Role of resistin in obesity, insulin resistance and Type II diabetes

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ABSTRACT

Resistin is a member of a class of cysteine-rich proteins collectively termed resistin-like molecules. Resistin has been implicated in the pathogenesis of obesity-mediated insulin resistance and T2DM (Type II diabetes mellitus), at least in rodent models. In addition, resistin also appears to be a pro-inflammatory cytokine. Taken together, resistin, like many other adipocytokines, may possess a dual role in contributing to disease risk. However, to date there has been considerable controversy surrounding this 12.5 kDa polypeptide in understanding its physiological relevance in both human and rodent systems. Furthermore, this has led some to question whether resistin represents an important pathogenic factor in the aetiology of T2DM and cardiovascular disease. Although researchers still remain divided as to the role of resistin, this review will place available data on resistin in the context of our current knowledge of the pathogenesis of obesity-mediated diabetes, and discuss key controversies and developments.

INTRODUCTION

Adipose tissue is known to produce a vast array of adipocyte-derived factors, known as adipocytokines. Under ‘normal’ physiological conditions adipocytokines may play an influential role in energy homeostasis, triacylglycerol (triglyceride) storage and mobilization of fat, with increased adiposity, specifically central adiposity. These processes can be substantially dysregulated [1]. Furthermore, it seems apparent that the pathogenesis of T2DM (Type II diabetes mellitus) is mediated through the concurrent progression of insulin resistance and subclinical inflammation, although the molecular mechanisms for this are less understood. It is, however, apparent that obesity represents one of the foremost contributory factors leading to diabetes, as such, the expression and functional properties of adipocytokines and their effects on metabolism have been the subject of intense research. Indeed, studies on adipocytokines and their potential effects in human obesity and T2DM have implicated them in the pathogenesis of the metabolic syndrome. Such factors include TNF-α (tumour necrosis factor-α), IL (interleukin)-6, angiotensinogen, leptin, PAI-1 (plasminogen activator inhibitor-1) and resistin (Table 1).

This review will address our current understanding of the pathophysiological role of resistin, and evaluate resistin as a pathogenic factor implicated in metabolic and inflammatory mechanisms leading to diabetes. In addition, we will highlight the continuing complexity of the biology of resistin and its role in the progression of...
### Table 1  Summary of factors implicated in the pathogenesis of obesity-related T2DM

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function/effect</th>
<th>Normal distribution</th>
<th>Effect of obesity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>Satiety and appetite; signals to the brain to regulate energy homeostasis and body weight.</td>
<td>Secreted by WAT, BAT, skeletal muscle, stomach and placenta; 2.5 times more leptin in Sc AT than Om AT; more leptin in adipocytes than pre-adipocytes.</td>
<td>↑ In human obesity and correlates with BMI, and ↓ after fasting or weight loss.</td>
<td>[38,112–114]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Reduces insulin secretion and insulin signalling in AT, pancreatic cells, liver and muscle.</td>
<td>Predominant in adipocytes in WAT; 1.67 times more TNF-α in Sc AT compared with Om AT</td>
<td>Correlates with BMI, ↑ in human obesity; 2-fold increase in obese compared with lean subjects; ↑ in a rodent model of obesity; ↓ adipose differentiation.</td>
<td>[112,115–120]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Affects glucose and lipid metabolism; improves insulin sensitivity and glucose tolerance; has anti-inflammatory properties.</td>
<td>35% of the basal supply is derived from WAT; produced by macrophages, fibroblasts, endothelial cells and skeletal muscle cells.</td>
<td>↑ In morbidly obese patients, ↓ after weight loss.</td>
<td>[38,121–125]</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Potent inhibitor of the fibrinolytic pathway.</td>
<td>Correlation with abdominal adiposity; pattern of adipose tissue distribution: ↑ in humans leads to ↑ thromboembolic complications.</td>
<td>↑ In humans.</td>
<td>[126]</td>
</tr>
<tr>
<td>AGT</td>
<td>Precursor of angiotensin II; regulator of blood supply and induces differentiation of pre-adipocytes.</td>
<td>mRNA greater in Om AT than Sc AT.</td>
<td>↑ In humans.</td>
<td>[127,128]</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Regulator of energy homeostasis; enhances insulin sensitivity and glucose uptake; has anti-inflammatory properties.</td>
<td>Exclusively secreted by adipocytes.</td>
<td>mRNA and protein greater in Sc AT than Om AT.</td>
<td>[76,78,103,129–131]</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Varied role in proliferation, differentiation, apoptosis and development.</td>
<td>Multifunctional, produced by variety of cells; inhibitor of differentiation.</td>
<td>↑ In mouse models of obesity and insulin resistance (ob/ob and db/db); ↓ in human obesity and T2DM; ↑ after weight loss.</td>
<td>[132]</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>Regulates adipose cell differentiation.</td>
<td>Sc adipocytes = Om adipocytes (BMI &lt; 28 kg/m²)</td>
<td>↑ In Sc WAT 2 times greater than in Om WAT (BMI &lt; 30 kg/m²).</td>
<td>[133,134]</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>Implicated in the coordination of energy balance and weight regulation.</td>
<td>Secreted by endocrine cells in the gastrointestinal tract.</td>
<td>↑ Circulating levels after chronic weight loss in humans.</td>
<td>[38,135–140]</td>
</tr>
<tr>
<td>Adiponutrin</td>
<td>Possible contribution to energy homeostasis, vesicular targeting and protein transport.</td>
<td>Non-secreted adipocyte protein.</td>
<td>mRNA (50-fold) in obese fa/fa rats; ↓ fasting mRNA levels; human gene expression may be regulated by changes in energy balance.</td>
<td>[141–143]</td>
</tr>
</tbody>
</table>

