Resistin and obesity-associated insulin resistance

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Obesity is a major risk factor for insulin resistance and type 2 diabetes mellitus. Adipocytes secrete numerous substances that might contribute to peripheral insulin sensitivity. These include leptin, tumor necrosis factor α, Acrp30/adiponectin/adipoQ and interleukin 6, the potential roles of which are briefly reviewed here. Thiazolidinedione (TZD) antidiabetic drugs regulate gene transcription by binding to peroxisome proliferator activated receptor γ, a nuclear hormone receptor found at its highest levels in adipocytes. A search for genes that are downregulated by TZDs in mouse adipocytes led to the discovery of an adipose-specific secreted protein called resistin. Resistin circulates in the mouse, with increased levels in obesity, and has effects on glucose homeostasis that oppose those of insulin. Thus, resistin is a potential link between TZDs, obesity and insulin resistance in the mouse. Future studies must address the mechanism of action and biological role of resistin and related family members in mice and humans.

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Insulin resistance is defined as a failure of target tissues (adipose, liver, skeletal and cardiac muscle) to respond normally to insulin [1]. At the molecular level, this resistance can occur anywhere in the insulin signaling pathway, from receptor binding to downstream signaling events. Obesity-associated insulin resistance is manifested by increased hepatic glucose output and reduced glucose disposal in peripheral tissues at a given level of insulin [2]. Quantitatively, skeletal muscle is the most important site of insulin-mediated glucose disposal [3]. Adipose tissue clearly plays a significant role in the pathogenesis of insulin resistance, as shown by the high correlation between obesity and insulin resistance [2]. Obesity-induced insulin resistance is affected both by the total amount of adipose tissue and its distribution [4]. Both visceral and deep subcutaneous adipose tissues are associated with insulin resistance [5]. Excessive free fatty acids (FFAs) released by lipolysis from adipose tissue have been implicated in non-insulin dependent diabetes mellitus. FFAs compete with glucose for oxygen and inhibit whole body glucose disposal via the 'Randle cycle' [6]. FFAs have a deleterious effect on insulin uptake by the liver and contribute to the increased hepatic glucose release [7]. The concept that signals emanating from adipose tissue regulate organismal glucose homeostasis has been reinforced by the observation that adipose-specific changes in gene expression alter insulin sensitivity in muscle and liver in the mouse [8].

Adipose tissue as an endocrine organ

Adipose tissue is the body's largest reserve of fuel, storing energy in the form of triacylglycerides. This energy can be rapidly mobilized during starvation and other times of need. The switch from energy storage to mobilization within adipocytes is regulated by hormonal signals from other tissues and organs, including the pancreas (insulin), the sympathetic nervous system (catecholamines) and the adrenal glands (glucocorticoids). Until recently, adipocytes were viewed as playing a passive role in fuel homeostasis, with obesity being a consequence of chronic positive energy balance. However, it is now recognized that the endocrine function of adipose tissue is a crucial determinant of energy balance. The discovery of leptin as an adipocyte-derived peptide hormone, the absence of which causes massive obesity in mice [9] and humans [10], conclusively established the endocrine function of adipose tissue. Characterization of the leptin receptor and its mutation in obese mice [11] and people [12] reinforced the notion of adipocytes as a key component of a classical endocrine system with the hypothalamus as a major target tissue [13]. The actions of leptin on the brain include not only regulation of satiety [14–16] but also efferent pathways controlling metabolism [17] and other endocrine systems involved in the starvation response [18].

The discovery of the leptin endocrine system triggered a conceptual shift leading to increased interest in other adipocyte products as signaling molecules. These include FFAs, the elevated levels of which in obesity are probably causally related to reduced insulin action in peripheral tissues [19]. In addition, increasing numbers of proteins are being found to be secreted by adipose tissue, including Acrp30/adiponectin/AdipoQ (herein referred to as Acrp30) [20–22], tumor necrosis factor α (TNF-α) [23], adipsin [24], plasminogen activator-inhibitor [25], acylation-stimulating protein [26], interleukin 6 (IL-6) [27], IL-8 [28], agouti protein [29], transforming growth factor β [30], angiotensinogen [31] and adipophilin [32]. Many of these cytokines and hormones are potential regulators of glucose homeostasis. This review of potential mediators of obesity-associated insulin resistance focuses on the polypeptides that have received the most recent attention, namely: leptin, TNF-α, Acrp30 and IL-6.

Potential mediators of obesity-associated insulin resistance

Leptin

Leptin regulates food intake, body weight, energy expenditure and neuroendocrine function [33,34].

