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REVIEW ARTICLE

The endocrine function of adipose tissue: an update

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Summary

Adipose tissue secretes bioactive peptides, termed 'adipokines', which act locally and distally through autocrine, paracrine and endocrine effects. In obesity, increased production of most adipokines impacts on multiple functions such as appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and haemostasis, all of which are linked with cardiovascular disease. Enhanced activity of the tumour necrosis factor and interleukin 6 are involved in the development of obesity-related insulin resistance. Angiotensinogen has been implicated in hypertension and plasminogen activating inhibitor-1 (PAI-1) in impaired fibrinolysis. Other adipokines like adiponectin and leptin, at least in physiological concentrations, are insulin sparing as they stimulate beta oxidation of fatty acids in skeletal muscle. The role of resistin is less understood. It is implicated in insulin resistance in rats, but probably not in humans. Reducing adipose tissue mass, through weight loss in association with exercise, can lower TNF-α and IL-6 levels and increase adiponectin concentrations, whereas drugs such as thiazolinediones increase endogenous adiponectin production. In-depth understanding of the pathophysiology and molecular actions of adipokines may, in the coming years, lead to effective therapeutic strategies designed to protect against atherosclerosis in obese patients

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Introduction

Marked central adiposity, one of the main characteristics of the insulin resistance syndrome and/or metabolic syndrome, is the basis of the portal/visceral hypothesis that states that increased adiposity, particularly in visceral depots, leads to greater free fatty acid (FFA) flux and inhibition of insulin action via Randle's effect in insulin-sensitive tissues.[1] Aberrantly high availability of nonesterified fatty acids reduces muscle use of glucose, strongly stimulates hepatic glucose and very low-density lipoprotein (VLDL) production and acutely potentiates glucose-stimulated insulin secretion. The longer-term lipotoxic effect of fatty acids on the pancreatic β-cell may also be part of the link between obesity, insulin resistance and development of type 2 diabetes.

As recent findings do not entirely support the portal-visceral hypothesis, the theories of the ectopic fat storage syndrome[2] and the endocrine paradigm[3] have been developed to explain the links between adiposity and disease.

Three lines of evidence support the ectopic fat storage syndrome. First, in mice and humans, failure to develop adequate adipose tissue mass, also termed lipodystrophy, results in severe insulin resistance and diabetes, which might be consequent to ectopic lipid storage in the liver, skeletal muscle and pancreatic insulin-secreting beta cell. Second, most obese patients shunt lipid into keletal muscle, liver, and probably beta cells and, as demonstrated by several studies, the degree of lipid infiltration closely correlates with insulin resistance. Third, increased fat cell size is associated with insulin resistance and diabetes. Large fat cells may underlie the failure of the adipose tissue mass to expand and accommodate a high energy influx. Altogether, these three observations support the acquired lipodystrophy hypothesis as the link between adiposity and insulin resistance.[2]

The endocrine paradigm was developed at the same time as the hypothesis of the ectopic fat storage syndrome. Adipose tissue was traditionally considered an energy storage organ, but over the last decade, it has emerged as an endocrine organ. It is now recognized that adipose tissue produces multiple bioactive peptides, termed 'adipokines', which not only influence adipocyte function in an autocrine and paracrine fashion but also affect more than one metabolic pathway through the bloodstream.[3]

The concept of white adipose tissue as an endocrine organ originated in 1995 with the discovery of leptin and its wide-ranging biological functions.[4] To maintain normal body functions, each adipocyte secretes diverse cytokines and bioactive substances into the surrounding environment. Although each adipocyte produces a small quantity of adipocytokines, as adipose tissue is the largest organ in the human body, their total amount impacts on body functions. Furthermore, as adipose tissue is supplied by abundant blood stream adipocytokines released from adipocytes pour into the systemic circulation.

So far, many adipokines have been identified (Table 1). They all integrate in a communications network with other tissues and organs such as the skeletal muscle, adrenal cortex, brain and sympathetic nervous system and participate in appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and haemostasis (Table 2).