Despite an incomplete current understanding of the role of resistin, this review will assemble and discuss the existing literature on resistin to evaluate the current metabolic, inflammatory and cardiovascular effects of this protein, with the intent of providing an insight as to where the role of resistin may lie.
Role of resistin in obesity, insulin resistance and Type II diabetes

Resistin, a putative adipocyte-derived signalling polypeptide, was originally identified by three independent groups using a variety of techniques [2–4]. Initial studies showed that resistin was up-regulated in rodent models of obesity and insulin resistance and down-regulated by an insulin-sensitizer, RSG (rosiglitazone) [3]; however, immunoneutralization of resistin reduced hyperglycaemia and improved insulin sensitivity [3]. These observations not only brought resistin to much scientific attention, but characterized it as a potential aetiological link between obesity and diabetes, with a clear functional role as a pathogenic factor contributing to insulin resistance. Additionally, this revealed possibilities of the mechanistic action for TZDs (thiazolidinediones) and their subsequent therapeutic applications.

The Structure of Resistin

Studies determined the resistin gene, referred to as Retn, encoded a 114-amino acid polypeptide [3], secreted as a disulphide-linked homodimer [5]. Recent X-ray crystallographic studies of resistin [6] have determined its complex hexameric structure (Figure 1). Resistin was shown to circulate in two distinct assembly states; the more predominant HMM (high-molecular-mass) hexamer and the substantially more bioactive LMM (low-molecular-mass) complex, which is unable to form intertrimer disulphide bonds [6]. This implied that regulated processing through disulphide cleavage is required to initiate bioactivity of the LMM form, and suggested further a potential target site for receptor interaction [6].

Figure 1  Ribbon diagram representations of resistin

The Discovery of Resistin

Resistin belongs to a family of cysteine-rich proteins, termed RELMs, which include RELM-α/FIZZ 1, RELM-β/FIZZ 2 and the recently discovered RELM-γ, with each member having an unique differential tissue distribution [2,7,8]. Resistin expression was first described in adipose tissue [3], with circulating levels detected in rodents and humans [9–12]. Our present knowledge of the expression of resistin and RELM-α, -β and -γ in human and rodent tissues is shown in Table 2. Although the expression of resistin in mice was originally restricted to adipocytes [3], the principle origin of human resistin has remained somewhat contentious. Unlike the mouse gene, the human homologue of resistin was sparsely detectable in human adipocytes [13]; this was confounded further by confusion over the proposed sites of resistin production [13–15]. These studies led to many of the current perceptions that resistin was an inconsequential factor in the progression of obesity-related T2DM, thereby contrasting rodent data. Conversely, McTernan et al. [16] detected resistin in adipose tissue, thus describing resistin as a potential pathogenic factor increased in central adiposity. The discrepancy between studies may have partially related to methodology of detection or quantification of resistin. However, to date, although a difference in resistin mRNA levels between adipocytes and macrophages is apparent [14,17,18], studies have not yet identified whether this difference is observed at the protein level. Determining the relative contribution of the adipocyte in obesity with regards to circulating resistin levels would also prove beneficial.

Homology of Human and Rodent Resistin

Although four genes for the family of RELMs have been identified in mice, only two homologues have been identified in the complete human genome [7,8]. Recently, an alternatively spliced variant of the Retn gene was identified in humans (resistin Δ2) [19], and another was identified in rats, termed ‘S-Resistin’ (short resistin) [20].

The human Retn gene is located on chromosome 19 in a region syntenic to the mouse Retn gene on chromosome 8. In relation to this, one group posed the view that...
Table 2  Distribution of mRNA and protein expression of resistin and RELM-β in rodents and humans, and RELM-α and RELM-γ in rodents