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Many effects of leptin are mediated centrally, but peripheral effects also exist [35]. Leptin-deficient *ob/ob* mice and leptin receptor-deficient *db/db* mice exhibit severe insulin resistance, which is reversed in the *ob/ob* mouse by leptin administration [14–16]. The degree of insulin resistance is greater than can be accounted for by hyperphagia and obesity, which suggests a direct role for leptin [2]. Leptin levels are low in mice that are severely insulin resistant owing to lack of adipose tissue, and leptin treatment restores insulin sensitivity in these models [36,37]. However, leptin levels are raised in most models of obesity-associated type 2 diabetes mellitus in mice and humans, ruling out leptin deficiency *per se* as the cause, although leptin resistance might play a role [38].

The long form of the leptin receptor is found in adipose tissue, skeletal muscle and liver, suggesting potential effects of leptin on these classical insulin target tissues [39]. Leptin administration in rats enhances insulin sensitivity and whole-body glucose disposal [40]. In normal mice, leptin administration results in a decrease in plasma insulin and a rise in plasma glucose [41]. By contrast, fasted mice experience a decrease in both plasma insulin and glucose levels upon leptin administration [40]. Overall, leptin appears to exert a net hypoglycemic effect that is at least partially independent of its weight-reducing effects. In hepatocytes, leptin has been shown to antagonize the effects of insulin [42]. Hepatocytes treated with leptin exhibit decreased insulin-stimulated tyrosine (Tyr) phosphorylation of insulin receptor substrate 1 (IRS-1), increased phosphoenolpyruvate carboxykinase synthesis and decreased glucokinase activity, therefore leading to increased gluconeogenesis and decreased glycogenolysis [43]. Leptin might also regulate peripheral glucose uptake into muscle and adipose tissue [44].

TNF-α

TNF-α was originally identified as a proinflammatory cytokine produced by macrophages and lymphocytes [45] and was subsequently found to be produced by adipose tissue [23]. TNF-α mRNA levels are elevated in adipose tissue in several animal models of obesity, namely *ob/ob*, *tub/tub* and KKA^{y} mice and the Zucker *fa/fa* rat [46]. Neutralization of TNF-α in obese insulin-resistant animal models results in increased peripheral uptake of glucose in response to insulin [23]. There is much evidence to indicate a role for TNF-α in obesity-related insulin resistance [47]. TNF-α-induced insulin resistance might result from inhibition of insulin-mediated Tyr phosphorylation of the insulin receptor (IR) itself in addition to IRS-1 [47]. TNF-α also downregulates glucose transporter *GLUT4* gene expression in adipocyte and myocyte cultures [46,48] in addition to GLUT4, IRS-1 and IR proteins in 3T3-L1 adipocytes [49]. Overnight infusion of recombinant human TNF-α in rats led to insulin resistance characterized by a decrease in hepatic glucose output and peripheral glucose utilization [50].

Mice lacking TNF-α have lower plasma insulin levels and body weights, and improved glucose tolerance relative to wild-type littermates after obesity induced by goldthioglucose [51]. TNF-α-deficient mice were also protected from obesity-induced insulin resistance on a high fat diet [52]. Studies of mice lacking all known TNF-α receptors have been somewhat discordant with regard to the effects on insulin sensitivity. Uysal *et al*. [52] found that mice lacking TNF-α receptors on the *ob/ob* background developed comparable obesity but less severe hyperglycemia and hyperinsulinemia than did *ob/ob* controls. By contrast, an independently generated mouse line deficient in TNF receptors was not protected from insulin resistance on a high fat diet or when crossed on to the *db/db* background [53].

Acrp30

Adipocyte complement-related protein of 30 kDa (Acrp30) was identified as a novel adipocyte-specific secreted protein with structural resemblance to complement factor C1q produced solely in adipocytes and secreted into serum [20]. Acrp30, also known as adipoQ, is induced during adipocyte differentiation and its secretion is stimulated by insulin [20,21]. The human homolog has been identified as adiponectin [54], gelatin-binding protein [55] and adipose most abundant gene transcript 1 [22]. Plasma levels are decreased in obese humans [54], and low levels are associated with insulin resistance and hyperinsulinemia in Japanese and Pima populations [56]. Thus, the reduced levels of Acrp30 in obesity might contribute to insulin resistance. Treatment of *db/db* mice with thiazolidinediones increased circulating Acrp30 levels [57,58]. The globular domains of Acrp30 form homotrimers and higher order structures via a collagen-like region [59], and a truncated form of Acrp30 corresponding to the globular domain circulates in human plasma [60]. Acute *in vivo* administration of this truncated Acrp30 decreased postprandial plasma FFAs following a high-fat meal or intravenous injection of Intralipid, and chronic administration resulted in body-weight loss without affecting food intake [60]. Administration of full-length Acrp30 to wild-type C57BL/6J and *ob/ob* mice resulted in a decrease in serum glucose levels without alterations in insulin levels [57]. In a mouse model of lipodystrophy, generated by the administration of the RXR antagonist, HX531, to *Pparg*$^{+/-}$ mice [*Pparg* encodes the peroxisome proliferator-activator receptor γ (PPARγ)], improvement in hyperglycemia and hyperinsulinemia were seen upon treatment with exogenous Acrp30 [58]. One site of action of Acrp30 is muscle, where Acrp30 increased muscle fatty acid oxidation *in vitro* [60] and the production of molecules involved in muscle fatty acid oxidation and energy dissipation in lipoatrophic mice [58]. Acrp30 also

