REVIEW

Adipose tissue as an endocrine organ: impact on insulin resistance

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ABSTRACT

It is well known that obesity is associated with insulin resistance and an increased risk for type 2 diabetes mellitus. Formerly it was postulated that increased lipolysis and consequently free fatty acid (FFA) production, from with triglycerides overloaded fat cells, would disrupt glucose homeostasis via Randle's hypothesis. Lipodystrophy, however, also leads to insulin resistance. Recently it has become clear that adipose tissue functions as an endocrine organ and secretes numerous proteins in response to a variety of stimuli. These secreted proteins exert a pleiotropic effect. The proteins that are involved in glucose and fat metabolism and hence can influence insulin resistance are discussed in this paper. They include leptin, resistin, adiponectin, acylation-stimulating protein, tumour necrosis factor-α and interleukin-6. The stimuli for production and the site and mechanism of action in relation to insulin resistance will be discussed. None of these proteins are, however, without controversy with regard to their mechanism of action. Furthermore, some of these proteins may influence each other via common signalling pathways. A theory is presented to link the interrelationship between these adipocyte secretory products and their effect on insulin resistance.

LIST OF ABBREVIATIONS

Acrp 30	Complement-related protein 30
AgRP	Agouti-related protein
AMPK	Adenosine monophosphate kinase
ADD1/SREBP	Adipocyte determination and differentiation factor/sterol regulatory element-binding protein
aP2	Fatty acid-binding protein
apM1	Adipose most abundant gene transcript-1
ASP	Acylation-stimulating protein
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BMI	Body mass index
CART	Cocaine-amphetamine-related transcript
C/EBP	CCAAT (is piece of DNA)/enhancer-binding proteins
CNS	Central nervous system
COS cells	Monkey cells immortalised with simian V40 virus
CRH	Corticotropin-releasing hormone
Cys	Cysteine
DAG	Diacetylglycerol
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
FAS	Fatty acid synthase
FFA	Free fatty acid
FIZZ	Found in inflammatory zone
Gdp 28	Gelatin-binding protein
GLUT-4	Glucose transporter-4
IL-6	Interleukin-6
IRS-1	Insulin receptor substrate-1
JAK	Janus kinase
α-MSH	Alpha-melanocyte-stimulating hormone
mRNA	Messenger ribonucleic acid
NEFA	Non-esterified fatty acids
NPY	Neuropeptide Y
PEPCK	Phospo-enolpyruvate carboxykinase
PI3K	Phosphatidylinositol-3 phosphate
POMC	Pro-opiomelanocortin
Ob-Rb	Long isoform of the leptin receptor
RELM	Resistine-like molecule
PPAR-γ	Peroxisome proliferator-activated receptor γ
RXR	Retinoid X receptor
STAT	Signal transducers and activators of transcription
TG	Triglycerides
TNF-α	Tumour necrosis factor alpha
TZDs	Thiazolidinediones
WAT	White adipose tissue

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INTRODUCTION

Type 2 diabetes mellitus is a chronic disease characterised by insulin resistance of the muscle, liver and adipose tissue and an impaired function of the β-cell of the pancreas.[1] The incidence of type 2 diabetes mellitus (type 2 DM) has increased dramatically over the last decades. Nowadays it is the most frequently occurring metabolic disease, affecting over 140 million people worldwide with an expected rise to about 300 million patients in 2025.[2] Epidemiological studies assessing the explanation for this explosion point to an excess caloric intake over metabolic demand and decreased physiological activity as plausible causes. A chronic imbalance between energy intake and energy expenditure eventually leads to obesity, a condition predisposing to insulin resistance and type 2 DM. Of type 2 diabetic patients, 80% are obese as defined by a body mass index >27 kg/m^2.[3]
In the past, adipose tissue was merely viewed as a passive organ for storing excess energy in the form of triglycerides. Recently, however, it has become clear that the adipocyte actively regulates the pathways responsible for energy balance and that this function is controlled by a complex network of hormonal and neuronal signals.
To discuss all the adipocyte secretory products (*table 1*) and all their effects is beyond the scope of this paper. In this review we will focus on the function of the adipocyte in relation to insulin resistance and obesity. First the differentiation process of the adipocyte will be discussed. Then some of the adipocyte secretory products that are involved in energy balance regulation and their function will be considered. Finally, some interactions between adipocyte-derived factors that could be involved in inducing insulin resistance will be described.

ADIPOCYTE DIFFERENTIATION

There are two forms of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). BAT serves primarily to dissipate energy, which is done via uncoupling protein 1 (UCP-1) in the mitochondria of BAT. Adult humans only have a small amount of BAT. WAT stores energy in the form of triglycerides. It has recently become evident that WAT also secretes a vast amount of so-called adipocytokines, which are involved in maintaining energy homeostasis. This will be discussed in this article.
In humans, the formation of white adipose tissue (WAT) begins during late embryonic development, with a rapid expansion shortly after birth as a result of increased fat cell size as well as fat cell numbers. Even in adults the potential to generate new fat cells persists. The origin of the adipose cell and adipose tissue are still poorly understood. Our current understanding indicates that a

Table 1
Proteins secreted by adipocytes

MOLECULE	EFFECT
Leptin*	Feedback effect on hypothalamic energy regulation; maturation of reproductive function
Resistin*	Appears to impair insulin sensitivity
Adiponectin*	Improves insulin sensitivity if administered to rodent models of insulin resistance; improves fatty acid transport and utilisation
Adipsin*	Required for the synthesis of ASP, possible link between activation of the complement pathway and adipose tissue metabolism
ASP*	Activates diacylglycerol acyltransferase, inhibits hormone sensitive lipase, stimulates GLUT-4 translocation to the cell surface
TNF-α*	Mediator of the acute phase response. Inhibits lipogenesis, stimulates lipolysis and impairs insulin-induced glucose uptake, thus leading to insulin resistance and weight loss
IL-6*	Increases hepatic glucose production and triglyceride synthesis, role in insulin resistance unclear
PAI-1	Potent inhibitor of the fibrinolytic system
Tissue factor	Initiator of the coagulation cascade
Angiotensinogen	Regulator of blood pressure and electrolyte homeostasis
PGI_2 and $PGF_2\alpha$	Implicated in inflammation and blood clotting, ovulation and menstruation, acid secretion
TGF-β	Regulates growth and differentiation of numerous cell types
IGF-1	Stimulates cell proliferation and mediates many of the effects of growth hormone
MIF	Involved in proinflammatory processes and immunoregulation
aP_2	Involved in intracellular trafficking and targeting of fatty acids
Agouti	Might be involved in inducing insulin resistance through increasing intracellular free calcium concentrations

* *Proteins discussed in this article.*

pluripotent stem cell precursor gives rise to a mesenchymal precursor cell, which has the potential to differentiate along mesodermal lineages of myoblast, chondroblast, osteoblast and adipocyte (*figure 1*).[4] Given appropriate stimuli the preadipocyte undergoes clonal expansion and subsequent terminal differentiation into a mature adipocyte. *In vitro*, adipogenesis follows an orderly and well-characterised temporal sequence.[4,5] Initially there is growth arrest of proliferating preadipocytes induced by the addition of a prodifferentiative hormonal mixture (including insulin, a glucocorticoid, an agent that elevates cAMP levels and foetal bovine serum). Growth arrest is followed by one or two rounds of cell division, known as clonal expansion. At about the second day after differentiation induction there is a second, permanent period of growth arrest. Growth-arrested cells are committed to becoming adipocytes and begin to express late markers of adipocyte differentiation at day 3. Cells eventually become spherical, accumulate fat droplets and become terminally differentiated adipocytes by day 5 to 7.

Most of the changes that occur during adipocyte differentiation take place at the gene expression level. Several reports[4,5] have attempted to schematise the stages of adipocyte differentiation as we have here in *figure 1*.

