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Title: The *in vitro* effects of resistin on the innate immune signaling pathway in isolated human subcutaneous adipocytes

Short title: Pro-inflammatory actions of resistin

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Abstract

Context: Obesity-associated inflammation is a contributory factor in the pathogenesis of type 2 diabetes mellitus (T2DM); the mechanisms underlying the progression to T2DM are unclear. The adipokine resistin has demonstrated pro-inflammatory properties in relation to obesity and T2DM.

Objective: To characterize resistin expression in human obesity and address the role of resistin in the innate immune pathway. Furthermore, examine the influence of lipopolysaccharide, recombinant human resistin (rhResistin), insulin and rosiglitazone in human adipocytes. Finally, analyze the effect of rhResistin on the expression of components of the NF-κB pathway and insulin signaling cascade.

Methods: Abdominal subcutaneous adipose tissue was obtained from patients undergoing elective liposuction surgery (n = 35, aged: 36-49 yr; BMI: 26.5 ± 5.9 kg/m^2). Isolated adipocytes were cultured with rhResistin (10-50 ng/ml). The level of cytokine secretion from isolated adipocytes was examined by ELISA. The effect of rhResistin on protein expression of components of the innate immune pathway was examined by Western blot.

Results: *In-vitro* studies demonstrated that antigenic stimuli increase resistin secretion ($P < 0.001$) from isolated adipocytes. Pro-inflammatory cytokine levels were increased in response to rhResistin ($P < 0.001$); this was attenuated by rosiglitazone ($P < 0.01$). When examining components of the innate immune pathway, rhResistin stimulated Toll-like receptor-2 protein expression. Similarly, mediators of the insulin signaling pathway, phosphospecific JNK1 and JNK2, were upregulated in response to rhResistin.

Conclusion: Resistin may participate in more than one mechanism to influence pro-inflammatory cytokine release from human adipocytes; potentially via the integration of NF-κB and JNK signaling pathways.

Introduction

The association between central obesity, insulin resistance and T2DM is established; however, the underlying mechanisms of this association remain unclear. Besides its metabolic functions, increased adipose tissue (AT) mass is recognised to have immunological characteristics, primarily through the secretion of adipokines, such as leptin, TNF-α and IL-6 (1). Within this context, AT is considered to integrate metabolic and immune functions. This duality of function may represent a conserved evolutionary mechanism, as suggested by observations examining the 'fat body' in *Drosophila* fruitfly; in which a single cell-type serves as a primary integrator for both pathogen and nutrient-sensing pathways (2).

It is acknowledged that with increasing adiposity there is profound macrophage infiltration into AT; macrophages may thus represent the site of an innate immune response. Alternatively, macrophage recruitment may arise from phenotypic change of pre-adipocytes (3, 4). Nevertheless, studies indicate interrelationships between excess AT mass, inflammation, insulin resistance and T2DM.

The adipokine resistin was originally described as a molecular link between obesity and insulin resistance in rodents; this has remained somewhat controversial in humans (5). Resistin is expressed primarily in adipocytes in rodents and employs a more metabolic role, by impairing glucose tolerance and inducing liver-specific antagonism of insulin sensitivity (6). In humans however, a more 'pro-inflammatory' function for resistin has been defined (7, 8). Although resistin gene expression is largely confined to macrophages (9, 10), recent studies have reported resistin protein expression and secretion from human adipocytes (11-14).

Serum profiles have highlighted increased circulating levels of resistin in obesity and T2DM; which further correlate with C-Reactive Protein (CRP) (13), a marker of inflammation and an established predictor of cardiovascular disease (15). Such a correlation

has been identified by subsequent studies on pre-diabetic, T2DM subjects (16) and individuals with acute rheumatoid arthritis (8). Circulating levels of resistin are associated with TNF-α receptor-2 (TNF-R2) and are predictive of coronary atherosclerosis, independent of CRP (17). Endotoxemia increases serum resistin levels, concurrently with soluble TNF-R2 levels in T2DM patients (18). Although the majority of studies report associations between resistin and inflammatory conditions, the precise mechanistic action of resistin in inflammation, particularly in concordance with components of the innate immune pathway, is unclear.