significantly repressed hepatic glucose output in the presence of insulin [57]. Together, these studies strongly suggest that Acrp30 might be functioning in multiple tissues to ameliorate insulin resistance. The Acrp30 receptor and downstream signaling pathway are currently unknown.

Interleukin 6

IL-6 is a cytokine that is produced by a variety of cell types, including immature immune cells, fibroblasts, endothelial cells, myocytes and endocrine cells [61]. In addition, it has been shown that both adipocyte and stromal-vascular constituents of adipose tissue secrete IL-6 [61]. Raised levels of IL-6 are seen in obesity [62]. Visceral adipose tissue released two to three times more IL-6 than subcutaneous adipose taken from severely obese, non-diabetic patients [61]. A direct positive correlation between insulin resistance and circulating IL-6 levels was detected in a comparison between lean and obese woman [63]. IL-6 increases hepatic triglyceride secretion and decreases adipose LPL activity [64]. Therefore, the mechanism by which IL-6 influences obesity-associated insulin resistance might be to increase circulating FFAs. The IL-6 receptor is a class I cytokine receptor [64]. In addition, a soluble form of the IL-6 receptor that is derived from the IL-6 receptor by a proteolytic cleavage has been identified, although its role is not understood [64].

Resistin: a recently discovered adipose-secreted polypeptide

Clearly, adipose tissue is an endocrine organ, secreting multiple humoral mediators of insulin resistance. One major challenge is to determine the full range of adipocyte-derived factors and their relative contributions to glucose homeostasis. Our laboratory has long been interested in the nuclear receptor PPARγ, which is abundant in fat cells, and we have been exploring the mechanism by which thiazolidinedione (TZD) ligands for PPARγ function as antidiabetic drugs *in vivo* [65]. These drugs induce adipocyte differentiation, including the induction of genes that increase fatty acid uptake into adipocytes and thereby lower FFA levels, which probably plays an important role in their antidiabetic efficacy [66]. We hypothesized that, in addition to inducing genes important for adipocyte differentiation and lipid storage, TZDs act on PPARγ in adipose tissue and differentially regulate a subset of genes related to insulin sensitivity that is independent of adipogenesis. A screen of 3T3-L1 adipocytes based on this notion led to the identification of a unique polypeptide termed resistin [67]. Resistin was independently identified by two other groups. Sul and colleagues used microarray analysis to identify the resistin polypeptide as adipose tissue-specific secretory factor [68]. Holcomb *et al.* identified the polypeptide as FIZZ3 by an expressed sequence tag (EST) database screen against a related protein induced during lung inflammation, known as 'found in inflammatory zone 1' (FIZZ1) [69]. Our group independently and reciprocally identified FIZZ1 by an EST database search against resistin, naming it resistin-like molecule α (RELMα) [70]. The international Committee on Standardized Genetic Nomenclature for mice has named the gene encoding resistin/FIZZ3/ADSF *Retn* and the gene encoding RELMα/FIZZ1 *Retn1a*.

The *Retn* mRNA encodes a 114-amino acid polypeptide containing a 20-amino acid signal sequence. The secreted 94-amino acid polypeptide contains 11 Cys residues. Resistin is secreted as a disulfide-linked dimer, with a single cysteine residue (Cys26) being required for dimerization. Mutation of this residue in recombinant resistin leads to secretion of the protein as a monomer. The remaining ten Cys residues are probably involved in intramolecular disulfide bonding, which determines the structure of the monomeric polypeptide [71].

Retn mRNA and the resistin protein are both induced during 3T3-L1 adipogenesis [67]. Sul and colleagues have reported that conditioned medium from COS cells expressing the gene encoding resistin has an inhibitory effect upon 3T3-L1 adipogenesis, suggesting a role for the secreted protein in regulating the differentiation of preadipocytes [68]. In both the mouse and rat, *Retn* is expressed almost exclusively in white adipose tissue [67–69]. The highest levels of *Retn* expression are seen in female gonadal adipose tissue. *Retn* mRNA levels are reduced in adipose tissue taken from obese mice ([72] and C.M. Steppan and M.A. Lazar, unpublished).

