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PROTEIN PIECES OF ADIPOSE TISSUE SECRETORY PUZZLE

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Over the past decade, the paradigm shift of adipose tissue as being far beyond its pivotal role in lipid and energy homeostasis has been increasingly recognized. Arguably, adipocytes as well as other adipose cells are at present considered bona fide *secretory cell types, using pleiocrine pathways for delivery of multiple signaling proteins designated adipokines. Transcriptomic and proteomic studies "upregulate" more than hundred adipokines that are synthesized, stored, and released by adipose tissue cells. However, the functional description of adipose-secreted proteins look like an incomplete puzzle. Here we describe only adipsin, adiponectin, leptin, resistin, visfatin, tumor necrosis factor-alpha, interleukin-6, plasminogen activator inhibitor type 1, nerve growth factor, brain-derived neurotrophic factor, and metallothioneins, and focus on their implications for the pathogenesis of various diseases besides obesity and related disorders. Accordingly, a horizon of the adipopharmacology of disease is outlined.* ***Biomed Rev 2007; 18: 27-43.***

Key words: adipokines, atherosclerosis, cardiometabolic diseases, inflammation, obesity

INTRODUCTION

Although the discovery of first adipose-derived endocrine factor, the serine protease adipsin, is traced back to 1987 (1), it was the discovery of leptin in 1994 (2) that centered many studies on the secretory function of adipose tissue, thus defining a new field of study, adipobiology: the study of the molecular and cellular biology of the normal and diseased adipose tissue and related disorders (3-13). These studies' results have indeed shifted the paradigm of adipose tissue from a simple energy storage to a major body's endocrine organ. The adipose-secreted products include an increasing number of signaling proteins, conceptually designated adipocytokines (14,15) or adipokines (3-13,16-20), the latter terminology being more accurate because not solely adipocytes, but even in a greater capacity, non-adipocytes of adipose tissue (such as matrix cells, stromovascular cells and associated macrophages and mast cells) are sources of adipose cytokines (adipokine) whereas adipocyte-derived cytokines (adipocytokines) should be considered members of the adipokine family of proteins, as defined from a cell-topological viewpoint. Functionally, adipokines are involved in the regulation of a wide range of biological processes far beyond lipid, glucose and energy metabolism.

In this review, we attempt to present the secretory nature of adipose tissue cells, and emphasis on the implications of

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adipokines in the pathogenesis of a large number of diseases. In addition to the many excellent reviews that have been written recently (3,5-20), including about perivascular adipose tissue (21-26) and obesity (27,28), the aim of our review is also to further describe how the knowledge of adipose tissue secretion may be explored in studying adipopharmacology of diseases, particularly cardiometabolic diseases such as atherosclerosis, hypertension, metabolic syndrome, obesity, type 2 diabetes mellitus, and lipodystrophy.

ADIPOSE TISSUE

In mammals including humans, there are two major subtypes of adipose tissue: white and brown adipose tissue, WAT and BAT, respectively (5,29-35). WAT has a couple of subdivisions, each with unique anatomic, metabolic and secretory properties: intra-abdominal or visceral and subcutaneous adipose tissue. Visceral adipose tissue is subdivided into intraperitoneal and retroperitoneal compartments. Intraperitoneal fat is itself composed of omental and mesenteric adipose tissues, comprising the majority of visceral fat. Importantly, in addition to the subcutaneous and visceral compartments, there are many small visceral depots associated with heart, blood vessels, major lymph nodes, ovaries, mammary glands, eyes, and bone marrow.

Although less developed in adult humans, BAT is recently also appreciated in adipobiology of disease (29-35). Along with its pivotal involvement in thermogenesis, a signature performance of brown adipocytes, white-to-brown adipocyte transdifferantiation may occur within the WAT. Likewise, various functions of WAT could be executed by brown adipose cells.

SECRETION BY ADIPOSE TISSUE CELLS

The endocrine role of adipose tissue was mentioned first in the late 1980's (1). Recent transcriptomic and proteomic analyses revealed that more than hundred secretory proteins are synthesized, stored, and released by adipose tissue including adiponectin, leptin, resistin, tumor necrosis factor-alpha (TNF-α), angiotensinogen, visfatin, adipsin, complement C3, complement B, acylation-stimulating protein, retinol-binding protein (RBP), interleukin (IL)-1β, IL-6, IL-8, IL-18, plasminogen activator inhibitor-1 (PAI-1), fasting-induced adipose factor, fibrinogen-angiopoietin-related protein, metallothionein-1-3, tissue factor (TF), haptoglobin, pigment epithelium-derived factor (PEDF), hippocampal cholinergic neurostimulating peptide, neutrophil gelatinase-associated lipocalin, and adiponutrin. Moreover, lipoprotein lipase (LPL), apolipoprotein E (apoE), cholesteryl ester transfer protein (CETP), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), hepatocyte growth factor (HGF), heparin-binding epidermal growth factor (HBEGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), monocyte chemoattractant protein (MCP)-1, apelin, zinc-alpha2 glycoprotein (also named lipid mobilizing factor), *agouti* protein and various extracellular matrix proteins including entactin/nidogen, collagen VIα3, fibronectin, and sulfatase 2, a member of the family of matrix endosulfatses that are able to remodel heparan sulphate proteoglycans (34), which altered function is implicated in diabetes (34 and references therein). These adipokines provide communication between adipose tissue and the rest of the body, and thus exerting many biological effects on various organs including the brain (27), immune cells (9,13,19,20), heart and blood vessels (14,15,21-26), liver, reproductive system, skeletal muscles, bone, and also related to the pathogenesis of many diseases (3-28).