The innate immune system is a candidate for the production of elevated levels of cytokines in obesity and T2DM. The innate immune pathway is activated when specific receptors, the Toll-like receptors (TLRs), bind certain antigens. For instance, TLR-4 binds the bacterial antigen lipopolysaccharide (LPS), through its co-receptor, CD14; alternatively, TLR-2 binds the fungal antigen, zymosan. Activation of TLR-4 by LPS can induce TLR-2 expression in 3T3-L1 adipocytes (19). TLR activation initiates an intracellular signaling cascade, causing NF-κB to initiate the production of inflammatory factors, such as IL-6 and TNF-α. Several serine/threonine kinases are activated during the innate immune response that influence insulin signaling (20). IκB kinase (IKK)-β mediates activation of NF-κB; whereas c-Jun N-terminal kinase (JNK), a central metabolic regulator, contributes to the development of insulin resistance in obesity (20). Activation of JNK and IKK-β within innate immunity highlights crosstalk between metabolic and immune pathways.

An integration of metabolic and immune systems may reflect the mode of resistin action within adipocytes and immune cells; exerting metabolic and immune functions in both cell-types. Resistin impairs insulin signaling via ‘suppressor of cytokine signaling-3’ (21) and inhibits glucose transport (22) in 3T3-L1 adipocytes; additionally, resistin promotes glucose-dependent lipogenesis and lipid accumulation in human macrophages (23). On the other

hand, the pro-inflammatory functions of resistin in human macrophages (7) and 3T3-L1 adipocytes (22) have also been described. Resistin may thus function in adipocytes to influence both metabolic and pro-inflammatory changes, suggesting that the effects of resistin are to some extent linked. Such a duality in function for resistin may be a consequence of the crosslink initially proposed between metabolic and inflammatory pathways in adipocytes and immune cells (3, 4). Where resistin may influence key factors in the sequential stages from one signal transduction pathway; this may consequently alter components from another.

The aims of this study were therefore to (1) establish the association between increasing adiposity and expression of resistin in human Abdominal Subcutaneous (Abd Sc) adipocytes and AT; (2) determine whether resistin levels are influenced by antigenic stimuli and inflammatory cytokines within adipocytes (3) examine the effect of rhResistin on the expression of components of the innate immune pathway and insulin signaling cascade within adipocytes (4) evaluate the combined effects of rhResistin, insulin and rosiglitazone (RSG) on the pro-inflammatory response (5) finally, examine the effects of NF-κB inhibitor and JNK inhibitor on the level of resistin secretion from adipocytes.

Subjects and Methods

Subjects

Abd Sc AT was obtained from a human non-diabetic population (n = 35, aged 36-49 yr; BMI: 26.5 ± 5.9 kg/m^2) undergoing elective liposuction surgery. Patients receiving endocrine therapy (steroids, hormone replacement therapy or thyroxine), anti-inflammatory therapy (aspirin, cyclooxygenase-2 inhibitors), statins, TZDs or any antihypertensive therapy were excluded. Studies were performed with the approval of the local ethics committee with informed consent being obtained from all subjects prior to enrolment.

Isolation of mature adipocytes

Abd Sc AT was digested in collagenase (2 mg/ml; Worthington Biochemical, USA) to isolate adipocytes, as previously described (13). Adipocytes were re-suspended in either 4% SDS or RIPA buffer (150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris) for extraction of protein. Cells were maintained in phenol red-free DMEM:F-12 medium containing 15 mM glucose, penicillin (100 U/ml) and streptomycin (100 μg/ml).