Three major classes of transcription factors that directly influence fat cell development have been identified: the peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer binding proteins (C/EBPs) and the basic helix-loop-helix family (ADD1/SREBP-1c).
The C/EBPs belong to the basic-leucine zipper class of transcription factors which function through homodimeric and heterodimeric complexes with C/EBP family members. Six isoforms have been identified with varying tissue distribution. C/EBP α, β and δ are expressed in both white and brown adipose tissue and are involved in the regulation of adipogenesis.[5]
The peroxisome proliferator-activated receptor (PPAR) belongs to the nuclear hormone receptor family. Three isotypes have been identified thus far, PPAR α, β and γ, each with a different tissue distribution, ligand and metabolic action. All PPARs form a heterodimer with the retinoid X receptor (RXR) and bind to a PPAR-RXR response element on the DNA. Their actions upon ligand binding, however, are completely different. PPAR-γ exists as three isoforms, γ1,γ2 and γ3. PPAR-γ2 is highly expressed in adipose tissue. The thiazolidinediones (a new class of oral blood glucose lowering drugs), which are high-affinity synthetic ligands for PPAR-γ, strongly induce adipogenesis and activate the expression of multiple genes encoding

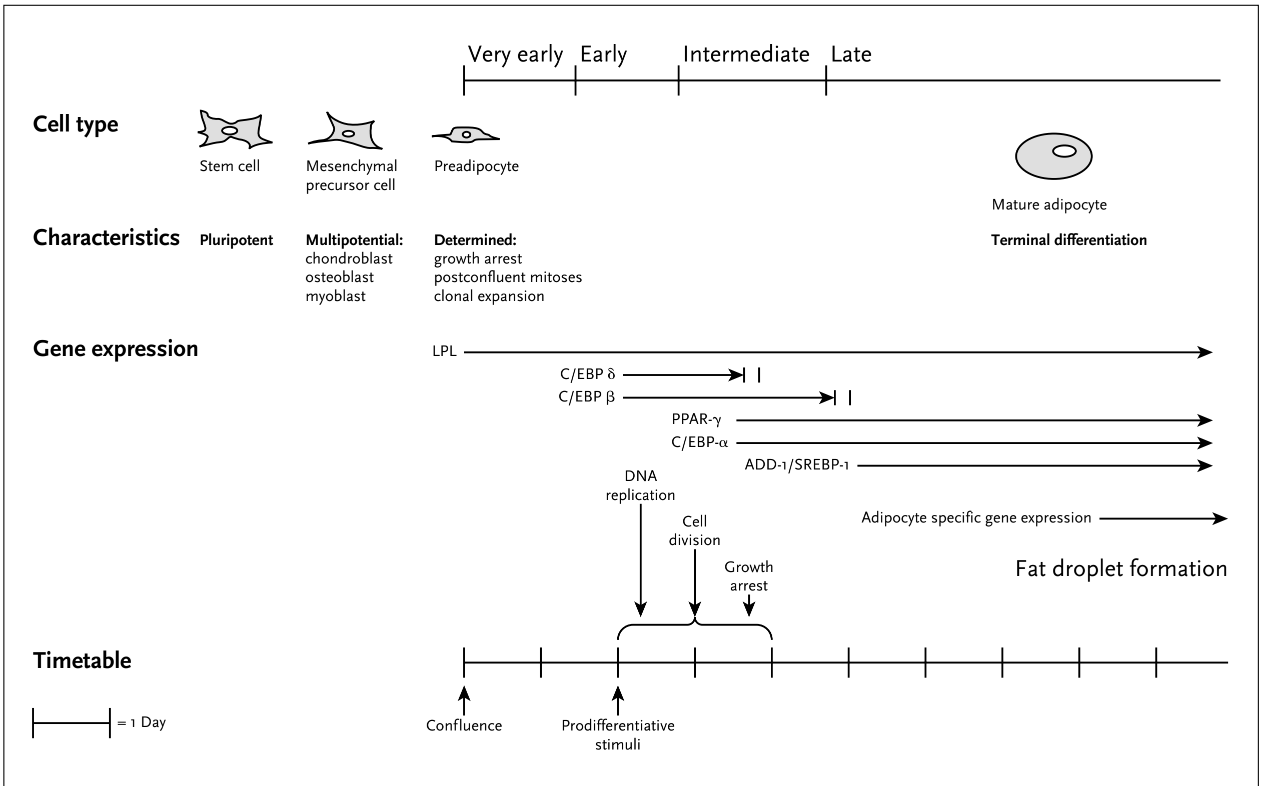

Figure 1
Addition of mitogens and hormonal stimuli to 3T3-L1 cells leads to a cascade of transcriptional events that account for the expression of most proteins-mediating adipocyte function
See text on the first three pages of this review for explanation.

for proteins involved in lipid and glucose metabolism.[6,7] Adipocyte determination and differentiation factor 1 (ADD1) and sterol regulatory element binding protein 1c (SREBP-1), which are rodent and human homologues respectively, belong to the basic helix-loop-helix (bHLH) family of transcription factors. ADD1/SREBP-1c is expressed in brown adipose tissue, the liver, WAT and the kidney.[5] The expression of ADD1/SREBP-1c is increased early during adipocyte differentiation.[4,5] The protein seems to exert its adipogenic effect through upregulation of PPAR-γ. Furthermore the protein might be involved in the production of an endogenous ligand for PPAR-γ.[8] In addition to its effect on adipogenesis, ADD1/SREBP-1c clearly stimulates many genes involved in fatty acid and cholesterol metabolism.[9] A summary of the molecular events of adipocyte differentiation, based on our current knowledge, is depicted in *figure 1* and *2*.

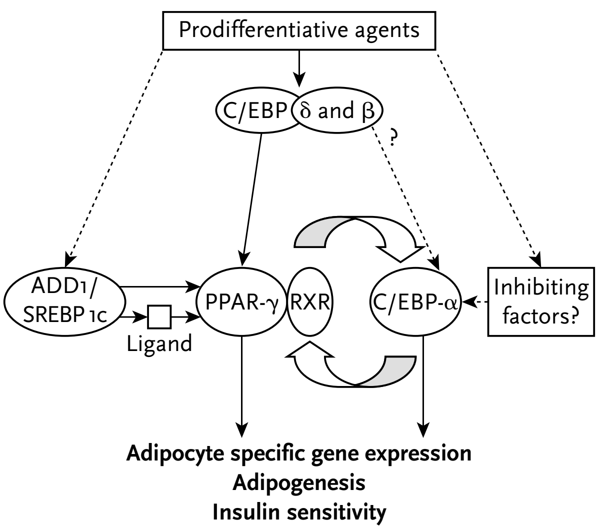

Figure 2[4,5]

Solid lines indicate direct or indirect transcriptional events. Broken lines indicate less clear interactions. The addition of prodifferentiative agents to 3T3-L1 cells leads to a significant and transient increase of the transcription factors C/EBP β and δ, which in turn mediate the expression of another transcription factor: PPAR-γ. PPAR-γ is also activated by ADD1-SREBP1c[8] although the events leading to the activation of ADD1/SREBP1c are not fully understood. PPAR-γ on turn activates C/EBP-α, these two proteins seem to cross regulate each other, thus maintaining their gene expression despite a decline in C/EBP β and δ. Activation of PPAR-γ and C/EBP-α leads to the expression of many adipocyte specific proteins involved in glucose and lipid metabolism (LPL, aP2, fatty acid synthase, etc), adipocyte differentiation and an increase in insulin sensitivity, either via a decrease in triglycerides and fatty acids or via a direct effect on proteins involved in glucose metabolism (PEPCK, GLUT-4).

ADIPOCYTE SECRETORY PRODUCTS

Leptin

Discovery, structure, genetic locus and sites of expression of leptin

The discovery of leptin (from the Greek *leptos* which means thin) in 1994[10] has led to a renewed and intensified interest in the adipocyte and its role in energy homeostasis. Leptin acts on hypothalamic neuropeptide-containing regions and increased leptin signalling leads to decreased food intake, increased energy expenditure and increased thermogenesis, all promoting weight loss. Apart from these effects, leptin is also involved in glucose metabolism, normal sexual maturation and reproduction, and has interactions with the hypothalamic-pituitary-adrenal, thyroid and growth hormone axes.
Leptin is a protein consisting of 167 amino acids and has a helical structure similar to cytokines. Leptin is the product of the *ob* gene, which is located on chromosome 7q31. Leptin is expressed mainly in white adipose tissue. The protein circulates as both free and bound hormone and is cleared among others by the kidneys.[11-13]

Modulators of leptin production[12,13]

Leptin levels are positively correlated with the amount of energy stored as fat, so leptin levels are higher in obese people.[14,15] Leptin levels rapidly decrease during fasting[16] and remain low until four to six hours after eating when they begin to rise again.[17] Plasma leptin levels show a diurnal pattern with a nocturnal peak shortly after midnight and a midmorning trough between 10 am and 12 noon.[18] Insulin also plays a role in the regulation of leptin secretion: prolonged insulin infusions markedly increase serum leptin levels.[19,20] Finally, even after adjustment for body fat mass, women have higher serum leptin levels than men.[15] At the gene promotor level, it is known that stimulation of PPAR-γ downregulates leptin production[21] whereas C/EBP-α stimulates leptin production.[22]

