponectine plasmatique. Chez la souris lipoatrophique où les taux circulants de leptine et d'adiponectine sont bas, l'administration des deux adipokines lève de façon synergique l'insulinorésistance. Leptine et adiponectine ont aussi des propriétés distinctes : la leptine, comme signal intégrateur à long terme des stocks énergétiques, et l'adiponectine, comme puissant agent anti-athérogène. Les substances antidiabétiques de type thiazolidinedione augmente la production endogène d'adiponectine chez le rongeur et l'humain, en faveur de l'idée selon laquelle le développement de nouvelles molécules ciblant les adipokines pourrait offrir une approche thérapeutique séduisante pour la protection des obèses de l'insulinorésistance et de l'athérosclérose.

Mots-clés : Tissu adipeux · Obésité · Sécrétion · Insulinorésistance · Thiazolidinediones.

Address correspondence and reprint requests to:

M Guerre-Millo. U 465 INSERM, Centre de Recherche des Cordeliers, Université Pierre et Marie Curie, 15, rue de l'École de Médecine, 75006 Paris France.
mguerre@bhd.c.jussieu.fr

Received: August 1st, 2003

U 465 INSERM, Centre de Recherche des Cordeliers, Université Pierre et Marie Curie, 15, rue de l'École de Médecine, 75006 Paris France.

In recent years, it has been recognized that adipose tissue (WAT) secretes a number of bioactive peptides and proteins, collectively termed "adipokines". These WAT-derived factors play a central role in whole body homeostasis by influencing a variety of biological and physiological processes, including food intake, regulation of energy balance, insulin action, lipid and glucose metabolism, angiogenesis and vascular remodeling, regulation of blood pressure and coagulation. The present review is focused on a restricted number of adipokines, which have been implicated in vascular (angiotensinogen, PAI-1) and energy and glucose homeostasis (ASP, TNF α , IL-6, resistin, leptin, adiponectin).

As a secretory tissue, WAT displays several unusual characteristics. First, instead of being confined at a specific location, WAT is found throughout the whole organism in individual pads that are not physically connected. How adipose secretion is coordinated between depots, through humoral or nervous pathways, is still unclear. Second, WAT is constituted of distinct cell types, including mature adipocytes, pre-adipocytes, fibroblasts and macrophages, all of which participate, to a greater or lesser extent, in WAT secretory function. Third, WAT is heterogeneous in term of metabolic capacities, depending upon the visceral or subcutaneous localization of fat depots. Similarly, certain depots might contribute more actively than others to the production of specific adipokines [1, 2]. Fourth, some adipokines are also secreted by non-adipose tissues and thus it is not always straightforward to determine the specific contribution of WAT to circulating levels of these factors. Finally, little is known regarding the molecular mechanisms involved in the biosynthesis and exocytosis of adipokines, although there is evidence that regulated and constitutive secretory pathways exist in adipose cells [3].

It is well known that WAT mass increases greatly in obesity or, conversely, substantially shrinks in lipoatrophic syndromes. The amounts of triglyceride stored within individual adipose cells is a major determinant of WAT weight. Virtually all known adipokines are markedly dysregulated in response to alteration of WAT mass, although the molecular link between the size of adipose stores and the rate of production a specific adipokine remains, in most cases, largely speculative. Both obesity and lipoatrophy are pathologic conditions highly associated with metabolic disorders, including hyperlipidemia, insulin resistance and type 2 diabetes (T2DM), and with increased risk of cardiovascular diseases. Thus, the idea has emerged that WAT might be instrumental in the pathogenesis of these complications, by virtue of its secreted factors. This review will discuss the arguments implicating adipokines in disease states linked to altered WAT mass, which provide a rationale for therapeutic strategies targeting adipose secreted factors in obesity or lipodystrophy.