Consistent with its secretion by adipocytes and its abundance in mouse adipose tissue, resistin is readily detectable in the mouse circulation. Serum resistin levels are raised in two different genetic models (*ob/ob* and *db/db*) and in a diet-induced model (DIO) of diabetes and obesity [67]. One potential explanation for this apparent paradox is that the net secretion of resistin protein from the increased number of adipocytes in obese mice overcomes the reduced amount of resistin per cell. The reduction in *Retn* mRNA per cell might reflect an inhibitory feedback mechanism. Alternatively, the correlation between the levels of adipocyte *Retn* mRNA and the resistin protein might vary in obesity. Of particular note is the fact that plasma adiponectin levels in rhesus monkeys do not correlate with mRNA levels in adipose tissue in the same monkey [73].

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Levels of *Retn* mRNA and the protein itself are downregulated by antidiabetic TZDs in 3T3-L1 murine adipocytes, consistent with the manner in which resistin was identified in our laboratory [67]. The effects of TZD on resistin expression *in vivo* are controversial. Way and colleagues [72] have reported increased mRNA levels in adipose tissue from obese mice and rats treated with rosiglitazone and other

Table 1. Comparison of adipocyte-secreted proteins and their properties related to insulin resistance and obesity[a]

Adipocyte-derived protein	Tissue distribution (mouse)	Protein levels in obesity	Protein levels in obesity-related insulin resistance
Leptin	Adipose, stomach and placenta	Increased[b]	Increased
TNF-α	Adipose, macrophages and lymphocytes	Increased	Increased
ACRP30	Adipose	Decreased	Decreased
Resistin	Adipose	Increased	Increased
Interleukin 6	Adipose, immune cells, fibroblasts, endothelial cells, myocytes and endocrine cells	Increased	Increased

[a]Abbreviations: ACRP30, adipocyte complement-related protein of 30 kDa; TNF-α, tumor necrosis factor α.
[b]Except in the *ob/ob* mouse, in which the gene encoding leptin is mutated.

PPARγ agonists. By contrast, we (C.M. Steppan and M.A. Lazar, unpublished) and others [74] have observed decreased *Retn* expression after rosiglitazone treatment of *ob/ob* and *db/db* mice, in addition to rats with diet-induced obesity. We have also found reduced circulating resistin levels in *ob/ob* mice treated with rosiglitazone [67]. The explanation for these differences is unclear.

The administration of anti-resistin antibody to mice with diet-induced obesity, insulin resistance and hyperglycemia partially corrected their blood glucose levels and improved their sensitivity to exogenous insulin. These data suggest that the raised circulating resistin levels might contribute to the hyperglycemia and insulin resistance seen in this model. Acute administration of recombinant resistin to normal mice led to a modest impairment of glucose tolerance. Recently, Yamauchi *et al.* [75] reported reduced adipocyte resistin expression associated with an increased insulin sensitivity in a mouse model of insulin resistance. Together, these data support the hypothesis that resistin plays a causative role in insulin resistance. Resistin blunted insulin-responsive glucose uptake in cultured 3T3-L1 adipocytes [67] and pretreatment of resistin-secreting 3T3-L1 adipocytes with a neutralizing antibody augmented insulin-stimulated glucose transport [67]. These data suggest that resistin has target cell actions that are antagonistic to those of insulin. However, the resistin receptor and downstream signaling pathway are unknown.

A putative human homolog of resistin has been identified, encoded by a gene on human chromosome 19 [67]. The deduced amino acid sequence of this protein is only 59% identical to that of mouse resistin, and the expression of this gene in human adipose tissue is much less than that observed for the *Retn* gene in mouse adipose tissue [76,77]. Moreover, although there are three mouse genes (*Retn*, *Retn1a* and *Retn1b*) encoding three distinct proteins (resistin/FIZZ3/ADSF, RELMα/FIZZ1and an intestine-specific RELMβ/FIZZ2), only two have been identified thus far in humans. These data raise the question of whether another as yet unidentified gene is the true human homolog of the murine *Retn* gene. The likelihood of this is decreased by the nearly complete sequence of the human genome. In addition, the human gene on chromosome 19 is located in a region syntenic to the location of the mouse *Retn* gene on chromosome 8, suggesting that these are indeed true homologs. In that case, it would appear that the mouse and human proteins differ greatly, not only in their sequence, but in their sites of synthesis, a potentially important difference between the endocrine functions of white adipose tissue in rodents and humans.