Although WAT and BAT both express many of the same adipocyte-specific genes for lipid synthesis and hydrolysis as well as secreted adipokines, significance and impact shows differences according to the type of adipose tissue, including between visceral and subcutaneous WAT. For example, leptin is expressed in brown adipocytes but only under condition of inactivity and atrophy (30). Conditions associated with activation of BAT, such as cold, decreases the leptin gene expression, often down to undetectable levels, whereas inactivating conditions increases it. The expression is accordingly negatively regulated via β_3-adrenoceptors (35). Adiponectin is also expressed in both WAT and BAT and similarly to the case for leptin, its expression is diminished by adrenergic stimulation (35). Visfatin is detected in the plasma and its concentration correlates with intra abdominal fat mass but not with subcutaneous fat mass (36). Subcutaneous adipose tissue presents higher TNF-α expression than omental fat depots and omental fat produce 3-fold more IL-6 than subcutaneous adipose tissue (37). Analysis of WAT $mRNA^{adiponectin}$ and protein suggest that subcutaneous adipose tissue expresses higher levels of hormone in non-diabetics, while the expression is reduced in omental fat. However, the subcutaneous fat depot of diabetic patients expresses less adiponectin irrespective of adiposity.

Excess and deficiency of adipose tissue is a dilemma, and interestingly leads to similar consequences, which are associated with severe insulin resistance, hypertriglyceridemia,

atherosclerosis and hepatic steatosis (38-40). Thus, the adipocyte is emerging as a major drug target due to its central role in a vast array of pathophysiological processes that include obesity, diabetes, inflammation, coronary artery disease (CAD) and cancer.

DISSECTING ADIPOSE SECRETORY PUZZLE INTO PIECES

The first discovered piece: adipsin (complement factor D)

In 1987, adipsin, a serine protease/complement factor D, was the first described adipocyte-derived endocrine factor (1). Onwards, it has been shown that murine 3T3-F442A adipocytes secrete complements D, C3, and B, the three essential components of the alternative pathway (41,42). Based on the study of adipsin in mice, Choy *et al* (43) and Choy and Spiegelman (44) suggested that there might be a link between the activation of the alternative complement pathway and adipose tissue metabolism. The reports on genetic (*ob/ob*, *db/db* and *fa/fa*) and drug-induced obesity showed that $mRNA^{adipsin}$ levels in adipose tissue as well as plasma adipsin concentrations were reduced (45). Contrarily, in diet-induced obesity in mice and rats and in human obesity, the levels of adipsin increased in a positive correlated manner with the body mass index (BMI). It is possible that adipsin may influence adipose tissue lipid metabolism indirectly (46). However, the exact role of adipsin in adipose tissue still remains unclear.

In addition to adipsin, there are several adipose tissue-derived complement components that suggest previously unsuspected links between energy balance and immunity. It has been shown that complement components C2, C3, C4, C7, and Factor B had higher expression in omental compared with subcutaneous adipose tissue. In addition, adipsin and the classical pathway components C1QB, C1R, and C1S were expressed in both depots (47). The activation of the alternative complement pathway results in the interaction of complement C3 with activated factor B and the enzyme adipsin, cleavage of the protein C3 into C3a and C3b and, ultimately, desaggregation of C3a to produce ascylation-stimulating protein (ASP), an adipose tissue-secreted factor that influences the rate of triacylglycerol (TG) synthesis in adipose tissue (48). Also, ASP increases glucose transport through its effects on glucose transporter translocation (GLUT-1, GLUT-3 and GLUT-4) (46).

Of note, a collagenous repeat containing sequence of 26 kD protein (CORS-26), dubbed "cartonectin", was recently identified in cultured adipocytes (49).

The most abundant and pleiotropic piece: adiponectin

Adiponectin, also known as APM1 (Adipose most abundant gene transcript), adipocyte complement related protein of 30 kD (Acrp30), or adipoQ, is an adipose tissue-specific protein of 244 amino acids sharing significant similarity with collagens type VIII and type X, and complement protein C1q (hence the name adipoQ) (50,51). Interestingly, the three-dimensional structure of the C terminal globular domain of adiponectin has homology to that of TNF-α (52). However, the physiological effects of the two proteins are very different from each other, and some of the effects of TNF-α are in fact the opposite of those of adiponectin. In addition, adiponectin reduces the production and activity of TNF-α (6,19,53).