Adipokines and vascular homeostasis

Angiotensinogen

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 angiotensinogen (AGE), the precursor of the vasoactive peptide angiotensin II. Although AGE is mainly produced by the liver, WAT is considered a major extrahepatic source of AGE, which could contribute to increased circulating levels in obese individuals. The pathophysiological importance of WAT production was brought to light by genetic manipulations in mice, where the AGE gene was introduced specifically into WAT. In wild-type mice, overexpression of AGE mRNA in WAT resulted in elevated plasma AGE, hypertension, and increased WAT mass. When the same manipulation was performed in AGE-null mice, which are hypotensive and lean, re-expression of AGE mRNA in WAT was sufficient to restore WAT mass and normal blood pressure [4]. In addition, AGE-deficient mice are partially protected from diet-induced obesity [5]. As reviewed in detail in [6], these experimental models support the idea that production of AGE by WAT increases circulating levels in obese subjects, thereby favoring hypertension. Increased AGE production could also contribute to enhancement of WAT mass, an effect which has been attributed to angiotensin II acting locally as a trophic factor for new adipose cell formation [7].

PAI-1

Impairment of the fibrinolytic system participates into the cardiovascular complications of obesity. This defect has been linked to high concentrations of plasma plasminogen activator inhibitor 1 (PAI 1), a factor which is the primary physiological inhibitor of fibrinolysis. WAT is the main tissue source of elevated plasma PAI-1 in obesity, with a major participation of visceral fat [8, 9]. Cells of the stromal vascular fraction and pre-adipocytes, which are more numerous in visceral than in subcutaneous fat, contribute to PAI-1 production by human WAT [10]. The biological role of PAI-1 extends beyond regulation of fibrinolysis, since PAI-1 has been shown to influence cell migration and angiogenesis by competing with integrin binding on extracellular matrix vitronectin. In WAT, PAI-1 could impair pre-adipocyte migration and attachment to vitronectin, as suggested by its potent inhibitory effect on these processes *in vitro* [11]. Through this function, PAI-1 has the potential to affect WAT growth. Accordingly, overexpression of PAI-1 mRNA in WAT attenuates WAT hypertrophy in mice submitted to a high-fat diet [12]. Surprisingly, however, disruption of PAI-1 gene reduced adiposity in genetically obese mice [13] and had no significant effect on WAT mass in diet-induced obesity [14]. Altogether these observations sug-

gest that increased secretion of PAI-1 by WAT in obesity, although deleterious in the regulation of fibrinolysis, could also exert a protective effect against excessive WAT growth, at least in diet-induced obesity.

Adipokines and insulin resistance

Acylation-stimulating protein (ASP)

WAT is known to produce multiple proteins from the alternate complement pathway. Adipsin is a secreted serine protease related to complement factor D, which was originally discovered as a adipose differentiation-dependent factor [15]. In humans, WAT releases substantial amounts of ASP, a protein derived from the interaction of complement C3, factor B and adipsin [16]. ASP appears to be inactive as an immune modulator and its best recognized bioactivity is to stimulate triglyceride storage in adipose cells. At the cellular level, this effect is achieved through different processes: stimulation of glucose transport, enhancement of fatty-acid re-esterification and inhibition of lipolysis [17, 18]. However, the receptor and signaling pathways mediating ASP effects are as yet uncharacterized. Most, but not all studies in humans report substantial increases in plasma ASP in obese subjects, along with moderate overexpression of C3 mRNA in WAT. It is not known whether increased circulating levels of the protein reflect increased activity or resistance to ASP. Resistance to ASP could promote the redirection of fatty acid flux away from WAT, toward the liver [16]. Mice deleted for the C3 gene represent a model of ASP deficiency. A delay in post-prandial triglyceride clearance was observed in male C3-null mice on the 129Sv genetic background, but not in C57Bl/6-deleted mice [16]. This suggests that ASP is not the only mechanism that influences this process in mice. Whatever the strain, the absence of ASP resulted in a moderate reduction of WAT mass, both on standard and high fat diet, indicating decreased triglyceride storage. In addition, ASP-deficient mice appear to be more sensitive to insulin, although this could be the consequence of their relative leanness. [19].