Leptin, TNF-α, Acrp30, IL-6 and resistin in obesity-associated insulin resistance

Obesity-associated insulin resistance is usually a polygenic disorder that is almost certainly multifactorial. In this context, it is of interest to compare the reported properties of leptin, TNF-α, Acrp30 and resistin (Table 1). Leptin, Acrp30 and resistin are found predominantly in adipose tissue, whereas TNF-α and IL-6 are also found in abundance in macrophages and lymphocytes. Leptin, TNF-α, IL-6 and resistin levels increase in obesity, whereas Acrp30 levels decrease. TNF-α and resistin appear to impair glucose tolerance, whereas leptin and Acrp30 have net hypoglycemic effects. Thus, although the relative contributions and mechanisms by which these adipocyte factors affect insulin sensitivity are not well established, it is interesting that each has distinct properties and might contribute differentially to the insulin resistance associated with different mouse models.

Concluding remarks

The discovery of resistin has provided a potential mediator of obesity, diabetes mellitus and the action of TZDs. The relative contribution of mouse resistin to the pathogenesis of insulin resistance in addition to its role(s) in normal physiology remain to be determined. Our current understanding of resistin is formed by studies performed in mice. Therefore, our knowledge of the biology of resistin is in its infancy. Future studies in humans will allow us to determine the function of human resistin. Identifying the similarities and differences between the properties of mouse and human resistin will also shed light on the mechanisms of obesity-related insulin resistance in these two species.

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Hyperplasia versus adenoma in endocrine tissues: are they different?

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The traditional view holds that hyperplasia of endocrine glands is secondary to oversecretion of a trophic hormone. However, in most cases, the mechanism underlying this growth is the spontaneous proliferation of benign neoplasias. Pathologists still depend on subtle morphological criteria to delineate and further classify these tumours. Owing to their variable architecture, a bewildering nomenclature has emerged for these tumours, exemplified by the many names applied to the goitrous thyroid gland: hyperplasia, adenomatous goitre, adenomatoid nodules, benign nodular thyroid disease, adenoma, etc. This article reviews the evidence suggesting that: (1) the varied types of benign neogeneration of endocrine tissue, the spectrum of which ranges from 'simple hyperplasia' to 'true adenoma', involve the same process; (2) even clonality of a growing lesion cannot distinguish hyperplasia from neoplasia; and (3) the basic processes in both cases are not different from those that cause benign tumours in other organs.

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Classically, it has been thought that non-neoplastic endocrine hyperplasia results from a long-lasting, excessive secretion of a trophic hormone. In the thyroid gland, endemic goitre caused by iodine deficiency, as well as glandular hyperplasia caused by thyrotrophin (TSH)-secreting pituitary tumours, peripheral thyroid hormone resistance or congenital defects of thyroid hormone synthesis, do involve TSH hypersecretion. In addition, bilateral adrenal hyperplasia in Cushing's disease or in patients with congenital defects of cortisol synthesis is the result of excessive corticotrophin. However, in most endocrine gland hyperplasias, no stimulating trophic factor can be identified [1,2]. Moreover, if a trophic factor were to cause hyperplasia, diffuse and uniform enlargement of the target gland with identical changes in all its cells responsive to the trophic factor would be expected. However, autonomous growth, with or without suppression of the relevant trophic hormone, is a common phenomenon, as in toxic and euthyroid nodular goitre and in hormonally active or inactive adrenal adenomas. Furthermore, nodular rather than diffuse growth is a prominent hallmark of endocrine neoplasias, and the newly grown tissue is markedly heterogeneous, both with respect to morphology and to function [1,3]. Continuous extrinsic stimulation cannot account for all of these characteristics, so the ultimate mechanisms that cause and modulate the most common forms of endocrine cell proliferation, in the thyroid and in other endocrine glands, must lie within the glandular cells themselves. These mechanisms are identical to those operating in non-endocrine benign tumours.

Autonomy of growth: a basic characteristic of proliferating endocrine tissue

'Autonomy of growth' indicates that proliferation of a tissue is driven by processes intrinsic to the cells themselves; although extracellular and environmental factors might accelerate or retard the growth process, they cannot prevent it [1,3]. Autonomous growth is the most important single characteristic of neoplasia, be it benign or malignant.

Autonomy of growth is also a hallmark of foetal and neonatal tissue. Among the endocrine glands, it is most striking in the remarkably high growth rate of the thyroid of newborn mice and of human foetal thyroid tissue grafted onto TSH-suppressed nude

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