The discovery of adiponectin occurred at about the same time as the discovery of leptin (1994-1996), but it did not receive major attention in the scientific community those days. Nevertheless, to date, it is the most promising adipokine with a sincere potential for developing novel intervention strategies for various disorders (5,17,54). Adiponectin exerts pleiotropic beneficial effects including anti-inflammatory, anti-atherogenic, anti-diabetic**,** and anti-fibrotic. The cloning of two adiponectin receptors, AdipoR1 and AdipoR2, was recently reported (55-61). AdipoR1 is abundantly expressed in muscle, whereas AdipoR2 is predominantly expressed in the liver. It has been shown that there is a fat depot specific regulation of AdipoR1 and AdipoR2 gene expression by fasting in both WAT and BAT. In addition, it has been identified a coordinated circadian pattern of AdipoR1 and AdipoR2 gene expression in these tissues. The expressions of AdipoR1/R2 in insulin target tissues appear to be inversely correlated with plasma insulin levels. Insulin negatively regulates the expression levels of adiponectin receptors via the PI3-kinase/Foxo1 pathway. The data also suggest that not only the agonism of AdipoR1/R2 but also strategies to increase AdipoR1/R2 may be logical approaches to provide a novel treatment modality for type 2 diabetes, inflammation (60-65) and liver cirrhosis (66,67)**.** Because decreased levels of adiponectin may contribute to several severe complications in type 2 diabetic individuals. For instance, chronic treatment with recombinant adiponectin increases insulin sensitivity in insulin resistant mice (61-63), whereas treatment with thiazolidinedione (TZD) anti-diabetic drugs, ligands of the nuclear transription factor peroxisome proliferator-activated receptor-gamma (PPARγ), also results in an enhanced adiponectin secretion (64). Notably, adiponectin is one of the major endogenous insulin sensitizing factor.

Besides this function, recent evidence supports adiponectin's anti-inflammatory and anti-atherogenic actions. Anti-inflammatory activity of adiponectin is mediated by inhibition of a pro-inflammatory cascade involving TNF-α and IL-6 and/or by induction of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (61,65). These effects are paralleled by specific effects on endothelial cell functions including inhibition of TNF-α-stimulated adhesion of monocytes on endothelial cells (57).

As indicated above (66,67), adiponectin also exerts anti-inflammatory and anti-fibrotic effects in the liver, and its deficiency might be related to liver disease progression, for example, in non-alcoholic steatohepatitis. An anti-fibrotic effect of adiponectin has been suggested on the basis of AdipoR gene expression in hepatic stellate (Ito's) cells, and the inhibition of these cell proliferation and migration after adiponectin treatment.

Adiponectin suppresses TNF-α–induced nuclear factor kappa B (NF-κB) activation in human aortic endothelial cells via a cAMP-dependent pathway (68). However, angiotensin II-induced human endothelial cell apoptosis can be prevented by adiponectin through promotion and stabilization of the association between eNOS and Hsp90 (69). Further, adiponectin exerts a preventive effect on vascular stenosis in the injured artery (70-72). Growing body of evidence indicates that adiponectin has anti-atherogenic role. It has been shown that hypoadiponectinemia increases prevalence and magnitude of systemic atherosclerosis including ischemic heart disease and arteriosclerosis obliterans. Related *in vivo* studies have shown that adiponectin can suppress the development of atherosclerosis in susceptible mice. Indeed, ApoE-deficient mice treated with recombinant adenovirus to increase the circulating levels of adiponectin demonstrated a 30% decrease in lesion formation compared with mice expressing a control protein (72). Adiponectin associated with foam cells in the fatty streak lesions, suppressed the expression of vascular cell adhesion molecule-1 and class A scavenger receptors, and tended to reduce levels of TNF-α.

Adiponectin is as a negative regulator of angiogenesis. It is a direct angiogenesis inhibitor that induces apoptosis in activated endothelial cells (73). *In vitro*, it inhibits endothelial cell proliferation and migration. Further, endothelial-related mechanisms involve activation of caspase mediated apoptosis. Adiponectin induces a cascade activation of caspases-8, -9 and –3, which leads to cell death. In mouse tumor model, adiponectin inhibits primary tumor growth. Recently, it has been shown that adiponectin decreases breast cancer cell proliferation by inhibiting the entry of cells into S-phase without inducing apoptosis (74). In this regard, adiponectin and its receptors may be effective anti-cancer factors with therapeutic implications for angiogenesis-dependent diseases.

Adiponectin expression and circulating protein levels are decreased in obesity (75). Plasma adiponectin levels have been previously reported to be correlated negatively with BMI and waist-to-hip ratio (WHR) in human studies (76,77). However, clinical studies have suggested that plasma adiponectin concentrations and insulin sensitivity are more closely correlated with intra-abdominal than with subcutaneous adipose tissue (78), and it was recently reported that low plasma adiponectin levels are most closely correlated with accumulation of posterior subcutaneous abdominal adipose tissue (79).

It has been demonstrated that the adiponectin gene is highly expressed in differentiated brown adipocytes and provided new insights into the regulation of this key hormone. Adiponectin transcript is not detected in undifferentiated T37i cells suggesting that adiponectin gene activation occurs during the adipocyte differentiation process (80). Adiponectin gene expression could also be considered as markers of differentiation, together with the inner mitochondrial membrane uncoupling protein-1 (UCP-1), in the T37i cell line, since these genes were expressed exclusively in fully differentiated cells. These data therefore suggest that adiponectin plays important roles in brown adipocyte biology. The results are comparable to those obtained in the WAT since expression of adiponectin transcript was detected in fully differentiated murine and human white adipose cells. Regulatory effects of various stimuli on adiponectin gene expression in brown and white adipocytes are found also strikingly different. For instance, insulin increases the adiponectin expression in brown adipocytes but not white adipocytes (6). These findings bring additional support for major functional differences between the two cell types and suggest their distinct involvement in various physiologic and pathologic events. Given the major role played by adiponectin as factor regulating insulin sensitivity and also other biological processes, BAT could be seen as a novel endocrine source of adipokines, not only in rodents but also in humans.