TNF α

WAT produces a number of pro-inflammatory cytokines, which emanate from both adipose cells and cells of the stromal vascular fraction, in the absence of acute inflammation. This includes tumor necrosis factor α (TNF α) and several interleukins (IL) [20, 21]. TNF α was the first adipose secreted product proposed to represent a molecular link between obesity and insulin resistance [22, 23]. Indeed, TNF α is overexpressed in WAT in obesity and decreases with weight loss and improvement of insulin sensitivity. A number of studies have demonstrated that TNF α alters insulin signaling in cultured cells and *in vivo*. Anti-TNF α antibodies ameliorate insulin sensitivity in obese rodents and TNF α -deficient mice are protected from obesity-induced

insulin resistance on a high fat diet. Finally, TZDs repress TNF α gene expression in WAT and prevent TNF α -induced insulin resistance in the rat. However, there is no evidence that WAT is a net exporter of TNF α in humans, which suggests an autocrine-paracrine mode of action of the cytokine rather than an endocrine effect. Locally, TNF α increases PAI-1 and C3 gene expression and decreases adiponectin (see below) in WAT [24]. Thus, TNF α -regulated pathways in WAT may mediate, at least in part, the obesity-induced alteration in circulating levels of certain adipokines.

IL-6

Human WAT produces substantial amounts of IL-6 and this secretion might represent 10-30% of circulating levels [21]. Plasma IL-6 is highly correlated with body mass and inversely related to insulin sensitivity [25, 26]. Recent data suggest that IL-6 plays a direct role in insulin resistance by altering insulin signaling in hepatocytes [27]. This effect is mediated by the induction of SOCS-3 (suppressor of cytokine signaling-3) which inhibits insulin-dependent insulin receptor autophosphorylation [28]. Paradoxically, IL-6-deficient mice developed mature-onset obesity associated with glucose intolerance [29]. Moreover, intracerebroventricular administration of the cytokine decreases body fat in rats [30]. 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.

Resistin

A new-comer to the dark side of adipose secretion is resistin [31], also known as ADSF for "ADipose tissue-specific Secretory Factor" [32]. This adipokine belongs to a family of cystein-rich secreted proteins named FIZZ (found in inflammatory zone), now renamed Retn. In the initial report of Steppan [31], immunodetected resistin was increased in plasma of mice with diet-induced and genetic forms of obesity. Later on, however, mRNA levels were reported to be decreased in WAT of obese rodents [33, 34]. Recombinant resistin promoted systemic insulin resistance in mice and decreased insulin-stimulated glucose transport in adipose cells, while an anti-resistin antibody produced the opposite effect [31]. More recently, infusion of resistin in the rat was shown to induce severe hepatic insulin resistance, accounted for by an increased rate of glucose production [35]. Although resistin was originally cloned on the criteria of its expression being reduced by TZDs, treatment of animals with insulin-sensitizing drugs have produced inconsistent pattern of resistin regulation. Finally, a human homolog of murine resistin has been identified. However, since its sequence and expression in WAT are quite different from that in rodent, it is not yet clear whether this protein plays a significant role in the development of insulin resistance in humans [36]. Identification of the receptor and signaling pathways and analysis

of the phenotypes resulting from deletion or overexpression of resistin in transgenic mice, will help to further define the biological roles of this adipokine.

Adipokines and insulin sensitivity

Leptin

The discovery of leptin as a crucial factor involved in long-term regulation of food intake, body weight, energy expenditure and neuroendocrine functions has significantly broadened our understanding of the mechanisms underlying the development of obesity and its complications. The tremendous amount of information currently available on leptin has been extensively reviewed and only some important elements will be summarized here.

Demonstration of the role of leptin in body weight homeostasis was provided by a mutation (*ob*) which occurred spontaneously in mice more than 50 years ago. Since then, *ob/ob* mice have been bred in animal facilities allowing the discovery of the leptin [37] and leptin receptor genes [38]. The potent effect of recombinant leptin to reduce food intake, body weight and WAT 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. Similarly, three massively obese children with no functional leptin, are currently successfully treated with leptin [39]. By contrast, rodents and three known individuals carrying mutations in the leptin receptor gene are resistant to the food-reducing effect of leptin. However, the fact that adipose leptin production is increased in obese individuals, except in leptin-deficient subjects, led to the concept of leptin resistance. The molecular basis of leptin resistance, apart from mutations in the receptor gene, are yet to be determined. Adenoviral or transgenic overexpression the leptin gene reduced food intake and body weight in rodents. Attempts to obtain the same effect in humans through daily administration of recombinant leptin were frustrating, since only very high doses of leptin induced a reduction of body weight in a subset of individuals [40]. Thus, although leptin is absolutely necessary for body homeostasis, increasing circulating leptin is not the panacea for common obesity.