| Tissue               | Resistin |  |  |  |  |  |  |  |  |  | References |
|----------------------|----------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|                      | Rodent R | Human P | Rodent R | Human P | Rodent R | Human P | Rodent R | Human P | Rodent R | Human P |                      |
| WAT                  | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [3,7,8,16,18,19,23,25,86,144,145] |
| Pre-adipocytes       | ×××      | ×          | ×××      | ×          | ×××      | ×          | ×××      | ×          | ×××      | ×          | [14,25] |
| Adipocytes           | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [3,15,25,29] |
| Mononuclear blood cells | ××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [8,13,15,17,68,86,88] |
| Hypothalamus         | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [19,145,146] |
| Pituitary gland      | ×××      | ×          | ×××      | ×          | ×××      | ×          | ×××      | ×          | ×××      | ×          | [145,146] |
| Adrenal gland        | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [19,72] |
| Spleen               | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [8,19,147] |
| Skeletal muscle      | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [19,72,148] |
| Pancreas             | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | ××××     | ×          | [19,149] |
| Placenta             | ×××      | ×          | ×××      | ×××××     | ×          | ×××      | ×××××     | ×          | ××××      | ×          | [7,19,150] |
| Gastrointestinal tract | ××      | ×          | ××××     | ×          | ×××      | ×          | ×××      | ×××      | ×××      | ×××      | [7,8,19,29,72] |
| Lung                 | ×××      | ×          | ×××      | ×××××     | ×          | ×××      | ×××××     | ×          | ××××      | ×          | [7,8,19,29] |

Figure 2  Amino acid sequence identities of rodent, porcine and bovine resistin, other RELM family members and the recently identified human alternatively spliced variant of resistin, resistin Δ2

Percentage sequence identities were obtained from the EMBL-EBI (European Bioinformatics Institute) server (http://www.ebi.ac.uk). The different colours represent the different RELM family members. b, bovine; h, human; p, porcine; r, rodent.

orthology existed between human and mouse genes [21], whereas another questioned the relevance of resistin, due to the absence of a true homologue for the murine Retn gene in humans [22]. Contrasting opinions regarding the orthology may relate to how genomic, transcribed and translated sequence identities are viewed. However, it is evident that human and mouse resistin have diverse genomic organizations [22]. Moreover, at the protein level, human resistin is only 55% identical with its murine counterpart (Figure 2), and this could, in part, explain such diverse expression and regulatory patterns of this protein, or simply suggest resistin may not be evolutionary well conserved across species.

THE METABOLIC ROLE FOR RESISTIN: AN ONGOING DEBATE

Resistin and obesity

Initial observations

Since the initial investigation of resistin in numerous rodent models of obesity and insulin resistance [3,23,24], ongoing experimental data has generated further inconsistency. Human studies have highlighted increased resistin expression in adipose tissue [15], particularly abdominal depots [16,25]; furthermore, positive correlations between serum resistin and body fat content have also been reported [26]. On the contrary, several studies have failed to demonstrate such correlations in rodents, with groups also reporting either reduced [27–30] or no alteration [31,32] of resistin levels in various models of obesity. Although it is difficult to address such diverse findings using similar, and in some instances the same, rodent models, inconsistencies may depend upon methodological differences [18]. The following sections will review cases for and against the role for resistin as an important pathogenic factor in obesity, insulin resistance and T2DM in light of recent studies.

Recent observations supporting a role for resistin in obesity

Studies by Lee et al. [33] showed that various murine models of obesity had higher circulating resistin levels...
compared with their lean counterparts. These observations coincided with rodent studies by Rajala and co-workers [34], showing circulating resistin levels were significantly elevated and concordant with increasing levels of insulin, glucose and lipids; thus substantiating the initial evidence that addressed the aetiology of resistin with increasing adiposity [3]. Recently, Asensio et al. [35] determined that high-fat-fed mice had induced adipocyte differentiation, denoted by fatty acid binding protein (AP-2) gene expression, a surrogate marker of differentiation, which positively correlated with resistin gene expression. Subsequently, in view of this and previous studies [4,36], it was suggested that elevated resistin expression was a result of adipocyte differentiation [35]. Moreover, the increase in adipocyte number may have caused a rise in local resistin production, inhibiting insulin action on glucose uptake in adipose tissue and, thus, preventing further adipocyte differentiation [35]. Therefore, at least in rodents, a regulatory feedback mechanism for resistin in adipogenesis may occur, acting as an adipose sensor for nutritional status. In accordance with these observations, Kim et al. [37] generated transgenic mice overexpressing a dominant inhibitory form of resistin which functioned to block the inhibition of resistin-mediated adipocyte differentiation. These transgenic mice developed obesity, possibly owing to enhanced adipocyte differentiation and adipocyte hypertrophy, as indicated by increased circulating levels of adiponectin and leptin [37].

Recent investigations of human resistin in relation to obesity have shown higher serum resistin levels in obese subjects compared with lean subjects [38–40], which positively correlated with the changes in BMI (body mass index) and visceral fat area [11,40–42]. The implication that resistin is important in human adipose tissue has been corroborated by studies showing increased protein expression with obesity [40], as well as protein secretion from isolated adipocytes [43]. These recent observations are concomitant with initial studies that showed increased serum resistin levels [26] and gene expression levels in abdominal depots [16,25] in states of increased adiposity. A further study has shown a significant reduction in circulating resistin levels following moderate weight loss [12] and post-gastric bypass [38]. Collectively, these observations suggest resistin could indirectly be subjected to nutritional regulation in humans.