Treatment of isolated adipocytes

For antigenic stimuli studies, adipocytes were treated (14 h) with either bacterial endotoxin, LPS (100 ng/ml; Sigma-Aldrich Company Ltd., Poole, UK) or fungal antigen, zymosan (30 μg/ml; Sigma-Aldrich Company Ltd., Poole, UK). Dose and time-responses for LPS and zymosan were previously established (LPS: 1-100 ng/ml; 14, 24 and 48 h; zymosan: 1-100 μg/ml; 14, 24 and 48 h) (data not shown). Cytokine secretion studies involved treatment of adipocytes with rhResistin (30 ng/ml, 48 h; Phoenix Pharmaceuticals, Belmont, CA, USA) (endotoxin concentration below 0.1 ng/μg, at final concentrations of 10-50 ng/ml). Isolated

adipocytes were also treated with insulin alone (10 nM Sigma-Aldrich Company Ltd., Poole, UK) or combined with RSG (10^{-8} M; GlaxoSmithKline, Harlow, UK). rhResistin, insulin and RSG concentrations and time-points were chosen based on data previously described (13). Adipocytes were further treated with rhTNF-α (10, 50, 100 ng/ml; Biosource Europe, S. A., Belgium) or rhIL-6 (10, 50, 100 ng/ml; Sigma-Aldrich). For inhibitor studies, adipocytes were treated with NF-κB inhibitor (SN50, CalBiochem, Nottingham, UK) (50 μg/ml; 24 h). Dose and time-course studies were performed to assess resistin secretion at 14, 24, and 48 h with control and NF-κB-treated adipocytes (10, 25, 50 and 100 μg/ml). Adipocytes were also treated with JNK inhibitor (SP600125, A. G. Scientific, Inc., San Diego, USA) (10 μM/ml); conditions based on previous data (24). For protein expression analysis, adipocytes were treated with increasing concentrations of rhResistin, using previously established time-points (10, 30, 50 ng/ml; 48 h). Adipocytes maintained in untreated media were used as controls. A trypan blue dye exclusion method was used to assess the viability of the adipocytes, as previously documented (Sigma-Aldrich) (13). Following treatment, conditioned media were removed and stored at -80ºC. Adipocyte protein was extracted as previously described (13) then stored at -80ºC.

Protein determination & Western blot analysis

Human AT and isolated adipocytes were re-suspended in 4% SDS or RIPA buffer, as previously detailed (13). Protein concentrations were determined using the Bio-Rad DC (Detergent Compatible) protein assay kit (25). Western blot analysis was performed using a method previously described (14). Human resistin polyclonal antibody (1:3000, Linco Research, Inc., Missouri, USA) was used to assess resistin expression. rhResistin (1 μg/ml; Phoenix Pharmaceuticals, Belmont, CA, USA) was used to confirm the specificity of the primary antibody (data not shown). Resistin was developed using an anti-guinea-pig

horseradish-peroxidase (HRP) secondary antibody (Biogenesis Ltd., Poole, UK). Human TLR-2 monoclonal and TLR-4 polyclonal antibodies were utilized (1:500 and 1:1000, respectively; Insight Biotechnology Ltd., Wembley, UK). Polyclonal anti-JNK1 & 2 SAPK phosphospecific and MyD88 antibodies (1:1,750; Biosource UK, Belgium and 1:250; TCS Cellworks, UK respectively) were utilized. Protein expression of NF-κB, (1:250, TCS Cellworks, UK), IKK-β (1:500, TCS Cellworks, UK) and IKK-α (1:500, Abcam, UK) was assessed using mouse monoclonal antibodies. Equal protein loading was confirmed by examining α-tubulin (1:5000) (The Binding Site, Birmingham, UK) protein expression. No statistical difference was observed in α-tubulin expression for all samples analyzed. For reducing conditions, samples were mixed in a 1:2 ratio with sample buffer containing 20% β-mercaptoethanol. A chemiluminescent detection system ECL/ECL$^+$ (Amersham Pharmacia Biotech, Little Chalfont, UK) enabled visualization of bands, whilst intensity was determined using densitometry (Genesnap, Syngene, UK).