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Table 1. Adipokines and their main effects

Adipocytokines	Effects on
LPL	Lipid metabolism
HSL	Lipid metabolism
Perilipin	Lipid metabolism
aP2	Lipid metabolism
CETP	Lipid metabolism
RBP	Lipid metabolism
IL-6	Inflammation, atherosclerosis, insulin resistance
TNF-α	Inflammation, atherosclerosis, insulin resistance
Adipsin/ASP	Immune–stress response
Metallothionein	Immune–stress response
Angiotensinogen	Vascular homeostasis
PAI-1	Vascular homeostasis
Adiponectin	Inflammation, atherosclerosis, insulin resistance
PPAR-γ	Lipid metabolism, inflammation, vascular homeostasis
CRP	Inflammation, atherosclerosis, insulin resistance
IGF-1	Lipid metabolism, insulin resistance
TGF-b	Cell adhesion and migration, growth and differentiation
Monobutyrin	Vasodilation of the microvessel
Uncoupling proteins	Energy balance and theromoregulation
Steroid hormones	Lipid metabolism, insulin resistance
Leptin	Food intake, reproduction, angiogenesis, immunity
Resistin	Inflammation, insulin resistance
P450 arom	Lipid metabolism
Apelin	Insulin resistance
Visfatin	Insulin resistance
ZAG	Lipid metabolism, cancer cachexia

Abbreviations: LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; aP2, adipocyte lipid-binding protein; RBP, retinol-binding protein; IGF-1, insulin-like growth factor-1; TGF-b, transforming growth factor-b; PPAR-g, peroxisome proliferator-activated receptor g; ZAG, zinc-a2-glycoprotein.

Leptin

Leptin, a 16-kD adipocyte-derived cytokine, is synthesized and released from fat cells in response to changes in body fat. It is encoded by a gene called *ob* (from obesity mice), and was named leptin from the Greek word 'λεπτος', meaning thin. Leptin circulates partially bound to plasma proteins and enters the CNS by diffusion through capillary junctures in the median eminence and by saturable receptor transport in the choroid plexus. In the hypothalamus, leptin binds to receptors that stimulate anorexigenic peptides such as proopiomelanocortin and cocaine- and amphetamine-regulated transcript and inhibit orexigenic peptides, e.g. neuropeptide Y and the agouti gene-related protein.[5] Leptin reduces intracellular lipid levels in skeletal muscle, liver and pancreatic beta cells, thereby improving insulin sensitivity. In muscle, insulin sensitization is achieved through malonyl CoA inhibition, which increases transport of fatty acids into mitochondria for beta oxidation. These changes are partially mediated by central sympathetic activation of adrenergic receptors.[6]

There is strong evidence showing that the dominant action of leptin is to act as a 'starvation signal'. Leptin declines rapidly during fasting, and triggers a rise in glucocorticoids, and reduction in thyroxine (T4), sex and growth hormones.[7] Moreover, the characteristic decrease in thermogenesis during fasting and postfast hyperphagia is mediated, at least in part, through a decline in leptin.[5] Therefore, leptin deficiency was perceived as a state of unmitigated starvation, leading to compensatory responses, such as hyperphagia, decreased metabolic rate and changes in hormone levels, designed to restore energy balance.[8]

Chan *et al.*[9] examined the role of leptin in regulating neuroendocrine and metabolic function in fasting humans. Placebo, low-dose recombinant methionyl human leptin (r-metHuLeptin) or replacement-dose r-metHuLeptin was administered during a 72-h fast. Replacement-dose leptin prevented starvation-induced changes in sex hormones and partially prevented suppression of hypothalamic–pituitary–thyroid axis and IGF-1 binding capacity. However, unlike rodents, leptin replacement during acute fasting did not affect fuel utilization, glucocorticoid or growth hormone levels in humans.

In patients with lipodystrophy and leptin deficiency, leptin-replacement therapy improved glycemic control and decreased triglyceride levels. In a recent study, nine female patients (age range, 15–42 years; eight with diabetes mellitus) with lipodystrophy and serum leptin levels under 4 ng/ml (0·32 nmol/ml) received r-metHuLeptin (recombinant leptin) subcutaneously twice a day for 4 months at escalating doses, in order to achieve low, intermediate and high physiological leptin replacement levels. During treatment, serum leptin levels increased and glycosylated haemoglobin decreased in the eight patients with diabetes. Four months therapy reduced average triglyceride levels by 60% and liver volume by a mean of 28% in all nine patients and led to suspension of, or to a substantial reduction in, antidiabetes medication. Self-reported daily caloric intake and resting metabolic rate also decreased significantly. Overall, recombinant leptin therapy was well tolerated.[10]

Similar results were observed in three severely obese children with no functional leptin.[11] Leptin receptor mutations are rare in humans. Affected members of a French family have a single nucleotide substitution (G-to-A) in the splice donor site of exon 16, which results in encoding of a leptin receptor (LEPR) without either transmembrane or intracellular domains.[12] The mutant receptor circulates at high concentrations bound to leptin.[12] LEPR null humans are hyperphagic, morbidly obese and fail to undergo normal sexual maturation.[12] Furthermore, these patients did not respond to thyrotropin-releasing hormone and growth hormone releasing hormone testing, suggesting leptin plays a critical role in neuroendocrine regulation.[12]

The concept of 'leptin resistance' was introduced when increased adipose leptin production was observed in obese individuals,[13] who were not leptin-deficient. Apart from mutations in the leptin receptor gene,[14] the molecular basis of leptin resistance has yet to be determined. Although adenoviral or transgenic leptin gene over-expression reduced food intake and body weight in rodents,[15] attempts to obtain the same effect in humans through daily administration of recombinant leptin were frustrating, as only very high doses reduced body weight in a subset of individuals.[16] Thus, although leptin is essential for body homeostasis, increasing circulating leptin is not the 'panacea' for common obesity.