Site of action of leptin and its role as part of an adipostat

Leptin acts through binding at and activation of specific leptin receptor isoforms, which belong to the class I cytokine receptor family.[23] Only the long isoform (*ob-rb*) is able to activate the JAK-(Janus kinase)-STAT (signal transducers and activators of transcription) signal transduction pathway upon leptin binding (*figure 3*). The long form of the leptin receptor is found in several peripheral tissues and in many areas of the brain, including the arcuate, ventromedial and dorsomedial hypothalamic nuclei.[24] These hypothalamic regions are known to be involved in the regulation of appetite, food intake, temperature regulation and body weight. Intracerebral administration of leptin alters the expression of many hypothalamic neuropeptides.[25] By modulating these neurotransmitter systems, leptin has

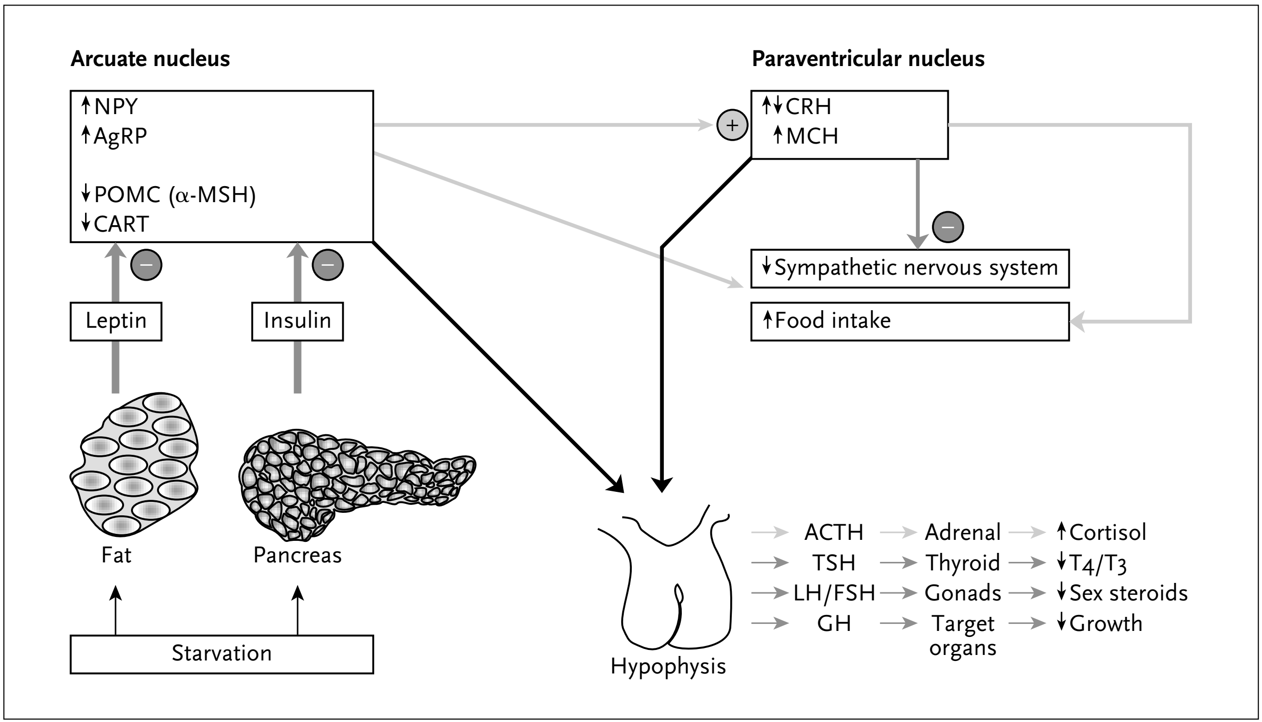

Figure 3

Starvation leads to a decrease in serum insulin levels and a decreased expression of the *ob*-gene leading to a decrease in serum leptin levels. This subsequently leads to an increased expression of neuropeptide-Y and agouti-related protein in the hypothalamus and a decrease in POMC and CART in the hypothalamus (see list of abbreviations for explanation). These hormones are involved in food intake and energy expenditure, leading to an increase in food intake and a decrease in energy expenditure. Furthermore, the hypothalamic hormones have either a direct or an indirect (via CRH and MCH) effect on various hormones secreted by the pituitary. Thus leptin has multiple effects, not only on food intake and energy metabolism but also on the hypothalamic-pituitary-adrenal axis, thyroid function and sex steroids. (Dark grey is inhibition, light grey is stimulation.)

a major role in maintaining energy balance and thus serves as part of an adipostat. During fasting, serum insulin levels fall and the uptake of glucose and lipids by the adipocyte diminishes. This leads to a decreased expression of the *ob*-gene, which is responsible for leptin formation and hence the plasma leptin concentration falls. Reduced leptin signalling leads to an increased expression of neuropeptide Y (NPY) and agouti-related protein (AgRP) in the arcuate nucleus of the hypothalamus. NPY and AgRP promote body weight gain by stimulating food intake and decreasing energy expenditure. Another neuronal cell type coproduces cocaine-amphetamine related transcript (CART) and pro-opiomelanocortin (POMC), from which α-melanocyte stimulating hormone (α-MSH) is cleaved. CART and α-MSH are both anorexigens and reduced leptin signalling inhibits the synthesis of CART and POMC (*figure 3*).[26,27] Finally, corticotropin-releasing hormone (CRH), which is also produced in the hypothalamus, might be important in mediating the effects of leptin, presumably via activation of sympathetic outflow to BAT, WAT, liver and muscle. Intracerebral injection of CRH stimulates thermogenesis and oxygen consumption and reduces food intake and body weight. CRH mRNA levels are increased by the intraventricular administration of leptin.[28]

Role of leptin in obesity

The initial conception of leptin as an anti-obesity hormone, whose primary role was to increase the metabolic rate and decrease food intake and appetite through action in the brain, was based on the following observations: 1) leptin deficient *ob/ob* mice and leptin receptor deficient *db/db* mice exert marked hyperphagia, decreased energy expenditure, morbid obesity and insulin resistance;[29,30] 2) administration of intravenous or intracerebroventricular leptin decreases body weight and fat mass through inhibition of food intake and increased energy expenditure in *ob/ob* but not in *db/db* mice;[31] 3) there is a threshold level of serum leptin (25-30 ng/ml) above which increases in serum levels are not translated into proportional increases in cerebrospinal or brain leptin levels, i.e. the transport system must be saturable;[32] 4) the discovery of leptin receptors in the hypothalamus, the region involved in regulation of food intake and energy balance.[27] However, in most obese humans the gene encoding leptin is normal: up till now only two families with a mutation in the leptin gene have been identified.[33,34] In contrast, most obese humans have increased serum leptin levels,[14,15] indicating that obesity is a leptin-resistant state. Such a resistance could theoretically occur at several levels of the leptin signal transduction pathway, but this has not been resolved yet.

Leptin and insulin resistance.

Since obesity is associated with insulin resistance, it is interesting to look at the role of leptin in the development of insulin resistance and diabetes. A strong correlation between serum leptin and insulin levels, independent of body fatness, has been demonstrated in human studies.[35,36] Hyperinsulinaemia induced by clamp techniques increases serum leptin levels, though not acutely.[19] Serum leptin levels are increased by insulin therapy as well, both in type 1 and type 2 diabetic patients.[36,37] Vice versa, a fair amount of evidence points to the fact that leptin has insulin- and glucose-lowering properties, although some studies find just the opposite. An extensive review on the association between leptin and insulin resistance has recently been published.[38] In both normal rodents[39] and rodents with obesity and insulin resistance[40-42] leptin therapy improves hyperinsulinaemia and hyperglycaemia. These effects are already apparent before weight loss occurs and are not due to energy restriction as was shown in pair-fed control studies.[41,43] Most obese humans have increased serum leptin levels[14,15] and thus far the overall effect of leptin therapy on weight loss and metabolic parameters has been modest.[44] It is likely that very high plasma levels of the hormone are needed to overcome the leptin-resistant state. A final point pointing to an antidiabetogenic effect of leptin is that both in lipodystrophic rodents[45] and humans (who have an extreme deficit of subcutaneous adipose tissue),[46] a condition associated with severe insulin resistance with hyperglycaemia, hyperinsulinaemia and hypertriglyceridemia, leptin therapy corrects all these metabolic abnormalities, independent of the accompanying reduction in food intake.