The most popular piece: leptin

Leptin is a pleiotropic protein/cytokine belonging to the family of long-chain helical cytokines and has structural similarity with IL-6, IL-12, IL-15, granulocyte colony-stimulating factor, and oncostatin M. Leptin expresses a broad variety

of biological actions, including reproduction, regulation of hypothalamic-pituitary-adrenal axis, glucose and insulin metabolism, lipolysis, sympathetic nerve activity, immune and inflammatory responses, hematopoiesis, angiogenesis, bone formation, blood pressure, and wound healing (81-86). Leptin (from the Greek *leptos* meaning thin) is a cytokine product of the obese (*ob*) gene that is produced by both white and brown adipocytes.

Leptin gene expression is regulated by several factors but mainly by food intake and hormones. For instance, insulin stimulates leptin secretion during feeding and a decrease of insulin levels precedes a fall in leptin concentrations during starvation in humans (81)**.** Glucocorticoids increase leptin levels and this response is more robust than the response induced by insulin (87). Other stimulators of leptin synthesis are acute infection, sepsis and pro-inflammatory mediators such as IL-1, TNF-α and leukemia inhibitory factor (84,88), whereas leptin levels were decreased by β_3-adrenergic activity, free fatty acids, growth hormone, and PPAR-γ agonists (89). Furthermore, the expression of this hormone is inhibited by testosterone, whereas it is increased by ovarian sex steroids (90,91).

Several lines of evidence suggest that hyperleptinemia is involved in the pathogenesis of obesity-associated cardiovascular diseases including arterial hypertension (92-94). First, chronic leptin administration increases blood pressure in experimental animals. Second, hypertension is observed in transgenic mice overexpressing leptin, although their body weight is lower than wild-type littermates (94). Third, obesity is accompanied by hypertension in hyperleptinemic agouti yellow obese mice but not in leptin-deficient *ob/ob* mice. Finally, plasma leptin concentration correlates with blood pressure in hypertensive humans, also in those who are not obese. It has been suggested that leptin induces hypertension by activating the sympathetic nervous system (94). However, this mechanism can not solely explain the hypertensive effect of leptin. Recently, it has been demonstrated that leptin increases the level of systemic and intrarenal oxidative stress, and decreases renal sodium excretion and nitric oxide production in the rat. These effects may also contribute to the development of leptin-induced hypertension in experimental models as well as in hyperleptinemic obese individuals (93).

Leptin plays an essential role in transmitting signals for energy status to the central nervous system (CNS). Leptin can cross the blood-brain barrier (BBB) to act at the arcuate nucleus. A saturable transport system exists at the vascular BBB and choroid plexus. Failure of the leptin transporter is a major contributor to the development of leptin resistance in obesity. In humans and diet-induced obesity of outbred rodents, BBB resistance likely precedes resistance at the arcuate nucleus. BBB resistance is acquired and to some extent reversible with reductions in body weight induced by either fasting or leptin treatment (95). The BBB transporter for leptin is regulated by a number of substances, including epinephrine, glucose, insulin, and triglycerides and by events such as starvation (96).

Leptin has potent effects on lipid metabolism, leading to a substantial reduction of fat mass within several days of administration (97). Leptin has also been shown to have numerous actions on sites other than the central nervous system through high-affinity receptors located in peripheral tissue cells. Actions on hematopoietic precursor cells, cultured hepatocytes, pancreatic islet cells, and human marrow stromal cells and the recently discovered effect on chondrocytes of skeletal growth centers have all been reported as parts of the biological action spectrum of leptin. A number of tissues other than adipocytes secrete leptin, including the hypothalamus, stomach, intestines, placenta and testes (81,98-101).

Leptin also acts acutely to increase glucose metabolism regardless of its weight-reducing actions, suggesting its spectrum of activity is not on lipid metabolism alone but also on glucose metabolism (102).

The importance of adequate nutrition for the maintenance of reproductive function has been well known for some time. Inadequate nutrition delays or prevents the onset of puberty, but the mechanism linking nutrition to the reproductive system has not been fully elucidated (103). Many studies have focused on leptin in the search for this possible link. Leptin receptor mRNA has also as been localized to human ovaries and testes and has also been found in immortalized rat GnRH neurons and ovarian granulosa cells. Thus it appears that leptin has some role in the regulation of reproduction, acting both centrally and peripherally (98,99,104).

Severe lipodystrophy, caused by a deficiency or destruction of adipose cells, is a state characterized by low leptin levels (39). Other abnormalities in this condition include hypertriglyceridemia and severe insulin resistance, which is usually accompanied by diabetes mellitus (40). There are several genetic and acquired forms of lipodystrophy in humans, and studies of a variety of genetically engineered animal models demonstrated that the metabolic abnormalities develop as a consequence of fat loss (38,105). Why is adipose tissue so vital to the prevention of these metabolic abnormalities? One

hypothesis is that leptin has a critical role in preventing the insulin resistance and hypertriglyceridemia of lipodystrophy. Interestingly, leptin-replacement therapy at a level meant to achieve physiologic levels led to a dramatic improvement in insulin resistance, hyperglycemia, hypertriglyceridemia, and hepatic steatosis in a mouse model of lipodystrophy (106). Recombinant human leptin therapy improves metabolic abnormalities in patients with lipodystrophy and is well-tolerated (39). Lee and colleagues have reported that hyperleptinemia corrects steatosis in a variety of organs that act as sites of lipid accumulation, such as liver, islet cells, and heart in diet-induced obesity (107).