Table 2. Adipokines and their metabolic effects in humans

Adipocytokines	Metabolic effects	Future Investigations into the
Adiponectin	Inhibition of monocyte adhesion to endothelial cells, macrophage transformation to foam cells, endothelial cell activation.	Detrimental effects of hypoadiponectaemia in obesity, type II diabetes mellitus, cardiovascular disease
Leptin	Satiety signal, inhibits lipogenesis, stimulates lipolysis, improves insulin sensitivity, angiogenic activity.	Effect on vascular structure
IL-6	Impairs appetite, lost fat tissue with no effect on lean mass, inhibits gluconeogensis, increases hepatic *de novo* synthesis of fatty acid and cholesterol.	Molecular mechanisms trough IL-6 can elicit proinflammatory or anti-inflammatory effects.
PAI 1	Inhibits activity of tissue-type plasminigen activator, an anticlotting factor.	Effects of tissue-type plasminogen activator, its inhibitor in type 1 and 2 diabetes mellitus
Adipsin	Stimulates triglyceride storage in adipose cells through stimulation of glucose transport, enhances fatty-acid re-esterification and inhibits lipolysis	Role on coronary artery disease
TNF	Stimulates release of FFA by adipocytes, reduces adiponectin synthesis and impaired insulin signalling.	Antifibrosis treatment for NASH
Resistin	Controversial effects on glucose metabolism Endothelial dysfunction?	Insulin resistance in muscle and liver
Angiotensinogen	Acts through vasoactive peptide angiotensin II, Correlates significantly with blood pressure.	Role on pharmacogenetic for hypertension
Aromatase	Converts androstenedione to estrone driving fat to subcutaneous and breast tissues.	Role in inflammation
11-Hydroxysteroid dehydrogenase	Regenerates metabolically active cortisol from cortisone in humans	Role in inflammation

A large prospective study – the West of Scotland Coronary Prevention Study (WOSCOPS) – showed, for the first time, that leptin might be an independent risk factor for coronary heart disease. At baseline, plasma leptin levels were significantly higher in 377 men (cases) who experienced a coronary event during the 5-year follow-up period than in 783 male controls, matched for age and smoking history who did not suffer a coronary event and who were representative of the entire WOSCOPS cohort.[17]

These data suggest leptin may affect vascular structure. In fact, *in vitro* and *in vivo* assays revealed that leptin has angiogenic activity[18] and contributes to arterial thrombosis through the platelet leptin receptor.[19] It also stimulates production of reactive oxygen species (ROS) as a result of monocyte activation *in vitro*.[20] Therefore, in an obese subject leptin may no longer be able to regulate caloric intake and energy balance, but may still exert its angiogenic activity and production of ROS, which affect vessel walls.[18–20]

Adiponectin

Adiponectin or, as it is also termed, adipocyte complement-related protein (Acrp 30) (because of its homology to complement factor C1q) is almost exclusively expressed in white adipose tissue. Circulating adiponectin concentrations are high (500–30 000 μg/l), accounting for 0·01% of total plasma protein. Adiponectin is present in serum as a trimer, hexamer or high molecular weight isoform.[21] Waki[22] reported that the high molecular weight isoform promotes adenosine monophosphate-activated protein kinase (AMPK) in hepatocytes. In contrast, Tsao *et al.*[23] who reported only trimers activate AMPK in muscle, whereas hexamers and the high molecular weight isoform activate NF-B. Differences in the tissue-specific expression patterns of two adiponectin receptors may contribute to these divergent activities.[24]

Adiponectin also has antiatherogenic properties, as shown *in vitro* by its inhibition of monocyte adhesion to endothelial cells, macrophage transformation to foam cells (through down-regulation of scavenger receptors, Ouchi *et al.* 1999)[25] and endothelial cell activation (through reduced production of adhesion molecules and inhibition of tumour necrosis factor α (TNF-α) and transcriptor factor nuclear factor kappa beta (NF-κβ), Tan KC *et al.* 2004).[26]