RNA extraction and quantitative RT-PCR

RNA was extracted from AT using the RNeasy Lipid Tissue Mini Kit (Qiagen, UK). RNA extraction was followed by a DN*ase* digestion step to remove any contaminating genomic DNA. 1 μg of RNA was reverse transcribed using RevertAid H Minus M-MuLV reverse transcriptase (Helena Biosciences Europe, Sunderland, UK) and random hexamers in 20 μl reaction volumes, according to the manufacturers' instructions. Messenger RNA levels were determined using an ABI 7500 real time PCR Sequence Detection system. The reactions were performed in 25 μl volumes in reaction buffer containing TaqMan Universal PCR Master Mix, 150 nmol TaqMan probe, 900 nmol primers and 50 ng cDNA (for CD45 expression) or 115 ng cDNA (for resistin expression). Previously determined quantitative primer and probe sequences for the resistin and CD45 genes were used (14). All reactions were multiplexed

with the housekeeping gene 18S, provided as a pre-optimized control probe (Applera, Cheshire, UK), enabling data to be expressed as delta cycle threshold (Ct) values (ΔCt = Ct of 18S subtracted from Ct of gene of interest) in order to correct for differences in the efficiency of reverse transcription. Measurements were carried out on at least three occasions for each sample.

Resistin secretion from treated adipocytes

Conditioned media from adipocytes treated with LPS or zymosan was assayed using a human resistin ELISA (Phoenix Europe GmbH, Germany). Conditioned media from rhTNF-α or rhIL-6 treated adipocytes was assessed using the human resistin ELISA from R&D Systems, UK. The R&D Systems human resistin ELISA (resistin range: 0-10 ng/ml) was further validated for recovery of resistin and cross-reactivity with resistin-like molecules (RELMs). Known concentrations of rhResistin (1, 5 and 10 ng/ml; R&D Systems, UK) were added to pooled serum (10.5 ng/ml). The recovery of spiked resistin was above 80% efficiency. Known concentrations of RELM-α or RELM-β partial-peptides (1, 2.5, and 5 ng/ml; Alpha Diagnostics, Eastleigh, UK) and rhResistin (5 ng/ml) were co-incubated with pooled serum (10.5 ng/ml), an aqueous solution or serum matrix containing rhResistin (5 ng/ml). The addition of RELMs to treatments did not interfere with the resistin assay or alter known and expected serum resistin concentrations. The human resistin ELISA previously validated (Phoenix Europe GmbH, Germany) was used in this study (13).

IL-6 and TNF-α secretion from treated adipocytes

Conditioned media from adipocytes treated with rhResistin, insulin, or insulin in combination with RSG was assayed for IL-6 and TNF-α (QuantiGlo ELISA, R&D Systems,

Abingdon, UK) (IL-6, intra-assay CV 3.1%, inter-assay CV 2.7%; TNF-α, intra-assay CV 6.7%, inter-assay CV 11.0%).

Statistics

Protein expression data between control and treatments were compared using an unpaired *t*-test. Data are presented as mean ± SEM. Analyses were carried out using SPSS (SPSS Inc. 12.0, Woking, UK) software. The threshold for significance was $P < 0.05$. Correlation analyses were calculated using a Pearsons Correlation Coefficient test.