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. 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 [41, 42]. 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 [43]. 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. Although of little help in leptin resistant obese patients, leptin administration has been proposed as a new treatment to ameliorate insulin sensitivity in lipotrophic diabetes, where low leptin levels prevail [44, 45]

Adiponectin

In the mid nineties, an adipose-secreted protein with homology with the complement factor C1q, was cloned independently by different groups and named adipoQ, Acrp 30, apM1, GBP28 or adiponectin. Adiponectin mRNA is highly expressed in adipose cells and the protein circulates at high concentrations in primates and rodents. Structurally, it consists of a collagenous tail and a globular head, which form trimer-dimers and high molecular weight complexes in the circulation. Different properties have been ascribed to various recombinant or processed forms (globular head) of the protein and the actual bioactive form(s) has not yet been unequivocally determined [46].

In sharp contrast to most adipokines, adiponectin expression and serum concentrations are not increased but reduced in a variety of obese and insulin-resistant states. In rhesus monkeys, plasma adiponectin decreases along with the development of insulin resistance associated with obesity and aging [47]. Similarly two case-control studies, in the obesity-prone Pima Indians and in the general population, suggest that individuals with high adiponectin concentrations are less likely to develop type 2 diabetes than those with low concentrations [48, 49]. Nutritional and therapeutic manipulations that ameliorate insulin sensitivity, like weight loss, caloric restriction and TZDs treatment, all increase adiponectin gene expression in WAT and plasma levels [50-54]. The stimulatory effect of TZDs is mediated *via* activation of the heterodimer PPAR γ /retinoid X receptor which binds to a PPAR responsive element (PPRE) present in the human adiponectin promoter [55]. Conversely, TNF α and IL-6 are potent inhibitors of adiponectin expression and secretion in human WAT biopsies or cultured adipose cells [53, 56]. This suggests that TNF α and IL-6-induced insulin resistance might rely, in part, on an autocrine-paracrine inhibition of adiponectin release.

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 [57, 58]. In addition, adiponectin has anti-atherogenic properties, as shown by its capacity to inhibit monocyte adhesion to endothelial cells and the macrophage-to foam-cell transformation *in vitro* [59, 60]. The phenotype of adiponectin-null mice confirmed the protective role of the protein against atherosclerosis and diet-

induced insulin resistance [61, 62], although in one study, adiponectin-null mice did not show aggravated insulin resistance on high fat diet as compared to wild-type mice [63]. 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 [64]. This suggests that adiponectin and leptin may work hand in hand to sensitize peripheral tissues to insulin. However, the two adipokines have both overlapping and distinct functions, since globular adiponectin ameliorates insulin resistance but not obesity in the *ob/ob* leptin-deficient mice [65].

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 [66, 67], similar to the action of leptin. Moreover, adiponectin also activates AMPK in the liver, resulting in reduced rate of hepatic glucose production [66, 68] and in isolated rat adipose cells, thereby increasing glucose uptake [69]. Although the signaling pathways evoked by adiponectin are not fully deciphered, two receptors have been recently cloned, Adipo R1 and Adipo R2, that are expressed predominantly in muscles and liver, respectively. These receptors are predicted to contain seven transmembrane domains, but do not seem to be coupled with G-protein [70].

Additional adipokines?

Finally, the list of adipokines influencing insulin sensitivity might not be complete. A clue in favor of this hypothesis was given recently by the outcome of knocking out the insulin sensitive glucose transporter GLUT 4 selectively in WAT in mice. As expected, these transgenic mice display markedly reduced rate of insulin-stimulated glucose uptake in WAT. Unexpectedly, however, they also developed insulin resistance in muscles (despite preservation of GLUT 4) and in liver *in vivo* [71]. This suggests that an adipose secreted factor capable of modulating insulin action in muscles and liver is altered when glucose utilization is reduced in the adipose cells. To our knowledge, this factor has not yet been determined. The development of molecular techniques of mass screening for new genes and proteins will undoubtedly contribute to the identification of new adipose secreted products.