Observations that argue against a major role for resistin in obesity

Contrary with the studies suggesting a role for resistin in obesity, Maebuchi et al. [44] have reported resistin was undetectable in serum of obese mice, with the same study indicating reductions of resistin mRNA and protein expression in obesity. Others have reported no association of resistin expression with increased adiposity, despite observing elevated circulating levels [33–35]. However, it has been suggested recently that resistin mRNA expression does not necessarily correlate with protein expression [34]. Possible explanations for such diverse observations include differences in post-transcriptional and post-translational modifications, consequently affecting secretory rates of resistin. Increased serum levels may enhance transcript degradation rates via negative feedback mechanisms, or the initiation and recruitment of inhibitors of translation. The secreted form of resistin is considered to have paracrine properties, and this may imply the majority of regulation occurs at the protein level. Similarly, Rajala and co-workers [34] have suggested that binding of serum cofactors to the resistin protein may prolong its half-life, thereby reducing clearance; this hypothesis is supported by studies showing protein interactions and tertiary alterations can influence adipocytokines, such as leptin and TNF-α [45–47]. Indeed, these studies demonstrate the limitations of attempting to derive all necessary information regarding resistin and obesity from gene expression studies.

Further recent human studies have shown no correlation of serum [48] or plasma levels of resistin with any markers of adiposity [49]. Heilbronn et al. [50] reported no relationship between resistin serum levels and percentage body fat, visceral adiposity and BMI. However, the authors [50] suggested that the lack of correlation of serum resistin and increased adiposity was partly due to the confounding variable of age, as non-obese subjects were significantly younger than obese subjects [50]. Another study showed similar levels of resistin expressed in gluteal femoral and subcutaneous abdominal depots in non-diabetic subjects [51]. However, no indication of the number of ‘gluteal’ subjects compared with ‘abdominal’ subjects was given. Moreover, the study used a high proportion of male subjects and, as previous studies had shown resistin to exhibit sexual dimorphism, with women having approx. 20% higher levels than men [42,48,49], this may explain these findings.

Although a high degree of discrepancy has emerged from publications regarding the association of resistin with obesity in the context of mRNA and serum levels, it is worth highlighting the importance of developing highly accurate methods of determining serum resistin concentrations. Methodological limitations may result in variations among serum concentrations, mRNA and protein levels, or may simply indicate that resistin does not play a significant role in the pathophysiology of obesity-mediated insulin resistance. However, with reference to the use of commercially available ELISAs, both rodent and human ELISAs have potential to cross-react with circulating RELMs. To date, not all studies using resistin ELISAs have assessed RELM cross-reactivity prior to analysis; therefore it may be that different ELISAs used may provide varying serum concentrations [52–55]. Similarly, assessment of serum resistin in rodents has
proved contentious [33,34]. Furthermore, due to recent advances in the understanding of the tertiary and quaternary structure of resistin [6], further studies are required to establish whether the complex distribution of the individual structural forms of resistin affect the validity of the currently available human and rodent assays.

**Resistin, insulin resistance and T2DM**

It is currently established that central obesity is a contributing factor to the pathogenesis of insulin resistance and consequently to T2DM. Although it is apparent that inconsistencies remain in the data for a role of resistin in obesity, there is a growing body of evidence suggesting a role for resistin in the aetiology of insulin resistance and T2DM.

**Regulation of resistin in models of insulin resistance and glucose intolerance**

Early rodent studies determined that reduced serum resistin levels in mice were associated with decreased adiposity and improved insulin sensitivity [56]. Rajala et al. [34] recently demonstrated that circulating resistin levels were significantly elevated and positively concordant with rising levels of insulin, glucose and lipids in Lep\textsuperscript{ob/ob} mice. This study also highlighted the potential interplay between resistin and lepitin, with leptin suppressing resistin mRNA and protein levels, concomitant with the reduction in glucose and insulin [34]. Furthermore, Asensio et al. [35] highlighted that lepitin administration in ob/ob mice improved insulin sensitivity, which was affiliated with a decrease in resistin gene expression. Collectively, these studies suggest leptin may exert insulin-resistance-ameliorating effects via counter-regulatory interactions and potentially suppressive mechanisms towards resistin. In contrast, Lee and co-workers [33] reported that neither transcriptional regulation of resistin nor circulating resistin levels correlated with serum insulin or glucose levels. Subsequent studies have denoted resistin expression was either suppressed [57] or unchanged [31] in rodent models of insulin resistance. Furthermore, although resistin mRNA levels were increased in insulin-resistant rats, no apparent change in insulin sensitivity was observed [24].