Interleukin (IL) 6 and TNF-α are potent inhibitors of adiponectin expression and secretion in human white adipose tissue biopsies or cultured adipose cells.[27] 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,[28] suggesting that adiponectin and leptin work together to sensitize peripheral tissues to insulin. However, because globular adiponectin improves insulin resistance but not obesity in *ob/ob* leptin-deficient mice,[24] adiponectin and leptin appear to have distinct, albeit overlapping, functions. Two receptors for adiponectin have been cloned. Adipo R1 and Adipo R2 are expressed predominantly in muscles and liver. Adiponectin-linked insulin sensitization is mediated, at least in part, by activation of AMPK in skeletal muscles and the liver, which increases fatty-acid oxidation and reduces hepatic glucose production.[29]

Unlike most adipokines, adiponectin expression and serum concentrations are reduced in obese and insulin-resistant states. *In vivo*, high plasma adiponectin levels are associated with reduced risk of myocardial infarction (MI) in men as demonstrated in a case control study that enrolled 18 225 subjects without cardiovascular disease who were followed up for 6 years.[30]

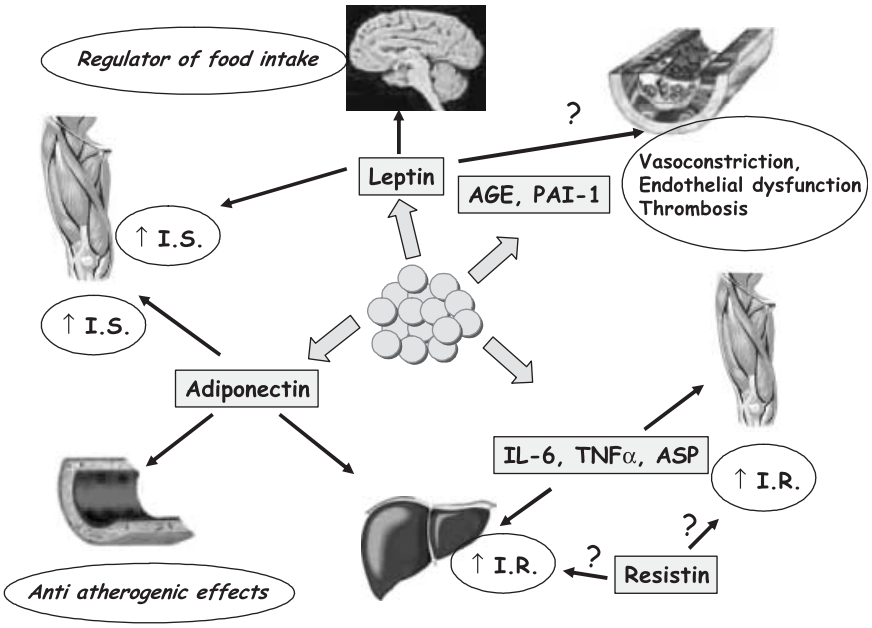

Fig. 1 Adipocytokines implicated in energy homeostasis, insulin sensitivity (IS), insulin resistance (IR) and atherothrombosis. Excessive production of interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), acylation-stimulating protein (ASP) deteriorates insulin action in muscle and/or in liver, whereas increased angiotensin (AGE) and PAI-1 secretion favours hypertension, endothelial dysfunction and thrombosis. The role of resistin on insulin resistance is still not clear. Leptin regulates energy balance and exerts an insulin sensitizing effect. Adiponectin increases insulin action in muscle and liver an exerts an anti atherogenic effect.

Although further studies are needed to clarify whether adiponectin independently predicts coronary heart disease events, in men with type 2 diabetes, increased adiponectin levels are associated with a moderately decreased risk of coronary heart disease. The association seems to be mediated in part by the effects of adiponectin on high-density lipoprotein (HDL) cholesterol, through parallel increases in both. Although many mechanisms have been hypothesized, exactly how adiponectin affects HDL cholesterol remains largely unknown.[31] In American Indians, who are particularly at risk of obesity and diabetes,[32] adiponectin does not correlate with the incidence of coronary heart disease.[33]

Two case control studies in obesity-prone Pima Indians and in Caucasians suggest that individuals with high adiponectin concentrations are less likely to develop type 2 diabetes than those with low concentrations.[34,35] Weight loss, caloric restriction and thiazoladinedione (TZD) treatment increase adiponectin plasma levels and gene expression in white adipose tissue.[27] TZD stimulates adiponectin gene expression via activation of the heterodimer peroxisome proliferator-activated receptor (PPAR)g/retinoid X receptor, which binds to a PPAR responsive element (PPRE) in the human adiponectin promoter.[36]

Tumour necrosis factor α (TNF-α)

TNF-α, a multipotential cytokine with several immunologic functions, was initially described as a cause of tumour necrosis in septic animals and associated with cachexia-inducing states, such as cancer and infection.[37] It is expressed as a 26-kD cell surface transmembrane protein that undergoes cleavage to produce a 17-kD soluble, biologically active form of TNF-α.