Hypotheses with regard to the glucose and insulin-lowering effect of leptin

As mentioned before, leptin seems to have an insulin-sensitising effect on the whole body level but conflicting results were reported when individual tissues were examined. Most *in vitro* experiments suggest a diabetogenic effect of leptin.[38] Beside the differences between animals and humans, sources of leptin and time of exposure to this hormone might also play a causative role in the differences found. Furthermore, the fact that leptin exerts a glucose- and insulin-lowering effect and improves insulin sensitivity *in vivo*, suggests involvement of centrally acting mechanisms. This concept is further supported by the observation that leptin fails to reverse insulin resistance and lipid accumulation in mice with ventromedial hypothalamic lesions.[47] The peripheral mechanism by which leptin exerts its glucose- and insulin-lowering effect might be via promoting fatty acid oxidation and triglyceride synthesis. Indeed, leptin administration activates 5'-AMP-activated protein kinase (AMPK) in skeletal muscle, leading to the inhibition of acetyl coenzyme A carboxylase and subsequently stimulation of fatty acid oxidation. The resulting intramyocellular lipid depletion will enhance insulin sensitivity.[48] Apart from insulin-sensitising effects, leptin diminishes hyperinsulinaemia, probably via inhibition of insulin secretion. Functional leptin receptors have been demonstrated on insulin-secreting β-cells of the pancreas.[49] Leptin inhibits glucose-stimulated insulin secretion both *in vitro*[50] and *in vivo*.[51] The mechanism involved is activation of the ATP-sensitive potassium channels in the β-cell. Finally, leptin shares intracellular pathways with insulin, both in peripheral tissues and in the central nervous system[52] Many effects of both insulin and leptin are mediated via activation of PI-3 (phospahtidylinositol-3-phosphate) kinase, so a degree of crosstalk between insulin and leptin may exist at the level of PI-3 kinase. Effects of leptin on insulin signalling have been studied and support an inhibitory effect of leptin on insulin signalling at the level of tyrosine phosphorylation of IRS-1 (insulin receptor substrate 1) and PI3-kinase binding to IRS-1.[38] The effect of hyperinsulinaemia on intracellular leptin signalling has rarely been addressed but in one study supraphysiological concentrations of insulin completely cancelled out the leptin-induced insulin response.[53]

Conclusion

Thus, leptin is an adipocyte secretory product that is not only involved in food intake and energy metabolism but clearly also has a role in glucose metabolism. Since plasma leptin levels are positively correlated with BMI, obesity seems to reflect a leptin-resistant state. Resistance for the action of leptin could promote obesity via decreased energy expenditure and a failure to diminish food intake. Furthermore, since leptin has a glucose- and insulin-lowering effect on the whole body level *in vivo*, resistance for this effect could induce insulin resistance. One explanation for the insulin resistance seen in obesity might be that the high leptin levels interfere with insulin signalling. Another possibility is that there is a diminished activation of AMPK in myocytes due to impaired leptin signalling. The resultant decrease in fatty acid oxidation will lead to an increase in intramyocellular lipids and thus to insulin resistance. Finally, both peripheral and central leptin resistance must be involved in insulin-resistant states since leptin treatment fails to correct insulin resistance in mice with ventromedial hypothalamic lesions.

Resistin

Discovery, structure, genetic locus, sites and modulators of expression of resistin

Resistin is a unique protein with cysteine-rich residues,[54] which belongs to a class of tissue-specific secreted proteins termed the RELM (resistin-like molecule)/FIZZ (found in inflammatory zone) family. Resistin/FIZZ 3 is specifically expressed and secreted by adipocytes. The gene encoding resistin in mice has been named *Retn*. The regulation of resistin gene expression is controversial, see *table 2*.

Resistin in obesity and insulin resistance

The initial report by Steppan *et al.*[54] suggested that resistin might constitute the link between obesity and insulin resistance. Resistin serum levels were increased in obese mice and resistin gene expression was induced during adipocyte differentiation. In addition, administration of resistin impaired glucose tolerance and insulin action in wild-type mice and *in vitro* in 3T3-L1 adipocytes whereas antiresistin antibody improved insulin sensitivity. The fact that thiazolidinediones suppressed resistin secretion led to the hypothesis that these insulin sensitisers exert their effect via downregulation of resistin gene expression. An increase in adipocyte gene expression during 3T3-L1 adipocyte differentiation[61] and after the induction of high-fat-diet induced obesity[57] was found in two other studies. Several other investigators, however, found a decreased resistin gene expression in WAT in different models of rodent obesity and insulin resistance,[59,64,65] and resistin did not seem to be involved in the aetiology of insulin resistance in Fischer 344 rats, a good model for the metabolic syndrome in humans.[66] Studies in humans are even more controversial. One study could not detect any resistin mRNA in human fat cells at all in subjects with varying degrees of insulin resistance and obesity.[67] Another investigator found increased resistin mRNA in adipose tissue of obese humans, compared with lean controls, but decreased mRNA in freshly isolated human adipocytes.[60] In addition resistin mRNA was undetectable in a severely insulin resistant subject. Janke *et al*. found an increased resistin gene expression in cultured human preadipocytes compared with mature adipocytes but again no relationship between resistin gene expression and either insulin resistance or body weight could be detected.[68] Although the higher resistin mRNA levels found in abdominal fat tissue compared with thigh could explain the increased metabolic abnormalities in abdominal obesity, the fact that resistin mRNA expression is very similar in subcutaneous and omental adipose tissue suggests that it is unlikely that resistin is the link between (visceral) adiposity and insulin resistance.[69]

Conclusion

The conclusion must be that many questions still have to be resolved. Conflicting results have been reported with regard to the factors regulating resistin gene expression (*table 2*). This is probably due to the difference between 3T3-L1 cell lines and *in vivo* models. Furthermore, the observed relation between resistin mRNA, serum resistin levels and insulin resistance in rodents cannot readily be extrapolated to humans. Murine resistin is only about 56% identical to human resistin at the amino acid level. Even in mouse models it is still unclear whether resistin plays a causal role in insulin resistance. Experiments in resistin knockout mice and in transgenic mice (which overexpress resistin) will be needed to solve this problem, but even then the relevance of resistin to human diabetes remains unclear, especially because some groups have found only minimal expression of the hormone in human fat.[69] Furthermore it would be interesting to know how resistin exerts its presumed insulin-antagonising effects and what its target organs are. For that purpose the resistin receptor would have to be found and downstream signalling pathways have to be unravelled.

Adiponectin

Discovery, sites of expression and stimuli leading to adiponectin production

Adiponectin is a recently identified[70,71] adipocyte-specific secretory protein of about 30 kD that appears to be involved

Table 2
Regulators of resistin expression

FACTOR	DECREASING RESISTIN	INCREASING RESISTIN	NO EFFECT
Thiazolidinediones	[54-56,58]	[59]	[60]
Insulin	[56,58]	[59,61]	
Glucose		[58]	
Dexamethasone		[56,58]	
β-adrenergic agonists	[62]		[56]
TNF-α	[58,63]		
Epinephrine	[58]		

Factors that have been reported to increase or decrease resistin expression with their references.

in the regulation of energy balance and insulin action and also seems to have anti-inflammatory and anti-atherogenic properties. Adiponectin is the product of the adipose tissue most abundant gene transcript-1 (apM1), which is exclusively expressed in WAT and is located on chromosome 3q27. Adiponectin is specifically expressed during adipocyte differentiation and is not detectable in fibroblasts. The expression of adiponectin is stimulated by insulin,[70,72] IGF-1[72] and the TZDs. Corticosteroids,[72] TNF-α[74] and β-adrenergic stimulation[75] inhibit adiponectin gene expression in 3T3-L1 adipocytes.

Serum and mRNA levels of adiponectin in obesity and insulin resistance

Serum adiponectin levels are decreased in humans with obesity[76,77] and type 2 diabetes[76,78] as well as in obese and insulin-resistant rodents.[79] In addition, adiponectin gene transcription is decreased in adipocytes from obese[71] and diabetic[80] humans and rodents.[71,79] Plasma adiponectin concentrations increase after weight reduction in obese diabetic and nondiabetic patients.[78] The degree of plasma hypoadiponectinaemia was more closely related to the degree of hyperinsulinaemia and insulin resistance than to the degree of adiposity.[76] Low plasma adiponectin concentrations predicted a decrease in insulin sensitivity[81] and an increase of type 2 diabetes[82] in Pima Indians as well as in a German population.[83] In nondiabetics plasma adiponectin levels are also positively correlated with insulin sensitivity.[84] A recent study confirmed that the relation between low adiponectin levels and insulin resistance is not determined by obesity since low plasma adiponectin levels at baseline did not predict future obesity.[85]

Finally, the fact that the insulin-sensitising thiazolidinediones strongly increase plasma adiponectin[73,86] further supports a role of adiponectin in insulin sensitivity.