Results

Resistin expression in AT

Results demonstrated that resistin gene expression positively correlates with increasing BMI in AT (ΔCT range, 25.0-30.7; $r^2 = 0.461$; $P < 0.001$) (BMI: 19.2-37.0 kg/m^2; n = 24). Analysis of CD45 expression with increasing adiposity showed a similar but weaker correlation (ΔCT range, 20.0-23.6; $r^2 = 0.226$; $P < 0.02$) (Fig. 1). Resistin protein data confirmed the mRNA data, as resistin protein expression was 1.5-fold higher in obese AT (BMI: 33.9 ± 4.6 kg/m^2, n = 8) compared with lean AT (BMI: 21.2 ± 1.4 kg/m^2, n = 8) ($P < 0.001$) (Fig. 2A). Furthermore, in adipocytes, a 2.2-fold higher level of resistin protein expression was observed in overweight subjects (BMI: 28.3 ± 2.7 kg/m^2, n = 4) in comparison with lean subjects (BMI: 23.2 ± 1.6 kg/m^2, n = 4; $P < 0.001$; Fig. 2B).

Effect of antigenic stimuli on the level of resistin secretion from adipocytes

LPS was shown to stimulate a 2.2-fold increase in resistin secretion (control: 1.24 ± 0.2 ng/ml; LPS: 2.75 ± 0.4 ng/ml; $P < 0.001$; n = 8) (Fig. 3). Similarly, zymosan stimulated a 2.5-fold increase in resistin secretion from adipocytes compared to control (control: 1.24 ± 0.2 ng/ml; zymosan: 3.1 ± 0.3 ng/ml; $P < 0.001$; n = 8) (Fig. 3).

Regulation of TNF-α and IL-6 secretion: effects of rhResistin, insulin & RSG

rhResistin alone, and in combination with insulin, significantly increases the level of TNF-α secretion from adipocytes (control: 74 ± 10 pg/ml; rhResistin: 435 ± 36.5 pg/ml; $P < 0.001$). Furthermore, RSG significantly reduces this resistin-stimulated increase in TNF-α secretion from adipocytes ($P < 0.001$). Following this reduction, TNF-α secretion levels remain higher than the control ($P < 0.01$) (Fig. 4A). Similarly, rhResistin and insulin significantly increase IL-6 secretion (control: 1962 ± 130 pg/ml; rhResistin: 2906.4 ± 297.0 pg/ml; $P < 0.01$); RSG

further reduces this resistin-induced increase in IL-6 secretion from adipocytes (Fig. 4B). Further analysis of cytokine secretion demonstrated that anti-resistin (10 μg/ml) antibody reduces the level of TNF-α (rhResistin: 89.2 ± 4.6 pg/ml; anti-resistin antibody (10 μg/ml): 71.5 ± 5.9 pg/ml; $P = 0.039$) and IL-6 (rhResistin: 1115.5 ± 40.6 pg/ml; anti-resistin antibody (10 μg/ml): 351.5 ± 55.9 pg/ml; $P < 0.01$) secretion from adipocytes.

Effect of rhTNF-α and rhIL-6 on the level of resistin secretion

To establish whether a cytokine feedback mechanism exists within adipocytes, we examined the level of resistin secretion from rhTNF-α and rhIL-6 treated adipocytes. Resistin secretion was unaffected by rhTNF-α, at any concentration up to 100 ng/ml (control: 135 ± 19 pg/ml; 10 ng/ml rhTNF-α: 129 ± 15 pg/ml; 50 ng/ml rhTNF-α: 141 ± 11 pg/ml; 100 ng/ml rhTNF-α: 116 ± 11 pg/ml; n = 12). Furthermore, rhIL-6 also had no significant effect on the level of resistin secretion (control: 129 ± 12 pg/ml; 10 ng/ml rhIL-6: 135 ± 13 pg/ml; 50 ng/ml rhIL-6: 123 ± 10 pg/ml; 100 ng/ml rhIL-6: 125 ± 12 pg/ml; n = 8).

Effect of rhResistin on TLR-2 and TLR-4 protein expression in adipocytes

For protein expression studies, rhResistin stimulated TLR-2 expression in adipocytes (control: 1.00 ± 0.11; TLR-2: 1.28 ± 0.10; $P < 0.001$, n = 6; BMI: 23.5 ± 3.8) (Fig. 5A). No significant change in TLR-4 protein expression was observed when compared with control (data not shown); this was expected, due to the known constitutive expression of TLR-4 in other tissues.