In 1993 it was the first product from adipose secreted tissue to be proposed as a molecular link between obesity and insulin resistance[38] and in fact, neutralization of TNF-α improves insulin resistance in obese rats.[39] A recent elegant hypothesis suggested that in obese rats TNF-α production from the fat cuff around the arteriole origin inhibits insulin-stimulated nitric oxide synthesis and results in unopposed vasoconstriction – a mechanism termed 'vasocrine' signalling.[40] These findings suggest a homology between vasoactive periarteriolar fat and visceral fat, which may explain relationships among visceral fat, insulin resistance and vascular disease.

In humans TNF-α is synthesized and secreted by adipocytes and stromovascular cells. Adipose tissue TNF-α mRNA correlates with body mass index, percentage of body fat and hyperinsulinaemia. Weight loss decreases TNF-α levels.[41] However, infusion of TNF-α–neutralizing antibodies to type 2 diabetic patients did not modify glucose levels or insulin sensitivity.[42] Adipose tissue TNF-α, which is not secreted in systemic circulation, acts in an autocrine and paracrine fashion. Several mechanisms could account for the effect of TNF-α on obesity-related insulin resistance – increased release of FFA by adipocytes, reduced adiponectin synthesis and impaired insulin signalling.[43] *In vitro* and *in vivo* studies show TNF-α inhibition of insulin action is, at least in part, antagonized by TZD, further supporting the role of TNF-α in insulin resistance.[44]

Acute ischaemia also increases TNF-α levels. A nested case control study in the Cholesterol And Recurrent Events (CARE) trial compared TNF-α concentrations at an average of 9 months after initial MI in 272 participants who subsequently developed recurrent non-fatal MI or a fatal cardiovascular event (cases) and in 272 age- and sex-matched participants who did not (controls). Overall, TNF-α levels were significantly higher in cases than controls. The excess risk of recurrent coronary events after MI was predominantly seen among patients with the highest TNF-α levels.[45]

The Health, Ageing and Body Composition study (Health ABC study) assessed the predictive value of several inflammatory markers on the incidence of cardiovascular events, i.e. coronary heart disease, stroke and congestive heart failure in well-functioning elderly people during an average follow-up of 3·6 years. Blood levels of IL-6, C-reactive protein and TNF-α were monitored. After adjustment for

potential confounders, IL-6 was significantly associated with all outcomes, TNF-α showed significant associations with coronary heart disease and congestive heart failure. C-reactive protein was significantly associated with congestive heart failure.[46]

In nested case control analysis, plasma levels of soluble TNF-receptor 1 (sTNF-R1) sTNF-R2, IL-6, and C-reactive protein were examined as markers of risk for coronary heart disease in women participating in the Nurses' Health Study and men participating in the Health Professionals Follow-Up Study. After adjustment for matching factors, high levels of IL-6 and C-reactive protein were significantly related to an increased risk of coronary heart disease in both sexes, whereas high levels of soluble TNF-α receptors were significant only in women. Further adjustment for lipid and nonlipid factors attenuated all associations; only C-reactive protein levels remained significant.[47]

Visceral body fat in obese women correlates with endothelial dysfunction, a marker of early-stage atherosclerosis, and the underlying mechanism may be inappropriate cytokine secretion. Fifty-six healthy premenopausal obese women (age range 25–44 years, body mass index 37·2, waist to hip ratio range 0·78–0·92) and 40 age-matched normal-weight women were compared. Obese women had increased basal concentrations of TNF-α, IL-6, P selectin, intercellular adhesion molecule-1, vascular adhesion molecule-1 and impaired vascular responses to L-arginine, the natural precursor of nitric oxide. Visceral obesity correlated positively with levels of TNF-α, IL-6 and adhesion molecules as well as with impaired response to L-arginine. After a 1-year multidisciplinary program of diet, exercise and behavioural counselling, all obese women lost at least 10% of their original weight (9·8 ± 1·5 kg, range 7·5–13 kg). Sustained weight loss was associated with lowered concentrations of cytokines and adhesion molecules and improved vascular responses to L-arginine. Weight loss is a safe method for down-regulating the inflammatory state and counteracting endothelial dysfunction in obese women.[48]

IL-6

IL-6, a pleiotropic circulating cytokine, is reported to have multiple effects ranging from inflammation to host defence and tissue injury. Secreted by many cell types, including immune cells, fibroblasts, endothelial cells, skeletal muscle and adipose tissue, IL-6 circulates as a glycosylated protein.[49]