Additional information about leptin and its mechanisms of actions should help the development of safe and effective pharmacological treatments of obesity and related cardiocascular disorders. Leptin has effects on vascular tissue and is associated with arterial wall thickness, decreased vessel distensibility, and elevated C-reactive protein levels (108). Leptin has also procoagulant and antifibrinolytic properties, and it promotes thrombus and atheroma formation, probably through the leptin receptors by promoting vascular inflammation, proliferation, and calcification, and by increasing oxidative stress.

Leptin promotes proliferation and differentiation of hematopoietic cells, alters cytokine production by immune cells, stimulates endothelial cell growth and angiogenesis, and accelerates wound healing. Leptin normalizes the suppressed immune function associated with malnutrition and leptin deficiency (109). It is well established that genetic defects in the leptin/leptin receptor system in mice are associated with thymus atrophy, impairment of the delayed-type hypersensitivity reaction a decreased number of circulating lymphocytes, intraepithelial lymphocytes in the intestinal mucosa and natural killer cells (NK) in the liver, spleen, lung and peripheral blood (110). An important role for leptin in bone development is supported by the observation that leptin-deficient *ob/ob* mice have skeletal abnormalities which can be prevented by central leptin administration. Hipothalamic leptin has permissive effect on normal bone growth (101).

It has been shown that BAT expresses only leptin, under conditions of inactivity and atrophy (30). Thus, conditions associated with activation of brown adipose tissue such as cold and increased sympathetic nerve activity, decrease leptin gene expression in brown adipose tissue. It might be reasonable to speculate that cold induced decreased leptin may involve the regulation of increased appetite during cold.

The hormonal regulation of brown adipocyte secreted leptin clearly differs from that observed in WAT (33). Insulin is a stimulator of leptin synthesis and exerts its effects mostly through a strong, rapid, and transient activation of leptin transcription. However, glucocorticoids inhibit insulin action in T37i cells, pointing to specific molecular mechanisms that account for the tissue specificity of leptin gene expression. It is suggested that leptin might exert direct and significant effects on brown adipocytes, most notably related to thermoregulation and energy expenditure, through an autocrine pathway.

The enigmatic piece: resistin

Resistin was discovered in 2001 and its precise biological role especially for humans remain to be settled (111). It appears to be involved in adipogenesis, and is hypothesized to be a link between obesity and insulin resistance- both conditions known to be associated with atherosclerois (112-114). Resistin is not only expressed in adipocytes, it is also highly expressed in monocytes/macrophages which makes it a pro-inflammatory molecule (113-116). It is a member of the newly-discovered family of cysteine-rich secretory proteins called resistin-like molecules (RELM) or found in inflammatory zone (FIZZ). The third member of the family (FIZZ-3), resistin, is expressed exclusively in white adipose tissue with the highest levels in gonodal fat. Resistin mRNA does not exist in any other mouse tissue including BAT. For the rats, resistin expression occurs mainly in white, but relatively in much lower amounts in brown adipose tissue (6,117).

Three major physiological processes have been proposed as targets for resistin actions: glucose metabolism, adipogenesis, and inflammation (118). Initial studies have demonstrated that obesity induced by a high-fat diet, mutation of the leptin gene *(ob/ob* mice) or leptin receptor gene (*db/db* mice), is associated with elevated circulating resistin concentrations. Intraperitoneally-administered resistin elevates blood glucose and insulin concentration in mice, and impairs hypoglycemic response to insulin infusion. In addition, anti-resistin antibodies decrease blood glucose and improve insulin sensitivity in obese mice. Resistin suppresses insulin-stimulated glucose uptake in cultured 3T3-L1 adipocytes, and this effect is prevented by anti-resistin antibodies. These data suggest that resistin induces insulin resistance and hyperresistinemia contributes to impaired insulin sensitivity in obese rodents (111). Resistin induces suppressor of cytokine signaling 3 (SOCS-3), in a time- and dose-dependent manner in cultured adipocytes as well as in adipose tissue and that inhibition of SOCS function

impairs resistin action. SOCS-3 may be a cellular mediator of the ability of resistin to antagonize insulin action in adipocytes (117). The suppressive effect of TZDs on resistin secretion found in some studies may contribute to the insulin-sensitizing effect of this class of drugs. However, other data do not confirm these results (118,119). In addition, TNF-α, which is upregulated in obesity, suppresses resistin gene expression and protein secretion by 3T3-L1 adipocytes (120).

Resistin, similar to leptin, besides its peripheral activities in the regulation of energy metabolism, could be involved in the CNS mechanisms of feeding, by inhibiting dopamine and norepinephrine release in the rat hypothalamus (121). Recently it has been shown that hypothalamic resistin induces hepatic insulin resistance in rats (122).