Conclusion

Through its secretory function, WAT lies at the heart of a complex network of factors capable of influencing several physiological processes. Some adipokines like leptin, and adiponectin exert a beneficial effect on energy balance, insu-

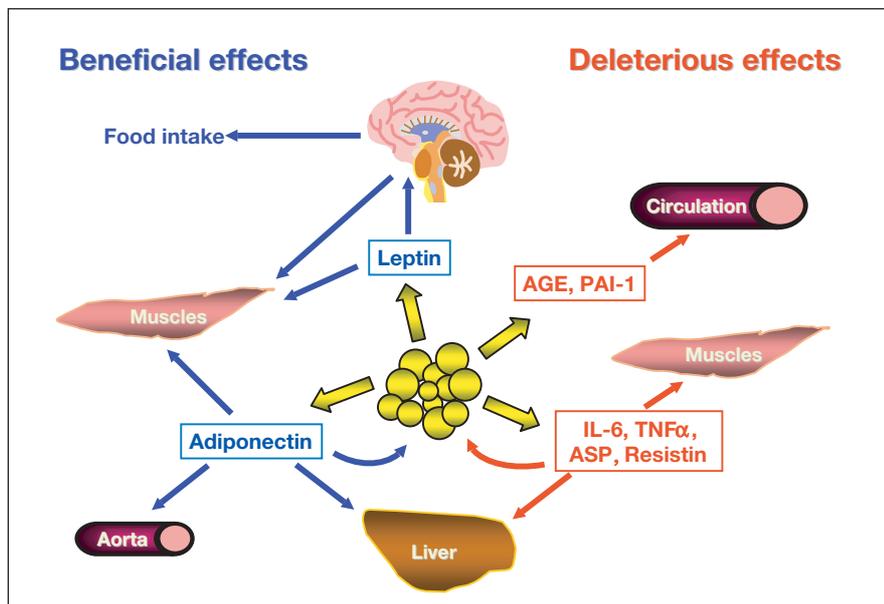


Figure 1

Adipose tissue secreted factors implicated in energy homeostasis, insulin sensitivity and vascular homeostasis.

Adipose tissue (WAT) secretes a number of bioactive peptides collectively termed "adipokines". Through its secretory function, WAT lies at the heart of a complex network capable of influencing several physiological processes. Dysregulation of adipokines production with alteration of WAT 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 (AGE) and PAI-1 secretion favors hypertension and impaired fibrinolysis. Leptin regulates energy balance and exerts an insulin-sensitizing effect. These beneficial effects are reduced in obesity due to leptin resistance. Adiponectin increases insulin action in muscles and liver and exerts an anti atherogenic effect. Adiponectin is the only known adipokine whose circulating levels are decreased in the obese state. The thiazolidinedione anti-diabetic drugs increase plasma adiponectin, supporting the idea that adipokine-targeted pharmacology represents a promising therapeutic approach to control type 2 diabetes and cardiovascular diseases in obesity.

lin action and vasculature. Conversely, excessive production of other adipokines is deleterious. For example, TNF α , IL-6 or resistin might deteriorate insulin action, while angiotensinogen and PAI-1 are likely to participate to the vascular complications linked to obesity (Fig 1). 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 WAT mass dysregulation, such as lipodystrophy and obesity.