Theory with regard to the possible mechanism of action of adiponectin

Administration of recombinant adiponectin to normal, obese and diabetic rodents led to acute normalisation of serum glucose levels.[79,87,88] Both decreased gluconeogenesis of the liver[87] and an increased fatty acid oxidation in muscle[79,88] have been proposed as underlying mechanisms. Recently, Yamauchi underscored his previous hypothesis.[89] Administration of adiponectin led to an increase in glucose utilisation and fatty acid oxidation in cultured myocytes and in soleus muscle of mice *in vivo*. In hepatocytes AMPK was activated as well, leading to a reduction in gluconeogenesis.

In addition, it has been shown that administering only the globular domain of adiponectin instead of full-length adiponectin is much more effective in improving insulin sensitivity because this fragment augments insulin-induced phosphorylation of insulin receptor substrate 1 (IRS-1) and protein kinase B in skeletal muscle.[79] Thus, adiponectin might exert its insulin-sensitising effect via the following mechanisms: 1) increased fatty acid oxidation leading to a lower muscle triglyceride content and lower plasma concentrations of free fatty acids which will both improve insulin signalling; 2) direct improvement of insulin signalling; 3) inhibition of gluconeogenesis, partly via reduced substrate delivery and partly via reduction of molecules involved in gluconeogenesis by activation of AMPK.

Disappointingly, no positive correlation between plasma adiponectin levels and 24-hour respiratory quotient (RQ) measurement (pointing to an increase in carbohydrate metabolism) could be demonstrated in healthy nondiabetic Pima Indians.[90] This does not rule out, however, that administration of adiponectin to subjects with low levels of this hormone will increase RQ and energy expenditure.

The acylation-stimulating protein (ASP) pathway

ASP production and site of action

Acylation-stimulating protein (ASP) is a 76 amino acid protein identical to C3adesArg, a cleavage product of complement factor 3 (C3) formed via interaction of C3 with factor B and adipsin. C3, factor B and adipsin are all components of the alternative complement pathway and are produced by the adipocyte in a differentiation dependent manner.[91]

The major site of action of ASP appears to be on the adipocytes themselves, which have a specific saturable receptor for ASP.[92] In human adipocytes there are differentiation and site-specific differences in ASP binding which are proportional to the ASP response: differentiated adipocytes bind more ASP and have a greater response to ASP than undifferentiated adipocytes.[93] Furthermore, subcutaneous adipose tissue has greater affinity and greater specific binding to ASP than undifferentiated adipocytes.[94]

ASP promotes triglyceride storage

ASP promotes triglyceride storage in adipocytes via three mechanisms. First, ASP increases fatty acid esterification in adipocytes by increasing the activity of diacylglycerol acyltransferase, which is the final enzyme involved in triglyceride synthesis.[91] Second, ASP stimulates glucose transport in human and murine adipocytes and preadipocytes.[93] This effect on glucose transport is accomplished via translocation of cell-specific glucose transporters to the cell membrane. Third, ASP decreases lipolysis via inhibition of hormone-sensitive lipase.[95] The effects of ASP are independent of and additional to the action of insulin.[95]

Stimuli leading to ASP production

In vitro studies in cultured adipocytes indicate that insulin[96] and even more so chylomicrons[96,97] increase ASP production. *In vivo*, plasma ASP concentrations seem to show little change after an oral fat load.[98] There is, however, postprandially an increased venoarterial gradient of ASP across a subcutaneous abdominal tissue bed with a maximum after 3 to 5 hours, indicating increased adipose tissue ASP production.[98] This increase in ASP postprandially is substantially later than the increase in insulin but shows a close temporal relationship with maximal plasma triacylglycerol clearance.[98]

Plasma ASP levels in obesity

An excellent review on the physiology of ASP in humans and rodents has recently been published.[99] Plasma levels of ASP are 225-fold lower (weighted average 28.3 nM) than its precursor C3. Studies measuring plasma ASP levels should therefore be interpreted with caution while it might very well be that ASP acts as a paracrine hormone.[99] Plasma ASP levels are increased in obese humans[100-103] and are reduced after fasting or weight loss.[101,103] ASP has also been shown to be significantly increased in type 2 diabetes[102,104] but since type 2 diabetes is often associated with obesity this might be a confounding factor. On the other hand, plasma ASP levels were inversely correlated to glucose disposal during a euglycaemic clamp in humans.[102] Adipocytes from obese humans are as responsive to ASP as adipocytes from lean people.[105] Thus the increased levels of ASP in human obesity in the face of a similar responsiveness to ASP compared with lean subjects, may promote energy storage, leading to adiposity.

Relation between ASP enhanced triglyceride clearance and insulin resistance

ASP production is increased in obese mice. Intraperitoneal (i.p.) administration of ASP to normal mice resulted in accelerated postprandial triglyceride (TG) and nonesterified fatty acid (NEFA) clearance after an oral fat load.[106] In addition, plasma glucose levels returned faster to basal levels. C3 knockout mice (KO), which are unable to produce ASP, showed delayed plasma triglyceride clearance after an oral fat load in the absence of any change in fasting plasma TG levels. Administration of exogenous ASP enhanced plasma TG clearance.[107] Remarkably these C3 KO mice were more insulin sensitive, had a reduced fat mass and yet an increased food intake. It was later shown that the hyperphagia/leanness was balanced by an increase in energy expenditure.[108]

Conclusion

In summary, ASP promotes storage of energy as fat. Decreased ASP production decreases lipid storage and induces an obesity-resistant state and improved insulin sensitivity. Plasma ASP levels are increased in obese humans; whether this is the effect or cause of the increased adipose tissue mass remains to be elucidated. Post or propter, increased ASP levels together with a continuing responsiveness of the ASP receptor will lead to further triglyceride storage. Although enhanced fatty acid trapping will decrease free fatty acid levels and hence diminish hepatic gluconeogenesis, increased ASP functioning in skeletal muscle will lead to an increase in skeletal muscle triglyceride storage leading to insulin resistance.

Tumour necrosis factor-α (TNF-α)

Structure of TNF-α, sites of production and receptor interaction[109]

TNF-α is a cytokine produced mainly by activated macrophages in response to invasive stimuli, but also by nonimmune cells such as muscle and adipose tissue. Furthermore, TNF-α has a variety of biological effects in various tissues and cell types, and can thus be considered a multifunctional cytokine.[109]

TNF-α is produced as a 26-kD membrane-bound precursor that is proteolytically cleaved to a 17-kD soluble form.[109] The cytokine interacts with two membrane-bound receptors, a 60-kD and an 80-kD subtype also called type I and type II receptor (TNFR-1 and TNFR-2). These receptors have different cellular and tissue distribution patterns and can bind other cytokines as well. TNF-α has a higher affinity for TNFR-1 than for TNFR-2.[109] Due to the high affinity for its receptor TNF-α can act either as an autocrine or paracrine cytokine at low concentrations or as an endocrine cytokine at high concentrations.

In addition to the membrane-bound receptors, soluble forms of the two receptors exist for which TNF-α has an even higher affinity. When TNF-α is bound to these soluble receptors no interaction can take place with the membrane-bound forms and thus TNF-α action is inhibited. Therefore, the physiological role of the soluble receptors may be to regulate TNF-α action.