Mice with a disruption of the IL-6 gene in both alleles develop normally, but, after ovariectomy or orchidectomy, are protected from the increased osteoclastogenesis and extensive bone loss exhibited by their normal littermates treated in the same way.[50] These mice exhibited small decrease in the absolute numbers of haematopoietic stem cells and progenitors,[51] and slightly impaired accumulation of leucocytes in subcutaneous air pouches.[52] Macrophage and neutrophil responses were severely impaired and consequently, the acute-phase response to infection by influenza pneumonitis,[53] *Candida albicans*[54] and vaccinia virus.[55]

Plasma IL-6 concentrations correlate positively with human obesity and insulin resistance, and high IL-6 levels are predictive of type 2 diabetes and MI.[56] Weight loss significantly reduces IL-6 levels in adipose tissue and serum.[57] However, only about 10% of the total IL-6 appears to be produced exclusively by fat cells.[58] Omental fat produces threefold more IL-6 than subcutaneous adipose tissue, and adipocytes isolated from the omental depot also secrete more IL-6 than fat cells from the subcutaneous depot.[59]

Administration of IL-6 to healthy volunteers increased blood glucose in a dose-dependent manner, probably by inducing resistance to insulin action.[60] Inhibition of insulin receptor signal transduction in hepatocytes might underlie the effects of IL-6 on insulin resistance. This could be mediated, at least in part, by suppression of cytokine signalling-3 (SOCS-3), increased circulating FFA (from adipose tissue) and reduced adiponectin secretion.[61,62]

IL-6 is related to insulin resistance in patients with high-grade inflammation as a result of cancer. Serum IL-6 concentration was detectable by conventional IL-6 enzyme-linked immunosorbent assay in 8/23 patients with different oesophageal, gastric, colon and lung cancers but could not be detected in any of the six healthy volunteer controls. Insulin resistance, evaluated by a euglycaemic hyperinsulinaemic glucose clamp, was significantly higher in the eight patients with detectable serum IL-6 than in the other cancer patients.[63]

Acylation-stimulating protein (ASP)/adipocyte trypsin (ADIPSIN)

Adipocyte trypsin (ADIPSIN) is a secreted serine protease related to complement factor D. In humans, adipose tissue also releases substantial amounts of acylation-stimulating protein (ASP), a protein derived from the interactions of ADIPSIN with complement C3 and factor B. Although ASP is known to stimulate triglyceride storage in adipose cells through stimulation of glucose transport, enhancement of fatty acid re-esterification and inhibition of lipolysis,[64] the receptor and signalling pathways mediating ASP effects have not yet been characterized.

Most, but not all studies in humans report substantial increases in plasma ASP in obese subjects[65] although it has still to be established whether these high circulating levels reflect increased ASP activity or resistance to ASP. Resistance to ASP could redirect fatty acid flux away from adipose tissue towards the liver.[66]

Hyperapobetaliproteinaemia, a familial dyslipidaemia characterized by increased hepatic release of LDL and VLDL, may result from impaired adipose tissue actions of ASP.[67] Interestingly, up to 25% patients with coronary artery disease have high ASP concentrations.[68]

Resistin

Human resistin is a dimeric protein containing 108 amino acids. Holcomb *et al.*[69] first described the gene family and its tissue-specific distribution, identifying a protein (FIZZ1) that was up-regulated in the asthmatic lung in bronchoalveolar lavages of mice with experimentally induced asthma. Found in inflammatory zone 1, FIZZ1 is also known as resistin-like molecule a (RELMα). One of two homologues, FIZZ2, also known as RELMβ, was localized in proliferating epithelia at the base of intestinal crypt.[70] A third homologue, FIZZ3, also known as 'resistin' or adipocyte-specific secretory factor was later identified. As TZD suppresses resistin production in 3T3-L1 adipocytes, Steppan *et al.* suggested resistin could be a link between obesity and insulin resistance.[70]

In murine models, obesity is associated with rises in circulating resistin concentrations.[71] Resistin increases blood glucose and insulin concentrations and impairs hypoglycaemic response to insulin infusion.[72] In obese mice, antiresistin antibodies decrease blood glucose and improve insulin sensitivity.[73] All these data support the hypothesis that in obese rodents, resistin induces insulin resistance and contributes to impaired insulin sensitivity.