In evaluating resistin and its association with insulin sensitivity in humans, several studies have identified positive correlations between resistin levels and insulin resistance \textit{in vivo} [49] and \textit{in vitro} [51]. Additionally, serum resistin levels were increased by approx. 20% in T2DM subjects [43], such findings have been re-affirmed by Fujinami et al. [52]. In contrast, other studies have reported no associations between serum resistin levels and markers of insulin resistance in T2DM patients [48,53,58] or insulin-resistant patients [59]. Moreover, serum and plasma resistin levels were either reduced or increased in T2DM patients with no significant correlation with HOMA-IR (homoeostasis model assessment for insulin resistance), waist circumference, BMI or total cholesterol [54,55]. Consequently, these studies suggest resistin is unlikely to play a critical endocrine role in insulin resistance or energy homoeostasis in humans. Nevertheless, a paracrine or autocrine manner of resistin to moderately affect metabolism cannot be ruled out.

**Effect of resistin on glucose homeostasis**

Recently, it has been reported that transgenic mice over-expressing resistin exhibited impaired insulin-mediated glucose transport [60]. This altered glucose metabolism appeared to occur without affecting insulin receptor signalling, therefore acting by reducing the intrinsic activity of cell-surface glucose transporters [60]. Lazar and co-workers [61] have recently shown resistin induced the expression of SOCS (suppressor of cytokine signalling)-3, a known inhibitor of insulin signalling. Moreover, the loss of SOCS function was shown to impair resistin from antagonizing insulin action in adipocytes [61]. This suggested that the insulin-independent action of resistin on adipocytes could partly be mediated by SOCS-3, which could have an impact on normal glucose homeostasis [61].

Rajala et al. [9] have shown that infusion of either resistin or RELM-β into rats decreased insulin sensitivity, primarily at the site of the liver. This worsening of glucose homeostasis was shown to be entirely attributable to the severely impaired insulin-mediated suppression of hepatic gluconeogenesis, rather than peripheral insulin resistance [9]. The study consequently suggested that fat- and gut-derived resistin and RELM-β may have clear and rapid effects on stimulating the rate of hepatic glucose production, as opposed to increasing glucose uptake or influencing peripheral insulin sensitivity [9]. In this regard, the secretion of RELM-β into the portal venous circulation appears to link the intestinal epithelium to the liver, enhancing changes in hepatic intermediate metabolism as a result [9]. Furthermore, this supported the notion of the existence of a feedback mechanism between adipose tissue and insulin-target organs, such as the liver. These findings have been reinforced by studies showing that the ablation of the resistin gene in mice lowering fasting glucose levels through reducing hepatic glucose production without significantly altering whole-body glucose disposal [62]. This study showed that improvement in glucose homeostasis was partly mediated via increased activation of hepatic AMPK (AMP-activated protein kinase) with reduced gene expression of the gluconeogenic enzymes G6Pase (glucose 6-phosphatase) and PEPCK (phosphoenolpyruvate carboxykinase) [62]. Conversely, the authors [62] also showed that resistin treatment in these knockout mice enhanced hepatic production by increasing glucose levels by approx. 25%. Furthermore, Rangwala et al. [63] documented that mice with chronic hyper-resistinaemia exhibited higher blood
Regulation of resistin by insulin sensitizers

Several studies have reported the down-regulation of resistin expression following RSG treatment in WAT (white adipose tissue) of db/db mice, diabetic fatty rats [3,64] and 3T3-L1 adipocytes [36]. Furthermore, resistin expression is down-regulated not only by RSG, but also by darglitazone [36] and troglitazone [65]. Conversely, subsequent rodent studies report that PPAR-γ (peroxisome-proliferator-activated receptor-γ) agonists [27], pioglitazone and troglitazone [28], and metformin [66] increase resistin gene and protein expression. However, human studies have shown that treatment of monocytes with PPAR-γ agonists failed to have any effect on resistin levels [15]. These initial observations suggest that down-regulation of resistin is not crucial for the antidiabetic effect of TZDs in all model systems. However, recent human studies have shed favourable light on the modulation of resistin by TZDs. Pioglitazone treatment suppressed plasma resistin concentrations in T2DM patients, which positively correlated with decreased hepatic fat content and improved insulin sensitivity [67]. Furthermore, RSG reduced resistin secretion from human adipocytes [43] and resistin expression in human macrophages [17,68]. To understand whether the down-regulation of resistin expression by RSG occurred through a direct PPAR-γ-mediated transcriptional mechanism, Patel et al. [17] identified five putative PPREs (PPAR-γ-response elements) in the resistin gene. One such response element, PPRE2, was shown to bind PPAR-γ [17]. However, how PPAR-γ exerts its suppressive actions on resistin expression remains to be elucidated, although it has been suggested that recruitment of co-repressors of transcription may play a role [69]. RSG has also been shown to have anti-inflammatory effects in human macrophages [70] by reducing inflammatory cytokine production, which may consequently affect resistin production [71]. Collectively, these human studies indicate that suppression of resistin expression may contribute to the insulin-sensitizing and glucose-lowering actions of the TZDs. Furthermore, the potential anti-inflammatory effects of TZDs on adipocytokine mediation may be of equal importance in the prevention of T2DM.