Effect of rhResistin on the insulin signaling and NF-κB pathway

rhResistin stimulated MyD88 expression in adipocytes (control: 1.00 ± 0.13; MyD88 50 ng: 1.80 ± 0.04; $\uparrow P < 0.01$, n = 6) (Fig. 5B). rhResistin further upregulated the expression of

phosphospecific JNK1 (control: 1.00 ± 0.03; JNK1-P 50 ng: 1.29 ± 0.05; ↑$P < 0.05$, n = 6) and phosphospecific JNK2 (control: 1.00 ± 0.08; JNK2-P 50 ng: 1.53 ± 0.03; ↑$P < 0.001$, n = 6) (Fig. 5B). Similarly, NF-κB (control: 1.00 ± 0.04; NF-κB 50 ng: 1.37 ± 0.02; ↑$P <$ 0.05, n = 4) expression was increased in response to rhResistin (Fig. 5B). Additionally, IKK-β and IKK-α were upregulated in response to rhResistin (control: 1.00 ± 0.04; IKK-β 50 ng: 1.17 ± 0.03 ↑$P < 0.01$, n = 4) (control: 1.00 ± 0.06; IKK-α 50 ng: 1.50 ± 0.02 ↑$P < 0.01$, n = 4) (Fig. 5C).

Effects of JNK or NF-κB inhibitor on resistin secretion

The level of resistin secretion from adipocytes was significantly reduced with NF-κB inhibitor treatment (control: 83.1 ± 20.5 pg/ml; NF-κB inhibitor: 61.6 ± 16.6 pg/ml; n = 7, $P < 0.05$) (Fig. 6A). However, no significant difference in resistin secretion was observed for JNK inhibitor treated adipocytes (control: 101.5 ± 29.3 pg/ml; JNK inhibitor: 77.0 ± 17.2 pg/ml; n = 4, P = N.S) (data not shown).

Discussion

Our study demonstrates the pro-inflammatory actions of resistin in human AT. We further establish that resistin can influence the secretion of pro-inflammatory cytokines from human adipocytes; this induction of cytokine secretion is attenuated by RSG. Furthermore, rhResistin stimulates the expression of TLR-2 and two central metabolic and inflammatory kinases, JNK and IKK-β respectively. Our findings implicate resistin in the stimulation of pro-inflammatory cytokine release from human adipocytes.

Whilst a more pro-inflammatory role for resistin is emerging in humans, the metabolic actions of resistin remain uncertain. Rodent studies implicate the liver as the major physiological target of resistin action; as exogenous resistin impairs glucose tolerance and hepatic insulin resistance (26). Similarly, adenovirus mediated hyper-resistinemia abrogates hepatic and peripheral insulin action (27). Conversely, resistin null mice exhibit low fasted blood glucose levels, due to reduced hepatic glucose production (28). *In vitro* adipocyte studies highlight that resistin impairs insulin-stimulated glucose uptake (21) and the insulin signaling cascade itself (29). Recent reports further highlight that human resistin has properties similar to its murine counterpart; whereby mouse and human resistin impair glucose transport (29). Here, we demonstrate the pro-inflammatory actions of resistin in human adipocytes. Collectively, these studies suggest an overlap between metabolic and immune functions for human resistin.

mRNA studies demonstrate that resistin is predominantly expressed in human macrophages (9, 10). Our initial and current studies demonstrate resistin protein expression and secretion from human adipocytes; this has been affirmed by recent observations (11, 12). Such quantitative differences in mRNA expression and circulating resistin levels have previously been highlighted (1, 30). The adipocyte may thus be an undervalued contributor to the circulating levels of resistin in obesity.