In humans, the physiological role of resistin is far from clear and its role in obesity and insulin resistance and/or diabetes is controversial. In humans, as resistin is primarily produced in peripheral blood monocytes and its levels correlate with IL-6 concentrations,[74] the question of its inflammatory role has been raised.[75,76]

Four genes encode for resistin in the mouse and two in humans.[77] The human resistin gene is localized on chromosome 19 and the mouse resistin gene on chromosome 8. Results of studies investigating genetic variations in the resistin gene, including single-nucleotide polymorphisms, are controversial. Some genetic case control studies demonstrated genetic variations in the resistin gene are associated with insulin resistance and obesity in humans.[78–80] Others show that the very low resistin mRNA expression in isolated human adipocytes does not correlate consistently with insulin resistance or obesity, making the role of human resistin in insulin resistance unclear.[81,82] No differences have been observed in resistin expression in adipocytes from normal, insulin-resistant, and type 2 diabetic individuals.[74,83,84] Mc Ternan *et al.* reported greater resistin mRNA expression in fat depots in the abdomen than in the thigh, suggesting human resistin could play a role in obesity-related insulin resistance.[85]

Plasminogen activating inhibitor-1

Plasminogen activating inhibitor (PAI)-1, synthesized in the liver and in adipose tissue, regulates thrombus formation by inhibiting the activity of tissue-type plasminogen activator, an anticlotting factor. PAI-1 serum concentrations increase with visceral adiposity, decline with caloric restriction, exercise, weight loss and metformin treatment.[86] Omental tissue explants secrete significantly more PAI-1 than subcutaneous tissue from the same subject.[87]

The Insulin Resistance Atherosclerosis Study examined the link between PAI-1 and the incidence of type 2 diabetes over a 5-year period and observed that PAI-1, which is known to be related to features of the insulin resistance syndrome, appeared to be an early inflammatory marker of type 2 diabetes. PAI-1 levels are higher in subjects converting from insulin resistance to diabetes and are independent of insulin sensitivity and body mass index.[88]

Angiotensinogen

Hypertension, a major risk factor for cardiovascular diseases, is frequently associated with obesity and insulin resistance. Epidemiological studies 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, adipose tissue is the major extrahepatic source of AGE and could raise circulating levels in obese individuals. The pathophysiological impact of adipose tissue production emerged when the AGE gene was specifically inserted into adipose tissue in murine models. In wild-type mice, over-expression of AGE mRNA in adipose tissue resulted in elevated plasma AGE, hypertension, and increased adipose mass. In AGE-null mice, which are hypotensive and lean, re-expression of AGE mRNA in adipose tissue restored adipose tissue mass and normal blood pressure.[89] In addition, AGE-deficient mice are partially protected from diet-induced obesity.[89] These experimental models support the hypothesis that adipose production of AGE increases circulating levels in obese subjects, thereby favouring hypertension.

Increased AGE production could also contribute to enhanced adipose mass because angiotensin II is believed to act locally as a trophic factor for new adipose cell formation.[90]

Aromatase

In human adipose tissue, aromatase activity is principally expressed in mesenchymal cells with an undifferentiated preadipocyte phenotype.[91]

P450 aromatase, a haem protein product of the *CYP19* gene, converts androstenedione to oestrone. Oestrogen production in fat rises with body weight and ageing.[92] Adipose tissue-derived oestrogens drive fat to subcutaneous and breast tissues, whereas androgens promote central or visceral fat accumulation.[93,94]

11-Hydroxysteroid dehydrogenase

11-β-hydroxysteroid dehydrogenases (11-β-HSDs) catalyse interconversion of active cortisol and inert cortisone. Two isoenzymes have been discovered, each with unique properties and powerful biological roles. 11-β HSD-1 regenerates metabolically active cortisol from cortisone in humans (and corticosterone from II dehydrocorticosterone in mice) and is increased in adipose tissue from obese subjects. 11-β-HSD-2 potently inactivates cortisol, protecting key tissues.[95] Both 11-β-HSD1 and 11-β-HSD2 are located at the endoplasmic reticulum (ER). 11-β-HSD1 has one short N-terminal transmembrane region with the catalytic domain protruding into the ER lumen; the N-terminus of 11-β-HSD2 is lumenal with the catalytic domain facing the cytoplasm.[96]

Compared with their lean littermates, *ob*/*ob* mice have reduced hepatic 11-β-HSD1 activity but a higher corticosterone level in liver because of their elevated plasma corticosterone. Consequently, liver phosphoenolpyruvate carboxykinase expression is elevated at least partly contributing to hyperglycaemia. In Zucker rats, 11-β-HSD1 activity is decreased in liver but increased in omental fat, a pattern similar to ob/ob mice.[97] However, hepatic 11-β-HSD1 activity is marginally increased in *db*/*db* mice.[98]

Corticosterone from adipose tissue is increased by 30% overproduced in transgenic mice modestly over-expressing 11-HSD in all adipose tissues. These mice accumulate visceral fat in adipocytes that are three times larger than controls and become hyperphagic, hyperglycaemic and hyperinsulinaemic. All had reduced levels of adiponectin and increased concentrations of leptin, TNF, angiotensinogen and FFA. These clinical and biochemical patterns mimic the human metabolic syndrome.[99]