Hormone and cytokine modulators of resistin expression

Studies investigating metabolic hormones and cytokines that are associated with insulin resistance have looked for their relationship with resistin. Such studies have produced data suggesting an interplay between hormones, cytokines and resistin, as shown in Table 3. Although the reported regulation of resistin by these factors appears intriguing, no underlying mechanistic principles are currently apparent. Therefore the physiological relevance of most of these factors with respect to resistin remain to be determined.

Gender effects on resistin regulation

In WAT, it has been shown that resistin mRNA expression is higher in male than female rats [72,73], in contrast with a similar study also undertaken in mice [74]. In human subjects, Yannakoulia and co-workers [42] reported that resistin concentrations were significantly higher in females compared with males, which was also confirmed by two other studies suggesting a significant gender difference [48,49]. However, a subsequent study examining both age and gender in relation to serum resistin indicated a lack of association in healthy subjects, as well as patients with Type I diabetes and T2DM [39]. As a result, the significance of gender on the degree of resistin expression in rodents and humans remains unclear, perhaps complicated by the issue of specificity of resistin ELISAs.

Adiponectin, insulin resistance and T2DM

Although resistin has been implicated in the pathogenesis of insulin resistance and T2DM, it is noteworthy to mention another novel adipocytokine proposed to have a role in the aetiology of human T2DM. Adiponectin expression and plasma levels, in contrast with resistin and other adipocytokines whose levels are increased in states of obesity, are reduced in insulin resistance and obesity [75,76]. Additionally, it has been reported that gastric bypass in morbidly obese patients has resulted in elevated levels of plasma adiponectin [77]. Interestingly, low serum levels of adiponectin have been shown to independently predict future risk of developing T2DM [78]. With regards to insulin sensitizers, adiponectin synthesis and secretion are up-regulated by TZDs in vitro and in vivo.
Effects of hormones and cytokines on the level of resistin expression and secretion

<table>
<thead>
<tr>
<th>Hormone/cytokine</th>
<th>Effect on resistin</th>
<th>References</th>
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<tbody>
<tr>
<td>Pituitary hormones</td>
<td>Growth hormone (somatotrophin; 1 mg·kg−1·day−1) ↑ resistin gene expression (720–950 %) in WAT of spontaneous dwarf rats and has moderate inhibitory effects on resistin transcript and protein (30–50 %) levels in 3T3-L1 adipocytes; ↑ gene expression levels in response to hyperprolactinaemia in mice; ↓ mRNA and protein expression (30–50 %) in 3T3-L1 adipocytes.</td>
<td>[65,151,152]</td>
</tr>
<tr>
<td>Steroid hormones</td>
<td>Dexamethasone ↑ mRNA and protein levels (2.5- to 3.5-fold) in 3T3-L1 adipocytes and approx. 70 % in mouse WAT.</td>
<td>[36,65]</td>
</tr>
<tr>
<td>Sex hormones</td>
<td>↑ In mice with elevated androgen levels; ↑ by hyperprolactinaemia and testosterone; administration of dehydroepiandrosterone ↑ gene expression in WAT of male Wistar rats; oestrogen ↓ adipose tissue mRNA levels in male rats.</td>
<td>[73,152–154]</td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td>Severely ↓ expression in hyperthyroid rats; ↓ serum levels in subjects with hyperthyroidism.</td>
<td>[73,155]</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>↓ Transcript and protein levels (30–50 %) in 3T3-L1 adipocytes.</td>
<td>[65]</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>Intracerebroventricular administration of neuropeptide Y ↑ gene expression in mice WAT.</td>
<td>[154]</td>
</tr>
<tr>
<td>β₁-Adrenoreceptors</td>
<td>The β₁-agonist isoproterenol ↓ gene expression levels in vitro by 20 % in 3T3-L1 adipocytes, which is reversible with the β₁-antagonist propranolol.</td>
<td>[157]</td>
</tr>
<tr>
<td>ET-1</td>
<td>ET-1 (100 nM) ↓ basal secretion by 59 % in 3T3-L1 adipocytes.</td>
<td>[158]</td>
</tr>
<tr>
<td>Insulin</td>
<td>↓ Gene expression (approx. 50 %) in 3T3-L1 adipocytes; ↑ secretion from 3T3-L1 adipocytes; ↑ mRNA synthesis (23-fold) in streptozotocin-diabetic mice or Zucker diabetic fatty rats; gene expression and protein concentration ↑ in fasted mice; ↑ resistin protein secretion in a concentration-dependent manner in human subcutaneous adipocytes.</td>
<td>[4,27,34,63,65,158]</td>
</tr>
</tbody>
</table>

[79–81] and suppressed by insulin-resistance-inducing hormones [82,83]. The precise function of adiponectin as a therapeutic target, improving glucose metabolism by increasing insulin sensitivity in humans, is currently of great interest.