We also show that LPS increases resistin secretion from isolated adipocytes. This coincides with recent studies, demonstrating that endotoxemia induces circulating resistin levels in healthy subjects (18); highlighting antigenic stimuli can increase resistin levels *in vivo*. We further demonstrate that resistin increases the level of TNF-α and IL-6 secretion from adipocytes; consistent with recent reports, whereby human resistin increases TNF-α and IL-12 secretion from macrophages (7). It is acknowledged that circulating levels of TNF-α and IL-6 are elevated in obesity (31). We further demonstrate that treatment with anti-resistin antibodies reduces the level of cytokine secretion; suggesting that resistin may directly contribute to an altered pro-inflammatory cytokine status by promoting inflammation. Additionally, we observed that LPS can directly stimulate TNF-α and IL-6 secretion from human adipocytes (32).

We additionally examined whether rhResistin influences the expression of key components of the innate immune pathway and observed that resistin upregulates the expression of TLR-2, MyD88 and NF-κB in adipocytes. When examining the key intermediate activating NF-κB, the IKK complex, rhResistin further increases the expression of the catalytic subunits IKK-β and IKK-α. Interestingly, JNK expression is upregulated in response to rhResistin, suggesting NF-κB activation may overlap into a JNK-mediated pathway. Such an overlap between JNK and NF-κB has been identified in macrophages and alveolar epithelial cells (33, 34); consistent with crosstalk between metabolic and inflammatory pathways. Alternatively, elevated TNF-α and IL-6 levels induced by resistin may activate JNK and NF-κB systems, rather than via a direct effect of resistin. To further examine the significance of NF-κB and JNK signaling on resistin action in human adipocytes, we treated cells with NF-κB or JNK inhibitors. Whilst NF-κB inhibition appeared to reduce resistin secretion, no affect was observed with JNK inhibitor. Although an overlap of JNK and NF-κB systems has been suggested, resistin may have more prominent effects on the NF-

κB pathway; the importance of the NF-κB pathway for resistin-induced inflammation has been highlighted (8).

Hyper-restinemia is known to contribute to an inflammatory response (22). rhResistin was shown to alter the level of cytokine release when compared to control. Insulin was utilized to observe the effects of RSG in this system; as such we demonstrated that the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, RSG, attenuates resistin-induced secretion of TNF-α and IL-6. Although the mechanisms for this are unclear, the resistin gene promoter contains a PPAR-γ binding site (10), through which RSG may coordinate the recruitment of transcriptional co-repressors (35), thereby suppressing resistin expression at the genetic level. However, this does not appear to be the mechanism through which our observations are being mediated, as we used exogenous resistin to stimulate cytokine production. This suggests that RSG may act downstream of the resistin promoter to mitigate resistin-mediated TNF-α and IL-6 stimulation, potentially via NF-κB.

Visceral adiposity, in addition to BMI, confers a high risk of insulin resistance and T2DM. Moreover, levels of resistin, interleukins and TNF-α differ between visceral and subcutaneous AT (36, 37). Whilst rodent studies have highlighted an increase in resistin expression in visceral AT (38), limited analysis has addressed this in humans. We previously reported higher levels of resistin expression in abdominal depots in comparison to thigh (14), consistent with a role for resistin in obesity-related insulin resistance. Further examination of resistin levels in human AT depots, particularly the pro-inflammatory actions of resistin in visceral AT in comparison to subcutaneous AT, may shed further light on the nature of resistin action in humans.

In conclusion, our study suggests that adipocytes may be a contributory source of resistin in human obesity. Furthermore, resistin responds to LPS treatment and can influence the secretion of inflammatory cytokines from human adipocytes. The intracellular mechanism

for such mediation of resistin on cytokine release appears to act primarily via the NF-κB pathway. Elevated levels of cytokines, induced by resistin, may thus contribute to the pro-inflammatory milieu proposed in obesity-related insulin resistance.

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