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. There is also controversy regarding the importance of adipocytes as a source of resistin in human (123-125). Resistin is expressed in stromovascular fraction of WAT and in peripheral blood monocytes, but its mRNA is not detectable in human adipose cells even by very sensitive RT-PCR method, either in lean or in insulin resistant, obese and diabetic patients. Species differences in cellular resistin distribution may be partially explained by recent observation that in humans, in contrast to rodents, resistin is highly expressed in cultured preadipocytes but barely detectable in mature adipose cells (6).

Resistin accumulates locally in the inflammated joints of patients with rheumatoid arthritis and its levels correlate with the intensity of inflammation as defined by the intra-articular white blood cell count and IL-6 levels. The studies on the role of resistin in the inflammatory process reveal that it exhibits potent proinflammatory properties and that resistin is able to induce arthritis when injected into healthy mouse joints. In addition, it is an important regulatory cytokine triggering the release of other proinflammatory cytokines such as TNF-α, IL-1β, and IL-6. It has been demonstrated that the proinflammatory effects of resistin are mediated through the NF-κB signaling pathway (115).

Resistin has been examined specifically in the context of cardiovascular disease and strongly suggested that resistin can play important role in development of atherosclerosis. It was observed that macrophages infiltrated atherosclerotic aneurysms and secreted resistin, and that resistin induced the production of PAI-1 and endotelin type 1 (ET-1) by endothelial cells and migration of vascular smooth muscle cells (112,113).

Differentiated brown adipocytes express resistin genes which is under strikingly different hormonal regulation than that of WAT (80). While glucocorticoids have inhibitory effect on resistin expression, insulin has stimulatory effect in the brown adipocytes, contrary happens in white adipocytes: TZDs suppress the adipose-derived resistin production, and their antidiabetic effect, may at least in part, be achived through this mechanism. However, this effect of TZDs can be seen only on WAT adipocytes and it has opposite effect on brown adipocyte. Thus, depending on the activity of BAT, the effect of TZDs on resistin production can be stimulatory in state of inhibitory.

The insulinomimetic piece: visfatin

Visfatin (standing for visceral fat-derived factor) was initially identified as pre-B-cell colony-enhancing factor, a growth factor for early stage B lymphocytes (126,127). Recently joining to the list of adipokines, it is detected in the blood plasma, visceral adipose tissue, skeletal muscle, liver, bone marrow and lymphocytes. Its expression regulated by molecules that promote insuline resistance, such as lipopolysaccharide, IL-1β, TNF-α, and IL-6 (36,128).The expression of $mRNA^{visfatin}$ in visceral adipose tissue was elevated by dual, PPAR-alpha and PPAR-γ agonist treatments (129). So far there are contradictory reports about visfatin plasma levels and visceral adipose tissue mass, one stating that they are not correlated there is no relation, and the other stating that they are and visfatin expression in the visceral adipose tissue was found to be increased in obesity and in these subjects plasma consentrations of visfatin correlated much more strongly with the amount of visceral than of subcutaneous adipose tissue (36). Thus, visfatin might carry a keyrole for understanding the biological differences between visceral and subcutaneous adipose tissue. Visfatin levels are also increased in acute lung inflammation and sepsis, which is accompanied by an insulin-resistant state. The biological properties of visfatin are similar to the growth factor-like properties of some of these cytokines (i.e. it is anti-apoptotic and promotes cell proliferation).

The most important and interesting aspect of visfatin is its exertion of insulin-mimetic effects that are dose-dependent and quantitatively similar to the effects of insulin itself in stimulating muscle and adipocyte endosome-plasma membrane translocation of GLUT-4, and inhibiting hepatocyte glucose production. Intravenous injection of recombinant visfatin in mice decreases plasma glucose in a dose-dependent fashion.

A high dose of visfatin is also capable of reducing hyperglycemia in obese, insulin-resistant mice. Chronic production of visfatin by adenoviral vector results in diminished plasma glucose and insulin levels in obese mice and lean controls. Mice heterozygous for a targeted disruption of the visfatin gene have slightly but significantly increased blood glucose and decreased glucose tolerance. In keeping with its insulin-mimetic effects, visfatin is as effective as insulin in reducing hyperglycemia in insulin-deficient diabetic mice (126). The striking fact is visfatin binds to and activates the insulin receptor, causing the receptor phosphorylation and induction of downstream signaling molecules. However, visfatin and insulin do not compete for binding to the insulin receptor, indicating that the two proteins are recognized by different regions of the receptor. Recently, McLaren *et al* found that insulin decreases and dexamethasone increases $mRNA^{visfatin}$ in both preadipocytes and adipocytes (130).

These observations are of great interest and trigger a burning question in mind: can visfatin be the expected miraculous molecule of treatment for type 1 and 2 diabetes mellitus in the future? However, the physiological role and pathological implications of visfatin must be considered with some caution. Plasma concentrations are lower than those of insulin, they do not fluctuate with the nutritional state, and it has been suggested that visfatin is released from fat cells during lysis rather than truly secreted (131). Visfatin facilitates adipogenesis and accumulation of fat in the intra-abdominal depot. Its paradoxical effects facilitating fat accumulation and promoting insulin sensitivity suggest that visfatin is either good news for diabetes treatment or bad news for obesity treatment (36,127). These reservations not withstanding, it will be of great interest to follow future developments regarding both the mechanisms of production and action of visfatin, and its possible implication in the metabolic syndrome.