**Potential role of resistin in inflammation**

Recent studies have highlighted the importance of inflammation in linking obesity and the occurrence of the metabolic syndrome, suggesting that the metabolic syndrome is a low-grade systemic inflammatory condition [84]. Although this emerging concept of inflammation has been recognized in the context of increasing adiposity, its molecular basis and physiological significance in terms of the pathogenesis of T2DM is yet to be fully elucidated. Thus adipose tissue may act as a site for mediation of inflammation in linking obesity and the occurrence of the metabolic syndrome, suggesting that the metabolic syndrome is a low-grade systemic inflammatory condition [84], whereas another reported LPS, IL-1, TNF-α and IL-6 strongly stimulated resistin expression in human [PBMCs (peripheral blood mononuclear cells)] [38,43,58]. Correlations between resistin expression and secretion and other inflammatory markers, including IL-6, leptin, sTNF-R2 (soluble TNF-receptor 2) and CRP (C-reactive protein), have also been reported in patients with severe inflammatory disease, obesity and T2DM [68]. Recently, Lehrke et al. [68] demonstrated that endotoxaemia caused a dramatic increase in resistin in primary human macrophages, and this was attenuated by aspirin and RSG. In this situation, RSG was suggested to possess dual insulin-sensitizing and anti-inflammatory properties. Additionally, LPS induced TNF-α, which preceded the increase in resistin, and therefore, in this study, the authors...
hypothesized that TNF-α was responsible for the later induction of resistin [89]. Thus cytokine induction of resistin may contribute to insulin resistance in endotoxaemia, obesity and inflammation.

Obesity is associated with adipose tissue infiltration by macrophages, thus contributing to resistin expression, and this may propagate further recruitment of more macrophages potentially interacting with adipocytes. Adipose tissue may therefore represent a site of an innate immune response in humans [90]. Alternatively, it is hypothesized that macrophage recruitment may instead arise from the trans-differentiation of pre-adipocytes into macrophage-like cells [68,88], highlighting the close interrelationship between adipocyte and macrophage lineages. This would be consistent with the emerging concept that adipocytes and macrophages share the property of secreting pro-inflammatory cytokines that regulate metabolic function. To date, resistin gene expression levels have been reported to be higher in human PBMCs [25]. However, comparative studies have not shown the relative resistin protein expression in macrophages compared with adipocytes; this could alter our perception of the contribution of resistin made by the adipocyte. Furthermore, studies have highlighted increased resistin protein expression in human abdominal fat, irrespective of the presence of mononuclear blood cells [91]. At the very least, it seems apparent that the findings on the pattern of resistin mRNA expression suggest that adipocytes contribute modestly to hyper-resistinaemia. Although, combined with the significant biomass that adipose tissue can contribute to whole-body weight, particularly in obese individuals, there is little doubt that the systemic contribution of adipose tissue is significant [92]. It has been suggested that obesity and T2DM are associated with chronic inflammation and activation of innate immune pathways [93,94]. In obesity, where pro-inflammatory cytokine levels are known to be elevated, it may be plausible that resistin could function as a signalling component of the innate immune response to circuitously induce the production of pro-inflammatory cytokines, such as TNF-α and IL-6, within macrophages and adipocytes. In turn, such immune dysregulation could contribute to the development of a pro-inflammatory milieu associated with insulin resistance. Overall, it is apparent that the relationships between obesity, inflammation and resistin expression are complex. Further studies are clearly required to explain the role of the adipocyte as a site of an immune response, potentially contributing to coupling of immunity and metabolic dysregulation.

In addition to resistin, adiponectin may possess anti-inflammatory properties. Recent studies have shown that adiponectin suppressed cytokine production in macrophages [95] and disrupted the activation of NF-κB (nuclear factor κB) in an aortic endothelial cell model [96]. Ajuwon and Spurlock [96] reported that adiponectin attenuated LPS-induced NF-κB activation and IL-6 production in 3T3-L1 adipocytes, and antagonized an endotoxin-induced increase of TNF-α expression [97]. These studies suggest a regulatory role for adiponectin in inflammation within adipose tissue, possibly acting via the regulation of NF-κB. Hypoadiponectinaemia may contribute to the pathogenesis of obesity-related insulin resistance, inflammation and T2DM, and therefore further studies should elucidate mechanisms of action.

Relationship of resistin with CVD (cardiovascular disease)

Adipocyte-derived hormones not only function prominently in the pathogenesis of T2DM, but may also serve as important vasoactive factors directly affecting endothelial function and vascular homeostasis [98]. Therefore these factors may represent an important point of convergence in linking insulin resistance to an increased risk of atherosclerotic vascular disease. Although the precise molecular mechanisms responsible for this convergence remain nebulous, resistin may have a role in its pathogenesis. A recent study by Reilly and co-workers [98] suggested resistin as a metabolic link between inflammation and atherosclerosis. This group reported that, in non-diabetic and diabetic subjects, plasma resistin levels were associated with metabolic and inflammatory markers, including sTNF-R2, IL-6 and LpPLA2 (lipoprotein-associated phospholipase A2) [98]. Furthermore, resistin levels correlated with CAC (coronary artery calcification), a quantitative measure of coronary atherosclerosis [98]. Notably, in metabolic syndrome subjects, resistin levels further predicted CAC, whereas CRP levels did not [99]. Such results imply resistin may be an independent marker of atherosclerosis in humans.