Several observations have associated adipose 11-β-HSD1 activity with obesity, insulin resistance and other features of the metabolic syndrome in different groups of obese men and women.[100] However,

no difference in 11-β-HSD1 activity was detected between obese type 2 diabetes patients and their obese controls, suggesting 11-β-HSD1 dysregulation probably associates more closely with obesity than with the diabetic phenotype.[101]

Adipokines, inflammation and atherosclerosis

Obesity, associated with unfavourable changes in adipokine expression such as increased levels of TNF-α, IL-6, resistin, PAI-1 and leptin, and reduced levels of adiponectin affect glycaemic homeostasis, vascular endothelial function and the coagulation system, thus accelerating atherosclerosis. Adipokines and a 'low-grade inflammatory state' may be the link between the metabolic syndrome with its cluster of obesity and insulin resistance and cardiovascular disease.

In fact, atherosclerosis is now recognized as an 'inflammatory' process of the arterial wall. Monocytes adhere to the endothelium and then migrate into the subendothelial space where they become foam cells loaded with oxidized lipoproteins. Foam cell production of metalloproteinases leads to rupture of the atherosclerotic plaque's fibrous cap and then to rupture of the plaque itself.[102] Thus, an 'inflammatory' process accounts both for the development and evolution of atherosclerosis.

In this inflammatory process, adipokines play multiple roles. TNF-α activates the transcription factor nuclear factor-κβ, with subsequent inflammatory changes in vascular tissue. These include increased expression of intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1,[103,104] which enhances monocyte adhesion to the vessel wall, greater production of MCP-1 and M-CSF from endothelial cells and vascular smooth muscle cells[105,106] and up-regulated macrophage expression of inducible nitric oxide (NO) synthase, interleukins, superoxide dismutase, etc.[107,108] Leptin, especially in the presence of high glucose, stimulates macrophages to accumulate cholesterol.[109] IL-6 exerts proinflammatory activity in itself and by increasing IL-1 and TNF-α.[110] Importantly, IL-6 also stimulates liver production of C-reactive protein, which, is considered a predictor of atherosclerosis.[111] IL-6 may also influence glucose tolerance by regulation of visfatin. Visfatin, a newly discovered adipocytokine in the human visceral fat, exerts insulin-mimetic effects in cultured cells and lowers plasma glucose levels in mice through activation of the insulin receptor.[112]

PAI-1 concentrations, which are regulated by the transcription factor nuclear factor-κβ, are abnormally high in hyperglycaemia, obesity and hypertriglyceridaemia,[113] because of the increased PAI-1 gene expression.[114] PAI-1 inhibits fibrin clot breakdown, thereby favouring thrombus formation upon ruptured atherosclerotic plaques.[115] In humans, circulating PAI-1 levels correlate with atherosclerotic events and mortality, and some studies suggest PAI-1 is an independent risk factor for coronary artery disease.[116] Angiotensinogen is a precursor of angiotensin II (AngII), which stimulates ICAM-1, VCAM-1, MCP-1 and M-CSF expression in vessel wall cells.[117] AngII also reduces NO bioavailability[118] with loss of vasodilator capacity and with increased platelet adhesion to the vessel wall.

In humans, endothelial dysfunction is indicative of the preclinical stages of atherosclerosis and is prognostic of future cardiovascular events.[119,120] High concentrations of proinflammatory adipokines may contribute to development of endothelial dysfunction. At this stage of disease, the role of resistin is particularly interesting. *In vitro* studies show resistin 'activates' the endothelial cell which, when incubated with recombinant human resistin, releases more endothelin-1 and VCAM-1·[121] Recombinant human resistin is also reported to induce higher expression of mRNA of VCAM, ICAM-1 and pentraxin-3 from endothelial cells[122] thus expressing a biochemical pattern of dysfunctional endothelium. Finally, resistin also induces proliferation of aortic smooth muscle cells.[123] In asymptomatic patients with a family history of coronary heart disease, plasma resistin levels are predictive of coronary atherosclerosis even after control for other established risk factors.[76,124]

In conclusion, the molecular effects of adipokines are a challenging area of research and in-depth understanding of their pathophysiology and molecular actions will undoubtedly lead to the discovery of effective therapeutic interventions. Reducing adipose tissue mass and consequently adipokine concentrations will prevent the metabolic syndrome and, if the hypothesis of adipokine-related linkage with atherosclerosis is proven, help prevent the development of atherosclerosis. Despite the new findings in the field of adipokines, researchers are still led to focus back on obesity as an essential primary target in the continued effort to reduce the risk of developing the metabolic syndrome and type 2 diabetes, with its associated cardiovascular complications.

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