Modulators of TNF-α production

In macrophages and monocytes, the expression and production of TNF-α is stimulated by endotoxins such as lipopolysaccharide (LPS). LPS resulted in a fivefold stimulation of TNF-α in human adipose tissue and isolated adipocytes *in vitro*, the latter indicating that it is unlikely that the response is entirely due to macrophages and monocytes in the stromal vascular fraction of adipose tissue. Insulin and glucocorticoids did not have a significant effect on TNF-α release from human adipose tissue or isolated adipocytes *in vitro*.[110] Thiazolidinediones reduced adipocyte TNF-α release in obese rodents[111] but no effect was seen in human adipose tissue *in vitro*.[110] Since high-fat diets resulted in a significant increase in TNF-α mRNA and protein in epidydimal and retroperitoneal fat pads in rats, free fatty acids and/or triglycerides may play an important role as inducers of TNF-α expression.[112]

Effect of TNF-α on glucose and lipid metabolism

Firstly, TNF-α inhibits preadipocyte differentiation by downregulating the expression of two important adipocyte transcription factors: PPAR-γ and CEBP/α.[113] Secondly, TNF-α reduces the expression of GLUT-4, glycogen synthase and fatty acid synthase, which are essential for insulin-mediated glucose uptake and the subsequent conversion of glucose to glycogen or fatty acids. Furthermore, genes involved in the uptake of free fatty acids and the subsequent conversion to triglycerides, such as lipoprotein lipase, long-chain fatty acyl-CoA synthethase and diacylglycerol acyltransferase, were also downregulated by TNF-α.[113] The above-mentioned changes in gene expression lead to a diminished insulin-stimulated glucose uptake and an altered lipid metabolism which can, via accumulation of triglycerides in various organ systems, eventually lead to insulin resistance of the muscle and liver. In addition, insulin resistance can be induced via a direct toxic effect of TNF-α on intracellular insulin signalling.[114] TNF-α reduces the insulin-stimulated autophosphorylation of the insulin receptor in a variety of cell types. It does so by phosphorylation of serine residues at the insulin receptor substrate-1 (IRS-1); this modified IRS-1 subsequently interferes with the insulin signalling capacity of the insulin receptor.[114]

Relation between TNF-α, obesity and insulin resistance
A positive relationship between obesity, insulin resistance and adipose tissue mRNA levels of TNF-α has clearly been established in rodent models.[115] Furthermore, mice with no functional copy of the TNF-α gene (TNF-α $^{-/-}$) although developing marked obesity on a high-fat, high-energy diet, remained highly insulin sensitive compared with their control litter mates (TNF-α $^{+/+}$).[116]
In contrast to rodents, the role of TNF-α in the induction of insulin resistance in humans is less clear. Although there seems to be a positive relationship between obesity and TNF-α mRNA and protein levels in adipose tissue in humans *in vitro*,[117-119] TNF-α is expressed at much lower levels in humans compared with rodents. In addition, no difference in TNF-α concentration was found in a vein draining subcutaneous adipose tissue compared with a peripheral vein, suggesting no or very low TNF-α production *in vivo*.[120] Furthermore, circulating TNF-α concentrations in obese diabetic and nondiabetic patients are not substantially elevated.[118,121] With regard to a direct relationship between TNF-α and insulin sensitivity *in vivo*, two studies found a strong and positive correlation between adipose tissue TNF-α mRNA levels and hyperinsulinaemia.[117,118] When the relation between adipose tissue TNF-α secretion and insulin-stimulated glucose transport was examined, a strong inverse relationship was found that was independent of fat cell volume, age and BMI.[122]
However, other studies[121,123] showed no significant relationship between adipose tissue mRNA for TNF-α and insulin sensitivity. Furthermore, treatment of insulin-resistant subjects with anti-TNF-α antibodies did not improve insulin sensitivity.[124] All these results implicate that TNF-α might have an effect on insulin resistance but that it must be a local factor. Interestingly, TNF-α is also produced by muscle, and muscle TNF-α production is increased in obesity.[125] Since adipose tissue dispersed within muscle is correlated with insulin resistance, the effect of fat cell secretory products on insulin signalling in skeletal muscle cells was recently studied in a model in which muscle cells were co-cultured with adipocytes. A disturbance of insulin signalling was found, but TNF-α did not seem to be involved.[126]

Conclusion
In conclusion, TNF-α is a multifunctional cytokine produced by adipocytes in proportion to the percentage body fat. TNF-α has a variety of metabolic effects, including increased lipolysis, decreased lipogenesis and decreased insulin-stimulated glucose transport, contributing to insulin resistance. These effects are induced by modulation of genes involved in glucose and lipid metabolism. Furthermore, TNF-α directly interferes with the early steps of insulin signalling. However, the role of TNF-α in obesity-induced insulin resistance in humans is not quite clear yet, as might be obvious from the contradicting results mentioned in the previous paragraph. The low plasma levels of TNF-α in humans indicate that the hormone most likely acts in a paracrine and or autocrine manner. This might be the reason why treatment with anti-TNF-α did not improve insulin sensitivity in humans *in vivo*.

Interleukin-6 (IL-6)

Structure, genetic locus and site of production of IL-6
IL-6 is a circulating, multifunctional cytokine that is produced by a variety of cell types including fibroblasts, endothelial cells, monocytes/macrophages, T-cell lines, various tumour cell lines and adipocytes. The protein has a molecular mass of 21 to 28 kD, depending on the cellular source and preparation. The gene encoding IL-6 is localised on chromosome 7p21 in humans.[127]
Although human adipocytes produce IL-6, adipocytes accounted for only 10% of total adipose tissue IL-6 production when IL-6 production by isolated adipocytes prepared from omental and subcutaneous fat depots was examined.[128] This means that cells in the stromal vascular fraction of adipose tissue have a major contribution in adipose tissue IL-6 release. The concentrations of IL-6 in adipose tissue are up to 75 ng/ml, which is well within the range to elicit biological effects.[129] Furthermore, plasma levels of IL-6 are markedly elevated in obesity and up to 30% of plasma levels could be derived from adipocytes.[130]

Modulators of IL-6 production
The stimuli leading to IL-6 production differ with the cell type; here only IL-6 production by adipocytes will be

discussed. Both in rodent and human adipocytes, IL-6 production is stimulated by catecholamines and inhibited by glucocorticoids, whereas insulin has no effect whatsoever.[128,131,132] Finally, another stimulator of IL-6 release is TNF-α, which has been reported to produce a 30-fold[113] increase in IL-6 production in 3T3-L1 adipocytes. Interestingly, IL-6 in turn inhibits the release of TNF-α!

IL-6 acts via receptor interaction

IL-6 acts through binding at and activation of a specific receptor, belonging to the class I cytokine receptors, which act through JAK-STAT signalling (see *figure 4* where leptin signalling is explained).[133] The IL-6 receptor consists of two membrane glycoproteins, a 80-kD ligand binding component and a 130-kD signal-transducing component (gp130). The 80-kD component binds IL-6 with low affinity; this complex subsequently binds with high affinity to gp130 after which signal transduction can take place.[127] Soluble forms of the IL-6 receptor have been found but neither their functional significance nor the regulation of their production is understood.

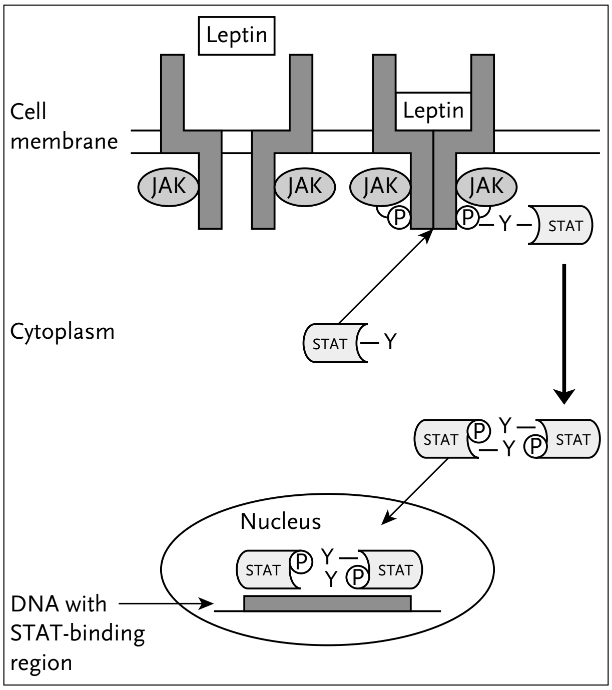

Figure 4

The leptin receptor is a transmembrane receptor belonging to the class I cytokine receptors. The receptor consists of two parts. The intracellular domain is associated with the Janus kinase, a tyrosine kinase. Binding of leptin to the receptor results in the fusion of the two receptor parts, which results in trans-phosphorylation of the JAK molecules, which subsequently phosphorylate the terminus of the leptin receptor. The phosphorylated receptor then forms a docking site for a variety of Src homology 2 (SH-2) domain containing proteins, including a novel family of cytoplasmatic transcription factors termed STATs (signal transducers and activators of transcription). STATs are then phosphorylated on a single tyrosine residue by JAKs, after which the STATs dimerise, migrate into the nucleus and regulate gene transcription.