Several studies have examined resistin as a cardiovascular risk factor and a potential contributor to endothelial dysregulation and atherosclerotic lesion formation [99]. Such studies have shown resistin stimulated factors, including ET-1 (endothelin-1), VCAM-1 (vascular cell adhesion molecule-1) and MCP-1 (monocyte chemoattractant protein-1), thus contributing to CVD [100]. Additional confirmation of the potential effects of resistin on the vasculature have arisen through studies examining human aortic endothelial cells. One study reported that resistin up-regulated the expression of adhesion molecules, including VCAM-1 and ICAM-1, as well as long PTX3 (pentraxin 3), a marker of inflammation [100–102]. Therefore, as adhesion molecules may represent the initial stages of atherosclerotic lesions and PTX3 expression is increased in atherosclerotic lesions, resistin may exert a pro-inflammatory effect on vascular endothelial cells, promoting the onset of atherosclerosis [100]. In contrast with resistin, adiponectin, known to enhance insulin sensitivity and reduce atherosclerotic plaques, suppressed a resistin-mediated rise in VCAM-1 and ICAM-1 [103]. These observations coincide well with a study showing...
that adiponectin suppressed the TNF-α-stimulated expression of E-selectin, VCAM-1 and ICAM-1 in human endothelial cells [104–106]. This suggests further that adiponectin may be vasoprotective and negatively modulate the atherogenic processes. These findings raise the possibility that resistin, in conjunction with other adipokines, may influence the degree of inflammation in the vasculature via direct modulation of endothelial function. However, further studies are required to fully elucidate the relationship of resistin and adiponectin with inflammatory-related CVD.

HUMAN GENETIC STUDIES OF RESISTIN

Several SNPs (single nucleotide polymorphisms) have been identified in the Retn gene, but only few have minor allele frequencies over 5% and are associated with disease risk [104–106]. Therefore further confusion ensues when reviewing genetic studies examining associations between resistin and disease. In a study of non-diabetic French Canadians in Quebec [104], two Retn 5’-flanking SNPs (−537 and −420) were associated with increased BMI. Furthermore, a resistin genotype at nucleotide +299 (IVS2 +181G → A) and obesity was a significant determinant of T2DM risk among Type II diabetic Caucasians in Boston (MA, U.S.A.) [107]. Additionally, the −420C → G SNP (−180 relative to putative transcription start site) was associated with higher resistin mRNA levels in abdominal fat of obese subjects [51]. Conversely, Mattevi et al. [105] showed an association between the −420C → G polymorphism with lower BMI in non-diabetic individuals from a Brazilian population of European descent, although, among non-diabetic Caucasians in Sicily and Gargano (Italy), an ATG triplet repeat in the 3′-untranslated region of the resistin gene was associated with a decreased risk of insulin resistance [108].

Genetic analysis of resistin using a Japanese population demonstrated that a −420G/G genotype was associated with T2DM and could accelerate the onset of disease by 4.9 years [106]; moreover, the genotype itself was a primary variant determining T2DM susceptibility [106]. Consistent with these findings, elevated levels of serum resistin were reported in T2DM subjects carrying the −420G genotype [109]. In contrast, studies in a Japanese obese population reported the −638G → A, −420C → G, and −358G → A SNPs, which although associated with serum resistin, did not confer any association with obesity or insulin resistance [110,111]. These genetic studies highlight the discrepancies in resistin SNP analysis examining the association with obesity-related insulin resistance; these may partly be explained by different genetic backgrounds or environmental conditions of the populations studied. Further studies on the physiology of resistin and genetic implications for the development of this disease are therefore crucial.

CONCLUSIONS

It is apparent that the pathogenesis of T2DM and CVD is complex and multifactorial in which several adipocytokines have been implicated. Over the last 4 years our understanding of the role of resistin in this process has led some to question its relative importance in man. Recent studies have shed more light on differences between rodents and humans, indicating the diversity of resistin action in these species. Further studies are required to establish the relevance of resistin to human diabetes, in particular its effects on the central nervous system and β-cell function that have not been characterized. It is also clear that further work is required to understand the basis for formation of different higher and lower molecular-mass forms of resistin in the circulation and their effects on function. Clearly, identifying a receptor for resistin would represent a major advance and could lead to a clearer understanding of its function. However, our growing knowledge of the role of pro-inflammatory molecules generated within adipose tissue and their link to diabetes means that interventions that reduce the production of pro-inflammatory cytokines, including resistin, may prove to have therapeutic potential.

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Role of resistin in obesity, insulin resistance and Type II diabetes