References

- Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab*, 1998, 83, 847-50.
- Dusserre E, Moulin P, Vidal H. Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. *Biochim Biophys Acta*, 2000, 1500, 88-96.
- Mora S, Pessin JE. An adipocentric view of signaling and intracellular trafficking. *Diabetes Metab Res Rev*, 2002, 18, 345-56.
- Massiera F, Bloch-Faure M, Ceiler D, *et al.* Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J*, 2001, 15, 2727-9.
- Massiera F, Seydoux J, Geloan A, *et al.* Angiotensinogen-deficient mice exhibit impairment of diet-induced weight gain with alteration in adipose tissue development and increased locomotor activity. *Endocrinology*, 2001, 142, 5220-5.
- Ailhaud G, Fukamizu A, Massiera F, Negrel R, Saint-Marc P, Teboul M. Angiotensinogen, angiotensin II and adipose tissue development. *Int J Obes Relat Metab Disord*, 2000, 24, S33-S35.
- Saint-Marc P, Kozak LP, Ailhaud G, Darimont C, Negrel R. Angiotensin II as a trophic factor of white adipose tissue: stimulation of adipose cell formation. *Endocrinology*, 2001, 142, 487-92.
- Mavri A, Stegnar M, Krebs M, Sentocnik JT, Geiger M, Binder BR. Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. *Arterioscler Thromb Vasc Biol*, 1999, 19, 1582-7.
- Mavri A, Alessi MC, Bastelica D, *et al.* Subcutaneous abdominal, but not femoral fat expression of plasminogen activator inhibitor-1 (PAI-1) is related to plasma PAI-1 levels and insulin resistance and decreases after weight loss. *Diabetologia*, 2001, 44, 2025-31.
- Bastelica D, Morange P, Berthet B, *et al.* Stromal cells are the main plasminogen activator inhibitor-1-producing cells in human fat: evidence of differences between visceral and subcutaneous deposits. *Arterioscler Thromb Vasc Biol*, 2002, 22, 173-78.
- Crandall DL, Busler DE, McHendry-Rinde B, Groeling TM, Kral JG. Autocrine regulation of human preadipocyte migration by plasminogen activator inhibitor-1. *J Clin Endocrinol Metab*, 2000, 85, 2609-14.
- Lijnen HR, Maquoi E, Morange P, *et al.* Nutritionally induced obesity is attenuated in transgenic mice overexpressing plasminogen activator inhibitor-1. *Arterioscler Thromb Vasc Biol*, 2003, 23, 78-84.
- Schafer K, Fujisawa K, Konstantinides S, Loskutoff DJ. Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. *FASEB J*, 2001, 15, 1840-2.
- Morange PE, Lijnen HR, Alessi MC, Kopp F, Collen D, Juhan-Vague I. Influence of PAI-1 on adipose tissue growth and metabolic parameters in a murine model of diet-induced obesity. *Arterioscler Thromb Vasc Biol*, 2000, 20, 1150-4.
- Cook KS, Min HY, Johnson D, *et al.* Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science*, 1987, 237, 402-5.
- Cianflone K, Xia Z, Chen LY. Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta*, 2003, 1609, 127-43.
- Cianflone K, Maslowska M, Sniderman AD. Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. *Semin Cell Dev Biol*, 1999, 10, 31-41.
- Van H, V, Reynisdottir S, Cianflone K, *et al.* Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. *J Biol Chem*, 1999, 274, 18243-51.
- Murray I, Havel PJ, Sniderman AD, Cianflone K. Reduced body weight, adipose tissue, and leptin levels despite increased energy intake in female mice lacking acylation-stimulating protein. *Endocrinology*, 2000, 141, 1041-9.
- Coppack SW. Pro-inflammatory cytokines and adipose tissue. *Proc Nutr Soc*, 2001, 60, 349-56.
- Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord*, 1998, 22, 1145-58.
- Hotamisligil GS. Molecular mechanisms of insulin resistance and the role of the adipocyte. *Int J Obes Relat Metab Disord*, 2000, 24, S23-S27.
- Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinol Metab*, 2000, 11, 212-7.
- Ruan H, Miles PD, Ladd CM, *et al.* Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor-alpha: implications for insulin resistance. *Diabetes*, 2002, 51, 3176-88.
- Bastard JP, Jardel C, Bruckert E, *et al.* Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab*, 2000, 85, 3338-42.
- Bastard JP, Maachi M, Van Nhieu JT, *et al.* Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *J Clin Endocrinol Metab*, 2002, 87, 2084-9.
- Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes*, 2002, 51, 3391-9.
- Senn JJ, Klover PJ, Nowak IA, *et al.* Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem*, 2003, 278, 13740-6.
- Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL *et al.* Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med*, 2002, 8, 75-79.
- Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun*, 2002, 293, 560-5.
- Steppan CM, Bailey ST, Bhat S, *et al.* The hormone resistin links obesity to diabetes. *Nature*, 2001, 409, 307-12.
- Kim KH, Lee K, Moon YS, Sul HS. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. *J Biol Chem*, 2001, 276, 11252-6.
- Way JM, Gorgun CZ, Tong Q, *et al.* Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem*, 2001, 276, 25651-3.