Effects of IL-6 on glucose and lipid metabolism

IL-6 has pleiotropic effects on various cell types. Here we will only focus on its role in glucose and lipid metabolism. Infusion of rhIL-6 to humans increased whole body glucose disposal and glucose oxidation but increased hepatic glucose production[134] and fasting blood glucose concentration in a dose-dependent manner.[135] With regard to lipid metabolism, IL-6 decreases adipose tissue lipoprotein lipase (LPL) activity[129] and has been implicated in the fat depletion taking place during wasting disorders, such as cancer, perhaps via an increase in plasma norepinephrine, cortisol, resting energy expenditure and fatty acid oxidation as was assessed in eight renal cancer patients.[134] In rats, IL-6 increased hepatic triglyceride secretion partly because the increase of adipose tissue lipolysis resulted in an increased delivery of free fatty acids to the liver.[136] This increased release of FFAs following rhIL-6 infusion was observed in humans as well.[134]

IL-6 in obesity and insulin resistance

In both mice[132] and humans, IL-6 mRNA in adipose tissue[137,138] but even more so plasma levels of IL-6 are positively correlated with BMI.[132,137,138] Weight loss is associated with a reduction in serum and IL-6 mRNA levels. After one year of a multidisciplinary programme of weight reduction, obese women lost at least 10% of their original weight and this was associated with a reduction of basal serum IL-6 levels from 3.18 to 1.7 pg/ml ($p<0.01$).[139] In another study, both IL-6 mRNA in adipose tissue and IL-6 serum levels were reduced with weight loss after three weeks of a very low calorie diet in obese women.[138] In this study, insulin sensitivity as assessed by the fasting insulin resistance index (FIRI= fasting glucose x fasting insulin/25) improved as well. The reduction in IL-6 levels could play a role in this improvement, since several studies found a significant correlation between circulating IL-6 levels and insulin sensitivity measured by either an intravenous glucose tolerance test[137] or the fasting insulin resistance index.[138] Recently this correlation between circulating IL-6 and insulin sensitivity was confirmed using the gold standard for insulin sensitivity: the hyperinsulinaemic euglycaemic clamp.[140] In addition, a high correlation between adipose tissue IL-6 content and insulin sensitivity was found, both *in vivo* and *in vitro*. Furthermore, for the first time IL-6 receptors were demonstrated in 60% of the subcutaneous adipocytes suggesting that IL-6 can alter adipocyte metabolism via autocrine or paracrine mechanisms and have a local influence on insulin sensitivity.[140] Further support for a relationship between IL-6 and insulin sensitivity comes from a genetic study. It appeared that subjects with an IL-6 gene polymorphism had lower IL-6 levels, a lower area under the glucose curve after an oral glucose tolerance test, lower glycosylated haemoglobin (HbA_{1C}), lower fasting insulin levels and an increased insulin sensitivity index compared with carriers of the normal IL-6 allele, despite similar age and BMI.[141] Finally, basal serum IL-6 levels are higher in type 2 diabetic patients.[142]

In contradiction with the above-mentioned positive correlation of IL-6 with BMI and inverse relation with insulin sensitivity is the observation that a lack of IL-6 also leads to obesity and a disturbed glucose tolerance, at least in mice.

Conclusion

Various studies show a clear relationship between increased IL-6 levels and obesity,[132,137,138] and between IL-6 levels and insulin resistance[137,138,140] even when corrected for BMI.[137] Furthermore, basal plasma IL-6 levels are higher in patients with type 2 diabetes[142] and subjects with an IL-6 gene polymorphism clearly have lower serum IL-6 levels and this is correlated with improved insulin sensitivity and postload glucose levels.[141] IL-6 does have different effects on the various end-organ tissues, however, with on the one hand improved glucose uptake in adipocytes and whole body glucose disposal, and on the other hand an increased hepatic glucose output, decreased LPL activity (leading to decreased triglyceride clearance) and increased hepatic triglyceride synthesis. How then does IL-6 fit in the insulin resistance syndrome? Is there a causal effect or are the increased IL-6 levels found in obesity and insulin resistance merely a reflection of the pathogenetic state or the increased adipose tissue mass? Is IL-6 detrimental to health or does it have a positive role in health. If we start from the principle that IL-6 production is increased in obesity and that it is involved in inducing insulin resistance, what would the mechanisms be by which IL-6 causes insulin resistance? Firstly, it has to be noted that omental fat produces threefold more IL-6 than subcutaneous adipose tissue.[128] Because venous drainage of omental tissue flows directly to the liver and IL-6 is known to increase hepatic triglyceride secretion[134,136] this might explain the hypertriglyceridaemia associated with visceral obesity. As mentioned before, increased triglyceride content of muscle and liver leads to insulin resistance. Secondly, IL-6 signal transduction is mediated via JAK-STAT signalling; it is possible that feedback mechanisms interfering with insulin signalling exist. Thirdly, IL-6 has opposing effects to those of insulin on hepatic glycogen metabolism[143] and increases hepatic glucose production.[135] On the contrary, despite an increase of IL-6 in obesity, insulin resistance and type 2 diabetes, there is evidence that IL-6 improves insulin sensitivity: 1) IL-6 increases glucose uptake in 3T3-L1 adipocytes;[144] 2) infusion of rhIL-6 to humans increased whole body glucose disposal and glucose oxidation;[134] 3) IL-6 inhibits TNF-α production, a cytokine with deleterious effects on insulin sensitivity; and 4) physical exercise, which is related to an improvement in insulin sensitivity, is coupled to an increased IL-6 secretion.[145] It might be that muscle derived IL-6 down-regulates TNF-α.[145] So, in conclusion, it is still not clear whether IL-6 has a positive or a negative metabolic role in health. One of the reasons for the contradicting results might be that there is a difference in the acute and chronic exposure to IL-6 with regard to health implications. Furthermore, there might be differences in local and CNS-acting effects of IL-6. More transgenic mice studies can help shed light on the role of IL-6 in insulin resistance. Up until now, it is quite possible that the increased IL-6 levels observed in adiposity and type 2 diabetes are the cause of an increased production by the enlarged adipose tissue mass and/or an attempt to overcome either insulin resistance or another metabolic defect, for example IL-6 resistance.

DISCUSSION

Obesity, defined as a BMI >27, is the consequence of a chronic imbalance between energy intake and energy expenditure. This is partly due to the modern society with excess ('fast') food intake and a sedentary lifestyle. The role that should be ascribed to primary defects in energy storage caused by adipocyte secretory products or impaired hypothalamic functioning remains to be elucidated. At the moment a combination of the two seems the most likely. It is well known that obesity is associated with insulin resistance and type 2 diabetes mellitus. An overwhelming amount of evidence indicates that visceral fat is associated with glucose intolerance and insulin resistance,[146-151] along with other facets of the metabolic syndrome such as dyslipidaemia. Therefore, in the past, the predominant theory used to explain the link between obesity and insulin resistance was the portal/visceral hypothesis,[152] which states that increased visceral adiposity leads to an increased free fatty acid flux into the portal system and inhibition of insulin action via Randle's effect.[153] However, several investigators have challenged the singular importance of visceral adiposity in inducing insulin resistance. They found an independent association between total fat mass and subcutaneous truncal fat mass and insulin resistance.[154-156] Furthermore, the observations that 1) triglyceride content within skeletal muscle cells is increased in obesity[157] and type 2 diabetes mellitus[157,158] and is a strong predictor of insulin resistance;[159] and 2) lipodystrophy is associated with insulin resistance as well[160,161] obviated the need to develop new theories to explain the link between adipose tissue and insulin resistance.[162] A well-accepted theory is that of ectopic fat storage.[162,163] A limitation in the capacity of adipose tissue to store triglycerides would divert triglycerides to be deposited in liver cells and skeletal muscle cells.[162,163] The cause of the ectopic fat storage is unclear. It might be due to impaired fat oxidation,[162] since inhibition of fat oxidation in rodents increased intracellular lipid content and decreased insulin action.[164] Furthermore, a mutation in the AGPAT2 gene encoding 1-acylglycerol-3-phosphate O-acyltransferase inhibits triacylglycerol synthesis and storage in adipocytes but not in hepatocytes, thus leading to hepatosteatosis, because the latter can accumulate triacylglycerol via AGPAT-1.[165] Another possibility is the central and/or peripheral action of leptin, since leptin therapy has been associated with the reversal of insulin resistance and hepatic steatosis in patients with lipodystrophy[46] and also with improvement of intramyocellular lipid content.[163] Finally, a defect in the proliferation and/or differentiation of adipocytes, whether or not due to alterations in the expression of transcription factors,[166] can lead either to impaired adipocyte triglyceride storage and/or adipocyte hypertrophy. This is where the third hypothesis emerges: the adipocyte as an endocrine organ.[162] Adipocytes secrete a large number of cytokines and hormones that act in a paracrine, autocrine and endocrine manner on adipocyte and whole body metabolism. It is plausible that these enlarged adipocytes are deregulated in their transcriptional setting and secrete a different pattern of hormones or different amounts of them compared with small adipocytes. On the other hand, enlarged adipocytes might merely be a manifestation of other, yet to be defined, pathogenethic factors.[162]