THE OLD PIECES OF THE ADIPOSE SECRETORY PUZZLE

Tumor necrosis factor-alpha

The biological actions of TNF-α have been studied for over a century. TNF-α is a cytokine initially described as an endotoxin induced factor causing necrosis of tumors and subsequently shown to be identical to cachexin, a factor secreted by macrophages in vitro (37,132). TNF-α is a 26-kD transmembrane protein, which is released into the circulation as a 17-kD soluble protein after extracellular cleavage by a metalloproteinase (133). TNF-α has two main receptors, type 1 and 2, which are expressed on many cells, including adipocytes (134,135). Thus, its actions occur in many cell types and include inflammation, mitogenesis, differentiation, immune modulation and antitumor immunity. TNF-α has also been implicated as an important modulator of energy metabolism, particularly in adipocytes. In these cells, TNF-α regulates the expression of many adipocyte genes and inhibits the differentiation program in adipocytes. It has also been demonstrated that it interferes directly to glucose homeostasis and lipid metabolism in adipose tissue and contributes to the development of insulin resistance in obesity and type 2 diabetes (136,137). Additionally, TNF-α causes reductions in the expression of genes involved in lipogenesis in adipocytes, likely through NF-κB mediated transcription (132). Although circulating TNF-α levels are relatively low and have no clear correlation with obesity or insulin resistance, tissue expression levels of TNF-α correlate positively with both conditions. In mice, chronic exposure of cells or whole animals to TNF-α induces insulin resistance, and treatment with TNF-α receptor antagonists neutralize this effect. Furthermore, mice with targeted gene deletion of TNF-α or its receptors showed increased insulin sensitivity and improved plasma free fatty acid levels (138).

Interleukin-6

Among the interleukines in the adipose tissue, IL-6 has been studied most extensively. Both IL-6 and its receptor (IL-6R) are expressed by adipocytes and adipose tissue matrix and one third of circulating IL-6 originates from adipose tissue. Expression and secretion of IL-6 are two to three times greater in visceral versus subcutaneous adipose tissue (6,37,139). Circulating levels of IL-6 are elevated in obesity and reduced after weight loss (140). Recently, it was reported that after removal of adipose tissue from mice there was a marked upregulation of $mRNA^{IL\text{-}6}$ in adipocytes. Other mediators of IL-6 gene expression in adipose tissue of humans are a meal or insulin administration (141,142).

IL-6 has different effects on energy homeostasis in the periphery and the CNS. Peripheral administration of IL-6 induces hyperlipidemia, hyperglycemia, and insulin resistance in rodents and humans. IL-6 also decreases insulin signaling in peripheral tissues by reducing expression of insulin receptor signaling components and inducing SOCS-3, a negative regulator of both leptin and insulin signaling (143). In rodent models of diabetes, IL-6 has been implicated in the development of muscle insulin resistance and β-cell apoptosis (143,144). In humans with type 2 diabetes, IL-6 levels are increased and correlate with the severity of glucose intolerance and with the

severity of inflammation, as indicated by the highly sensitive C-reactive protein serum concentration (145).

IL-6 also inhibits adipogenesis and decreases adiponectin secretion (143). Central administration of IL-6 increases energy expenditure and decreases body fat in rodents. Furthermore, transgenic mice overexpressing IL-6 have a generalized defect in growth, which includes reduced body weight and decreased fat pad weights (146).

Plasminogen activator inhibitor type 1

PAI-1, an inhibitor of tissue plasminogen activator, is an important inhibitor of fibrinolysis also contributes to remodeling of the vascular architecture (147-149). It plays role in the pathogenesis of atherothrombosis and cardiovascular disease. PAI-1 is expressed by many cell types within adipose tissue including adipocytes (150-152). The subcutaneous fat depot has been shown both to exhibit a higher PAI-1 gene expression and to secrete greater amounts of PAI-1 than visceral adipose tissue. Recently, the stromal-vascular fraction has been shown to be the main source of PAI-1 production in human fat with evidence of a 5-fold higher expression in the visceral than in the subcutaneous depots, which is in agreement with the strong relationship observed between circulating PAI-1 concentrations and visceral fat enlargement (153). Plasma PAI-1 is increased in patients with central obesity and it has been found that abdominal adipose tissue is an important source of plasma PAI-1 (153). Moreover, insulin resistance may be an important regulator of PAI-1 level. Recently, higher levels of plasma PAI-1 in HIV-infected patients with HIV-associated lipodystrophy syndrome (HALS) have been demonstrated (154). There are at least three kinds of cells that may contribute to the level of circulating PAI-1: liver cells, endothelial cells and adipocytes. Plasma concentrations of PAI-1 have been found to be correlated positively with the amount of PAI-1 produced by subcutaneous adipose tissue in vitro as well as $mRNA^{PAI-1}$ concentration in subcutaneous adipose tissue in HALS patients. The enhanced plasma PAI-1 may be to the result of increased production of PAI-1 in other cell types such as endothelial cells or hepatocytes, or increased production in other depots, for example, the enhanced visceral adipose depot (151).