34. Le Lay S, Boucher J, Rey A, *et al.* Decreased resistin expression in mice with different sensitivities to a high-fat diet. *Biochem Biophys Res Commun*, 2001, 289, 564-7.
35. Rajala MW, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. *J Clin Invest*, 2003, 111, 225-30.
36. Savage DB, Sewter CP, Klenk ES, *et al.* Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes*, 2001, 50, 2199-202.
37. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 1994, 372, 425-32.
38. Tartaglia LA, Dembski M, Weng X, *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell*, 1995, 83, 1263-71.
39. Farooqi IS, Matarese G, Lord GM, *et al.* Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest*, 2002, 110, 1093-103.
40. Heymsfield SB, Greenberg AS, Fujioka K, *et al.* Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*, 1999, 282, 1568-75.
41. Shimabukuro M, Koyama K, Chen G, *et al.* Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci U S A*, 1997, 94, 4637-41.
42. Muoio DM, Dohm GL, Fiedorek FT, Jr., Tapscott EB, Coleman RA, Dohn GL. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes*, 1997, 46, 1360-3.
43. Minokoshi Y, Kim YB, Peroni OD, *et al.* Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, 2002, 415, 339-43.
44. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature*, 1999, 401, 73-6.
45. Ebihara K, Ogawa Y, Masuzaki H, *et al.* Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipotrophic diabetes. *Diabetes*, 2001, 50, 1440-8.
46. Pajvani UB, Du X, Combs TP, *et al.* Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem*, 2003, 278, 9073-85.
47. Hotta K, Funahashi T, Bodkin NL, *et al.* Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes*, 2001, 50, 1126-33.
48. Lindsay RS, Funahashi T, Hanson RL, *et al.* Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet*, 2002, 360, 57-8.
49. Spranger J, Kroke A, Mohlig M, *et al.* Adiponectin and protection against type 2 diabetes mellitus. *Lancet*, 2003, 361, 226-8.
50. Maeda N, Takahashi M, Funahashi T, *et al.* PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes*, 2001, 50, 2094-9.
51. Combs TP, Wagner JA, Berger J, *et al.* Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. *Endocrinology*, 2002, 143, 998-1007.
52. Yu JG, Javorschi S, Hevener AL, *et al.* The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes*, 2002, 51, 2968-74.
53. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A *et al.* Regulation of Adiponectin by Adipose Tissue-derived Cytokines: In vivo and in vitro Investigations in Humans. *Am J Physiol Endocrinol Metab*, 2003, Epub ahead of print.
54. Combs TP, Berg AH, Rajala MW, *et al.* Sexual Differentiation, Pregnancy, Calorie Restriction, and Aging Affect the Adipocyte-Specific Secretory Protein Adiponectin. *Diabetes*, 2003, 52, 268-76.
55. Iwaki M, Matsuda M, Maeda N, *et al.* Induction of Adiponectin, a Fat-Derived Antidiabetic and Antiatherogenic Factor, by Nuclear Receptors. *Diabetes*, 2003, 52, 1655-63.
56. Fasshauer M, Kralisch S, Klier M, *et al.* Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochem Biophys Res Commun*, 2003, 301, 1045-50.
57. Tsao TS, Lodish HF, Fruebis J. ACRP30, a new hormone controlling fat and glucose metabolism. *Eur J Pharmacol*, 2002, 440, 213-21.
58. Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab*, 2002, 13, 84-9.
59. Ouchi N, Kihara S, Arita Y, *et al.* Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*, 1999, 100, 2473-6.
60. Ouchi N, Kihara S, Arita Y, *et al.* Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*, 2001, 103, 1057-63.
61. Maeda N, Shimomura I, Kishida K, *et al.* Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med*, 2002, 8, 731-7.
62. Kubota N, Terauchi Y, Yamauchi T, *et al.* Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem*, 2002, 277, 25863-6.
63. Ma K, Cabrero A, Saha PK, *et al.* Increased beta -oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin. *J Biol Chem*, 2002, 277, 34658-61.
64. Yamauchi T, Kamon J, Waki H, *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nat Med*, 2001, 7, 941-6.
65. Yamauchi T, Kamon J, Waki H, *et al.* Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem*, 2003, 278, 24618.
66. Yamauchi T, Kamon J, Minokoshi Y, *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med*, 2002, 8, 1288-95.
67. Tomas E, Tsao TS, Saha AK, *et al.* Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A*, 2002, 99, 16309-13.
68. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest*, 2001, 108, 1875-81.
69. Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ. Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes*, 2003, 52, 1355-63.
70. Yamauchi T, Kamon J, Ito Y, *et al.* Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, 2003, 423, 762-9.
71. Abel ED, Peroni O, Kim JK, *et al.* Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature*, 2001, 409, 729-33.