In obese humans and rodents there is, besides numerous other proteins and cytokines that have not been discussed here, overproduction of leptin,[14,15] IL-6,[132,137,138] TNF-α,[115,117-119] ASP[100,101] and resistin;[54,60] and a decreased production of adiponectin (see *figure 5*).[71,77,78,80] Of leptin,[23] TNF-α[74] and IL-6[127] it is known that they act via receptors on the cell surface and subsequent intracellular signalling cascades. As can be seen in *figure 5*, all three cytokines decrease food intake and increase energy expenditure and lipolysis together with a decrease in lipogenesis. These are well-adaptive mechanisms to prevent further weight gain. Since all these cytokines are increased in adiposity it is unlikely that they are the cause of adiposity unless there is an impairment in cytokine signalling. Interestingly, leptin and TNF-α have opposing effects with regard to insulin sensitivity. TNF-α interferes with insulin signalling and downregulates many genes encoding for proteins involved in glucose and free fatty acid uptake.[113] Leptin can act through some components of the insulin-signalling cascade as well.[52] The relation between TNF-α and leptin in humans is not clear. Infusion of TNF-α to patients has been reported to acutely raise serum leptin levels,[167] whereas chronic exposure of cultured human adipocytes to TNF-α resulted in a decrease in leptin production.[168] If TNF-α increases leptin production this might be an adaptive mechanism to com-

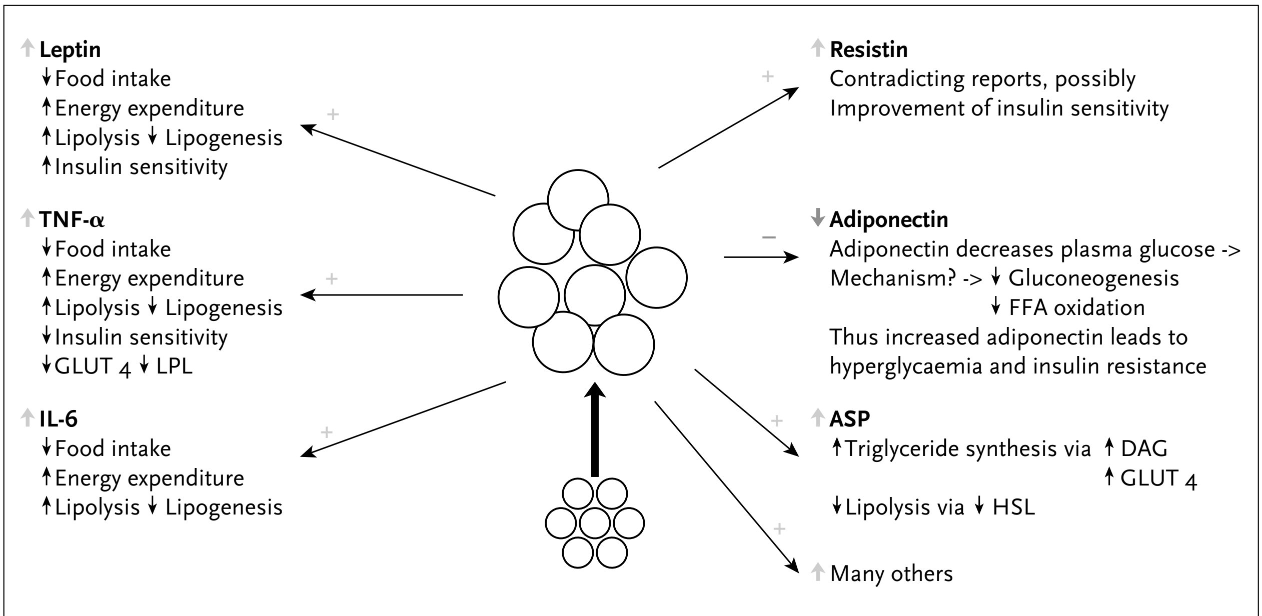

Figure 5

Hyperplasia and hypertrophy of adipocytes as seen in adiposity leads to an increased (light grey arrow) production of leptin, TNF-α, IL-6, resistin, ASP and many other proteins, and a decreased production (dark grey arrow) of adiponectin. The results of these increases, respectively decrease, are mentioned below each protein.

pensate for the TNF-α induced impaired insulin signalling. When we take a further look at the mutual coherence of the adipocyte secretory factors it is striking that both insulin and TNF-α are, somehow, involved in the regulation of all of the adipocyte secretory products. Insulin increases the production of leptin,[19,20,36,37] adiponectin[70,72] and ASP,[96] whereas no effect has been recorded with regard to TNF-α[110] and a potentially positive effect on resistin levels.[61] TNF-α downregulates resistin[58] and stimulates the production of leptin,[169] adiponectin[74] and IL-6.[113] The problem is that some of these factors lead to an improvement of insulin sensitivity whereas others have just the opposite effect. This makes it extremely difficult to elucidate which factors are most important in regulating insulin sensitivity. Furthermore, the time of exposure to a stimulus seems to be important. Thus it seems that leptin and insulin are long-term regulators with regard to food intake and energy expenditure whereas insulin has a direct effect on glucose uptake and lipolysis.
How do these adipocyte-derived factors mediate their effects? What they all seem to have in common is a change in the expression of genes encoding for proteins involved in glucose and protein metabolism. Transcription of genes can only take place if they are activated, which always occurs via some kind of ligand-receptor interaction followed by an intracellular signal transduction. Cytokine signalling proceeds in part via the JAK-STAT pathway.[170] The actions of leptin, TNF-α and IL-6 may influence each other via common signalling steps. Furthermore, it is known that leptin can signal through some components of the insulin signalling cascade such as IRS-1 and -2, PI3K and MAPK and can modify insulin-induced changes in gene expression *in vitro* and *in vivo*.[171] TNF-α can interfere with the early steps of insulin signalling as well.[114] So, more and more evidence exists that the adipocyte secretory cytokines leptin, IL-6 and TNF-α not only interact with each other but also with insulin on the level of intracellular signal transduction.
In the case of obesity and hyperinsulinaemia, there is an increase in hormones and cytokines produced by the adipose tissue. These hormones subsequently mediate a change in the expression of genes encoding for proteins involved in glucose and lipid metabolism. In case of ASP these changes promote triglyceride uptake. However, in case of IL-6, TNF-α and adiponectin there is a deleterious effect on glucose uptake and fatty acid oxidation leading to insulin resistance. The effect of increased serum resistin levels remains to be elucidated. Everything seems to come down to interference with intracellular signal transduction, not only of insulin but also of the various adipocyte secretory products, with a subsequent change in the expression of genes involved in glucose and lipid metabolism leading to a diminished glucose uptake and fatty acid oxidation. The latter will, via accumulation of triglycerides in liver cells and muscle cells, enhance insulin resistance, thus further impairing glucose uptake.

Concluding remarks

It is now well established that adipose tissue not only has an important function in the storage and release of triglycerides but also has an important effect on whole body metabolism and energy homeostasis via the production of various hormones and cytokines.
Adipose tissue not only responds to insulin, glucagon, cortisol and catecholamines but also to cytokines and products that it produces itself, thereby regulating its own metabolism and cell size. Some of the products produced by the adipocytes, such as TNF-α and leptin, are clearly involved in the induction of insulin resistance. The role of others (resistin, IL-6) has yet to be defined. Their increase in obesity is at least a manifestation of the increased adipose tissue mass itself. Further research is needed to come to a better understanding of the molecular pathways regulating the production of these hormones, their individual actions and target organs, and finally their mutual interaction and role in insulin resistance. These new insights provide the basis for the development of improved therapies for obesity and insulin resistance related diseases as type 2 diabetes and cardiovascular complications.

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