In human population studies, circulating PAI-1 levels correlate with atherosclerotic events and mortality, and some studies suggest that PAI-1 may be an independent risk factor for coronary artery disease. Hyperglycemia, AngII, and very low-density lipoprotein cholesterol, in addition to obesity, contribute to elevated serum PAI-1 levels. All of these factors increase PAI-1 gene expression. High levels of PAI-1 in patients with diabetes are a major contributor to the prothrombotic state in diabetes, which leads to enhanced atherosclerotic mortality (148,149). PAI-1 is also a potential target in the prevention of adipose tissue accumulation. Two independent studies show that PAI-1 knockout mice are fully protected against high-fat diet-induced or genetic obesity in an *ob/ob* background (155,156). Recently an *in vitro* study showed that PPAR-γ agonists inhibited PAI-1 expression in adipose tissue and that antithrombotic effect was mediated by adiponectin (157). In the light of those data, antagonising TNF-α, Il-6 and PAI-1 opens a new horizon in adipopharmacology.

THE METABOTROPHIC PIECES OF THE ADIPOSE SECRETORY PUZZLE

Nerve growth factor and brain-derived neurotrophic factor

The nerve growth factor, a prototypic member of the protein family of neurotrophins, was discovered by Rita Levi-Montalcini in 1951 (158). Studies in the past three decades initiated by Luigi Aloe *et al* have demonstrated that neurotrophins are not only promoters of nerve growth and survival, but also exert trophic effects over immune and other cell types (159). More recent studies demonstrate that NGF and BDNF exert metabotrophic effects on glucose, lipid and energy homeostasis. Thus these neurotrophins as well as other neurotrophic factors were considered metabotrophic factors (12,28,160-164). Note that NGF (5,7,12,27,28,163) and BDNF (Sornelli *et al*, this voume of *Biomedical Reviews*) have recently been incorporated in the list of adipokines. Further, low NGF levels were found in patients with acute coronary syndromes (165) and metabolic syndrome and obesity (28,160,166,167). Accordingly, a hypothesis has been raised that both NGF and BDNF might have therapeutic potentials for these diseases (12,28). Supportively, *(i)* NGF shares a striking structural homology with proinsulin and enhances glucose-induced insulin secretion, *(ii)* NGF improves pancreatic islet transplantation, *(iii)* NGF exerts antioxidant effects, *(iv)* NGF upregulates PPARγ expression, whereas PPARγ agonists stimulate the secretion of adiponectin, and *(v)* mutations affecting *Bdnf* (gene encoding BDNF) in mice or *Ntrk2* (gene encoding the high-affinity BDNF receptor TrkB) are associated an increase food intake and severe obesity (reviewed in 12,28,161,162,164).

Metallothioneins

Metallothioneins (MT) constitute a family of cysteine-rich metalloproteins, of which MT-1 and MT–2 are the best studied MT; they exert potent neurotrophic effects in various brain injuries (168). Metallothioneins (169,170) including MT-3 (171) are also produced by adipose tissue, and exert a number of metabotrophic actions, such as antioxidant, antiinflammatory, antidiabetic, and cardioprotecive (28,164).

Taken together, adipopharmacology of metabotrophic factors might be a novel approach that may lead to the development of new classes of anti-obesity, anti-diabetic and anti-atherosclerotic drugs, a combination targeting of NGF-BDNF-adiponectin-MT appearing to be a very promising approach at present.

CONCLUSION

Adipose tissue has a key role in mammalian biology, but unlike other tissues, its mass has an almost unlimited ability to vary. It is now known that both increased (in obesity and related disorders) and decreased (in anorexia, orthorexia, and lipodystrophy) adipose tissue mass exert effects on immune, inflammatory, nervous, cardiovascular, reproductive, hematopoietic and skeletal system. A better understanding of its implications for adipokine-mediated diseases will possibly open the door for discovering potentially effective therapeutic interventions especially for obesity and related diseases. It thus appears that the adipopharmacology of disease might be a rational scientific pursuit in future studies.

According to the growing evidence, brown adipocytes are significant for human physiology although there is no discrete BAT depots that can be found in human adults. Investigations directed towards understanding the physiological role of UCP-1 mediated thermogenesis in BAT have provided the foundation for an alternative approach to antiobesity research. Impaired BAT activity has been associated with the development of obesity. Mice with increased BAT activity due to overexpression of UCP-1 have enhanced energy expenditure and are resistant to diet-induced obesity. Transgenic mice with BAT ablation become obese, hyperlipidemic, and insulin resistant. BAT and UCP-1 is now considered as an anti-obesity weapon and cross-talk between BAT and WAT seems to open a new horizon for the treatment of obesity. An alternative approach exploits the plasticity of the adipose tissue by manipulating the expression of UCP-1 in other tissues, such as WAT or muscle. The observed conversion of BAT to WAT in the newborn had led some researchers to explore the possibility of converting white adipocytes back into brown in the adult.

It is quite likely that discrete functional specializations of BAT and subcutaneous and visceral WAT including organ-associated examples, such as perivascular and epicardial adipose tissue (21-26,172,173), should be considered in adipokine-tergeting pharmacotherapy. Thus, both “white” and “brown” approaches should be pursued in the adipopharmacology of disease. Several therapeutic agents are around the corner and still others are on the horizon of “the unfinished jigsaw puzzle” (174) of adipose tissue secretion-related cardiometabolic and other diseases.

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