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Manuela Good, Felicity M Newell, Larisa M Haupt, Jonathan P Whitehead, Louise J Hutley and Johannes B Prins

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# TNF and TNF receptor expression and insulin sensitivity in human omental and subcutaneous adipose tissue – influence of BMI and adipose distribution

MANUELA GOOD, FELICITY M NEWELL, LARISA M HAUPT, JONATHAN P WHITEHEAD, LOUISE J HUTLEY, JOHANNES B PRINS

## Abstract

**T**umour necrosis factor (TNF) $\alpha$  is implicated in the relationship between obesity and insulin resistance/type 2 diabetes. In an effort to understand this association better we (i) profiled gene expression patterns of TNF, TNFR1 and TNFR2 and (ii) investigated the effects of TNF on glucose uptake in isolated adipocytes and adipose tissue explants from omental and subcutaneous depots from lean, overweight and obese individuals.

TNF expression correlated with expression of TNFR2, but not TNFR1, and TNF and TNFR2 expression increased in obesity. TNFR1 expression was higher in omental than in subcutaneous adipocytes. Expression levels of TNF or either receptor did not differ between adipocytes from individuals with central and peripheral obesity. TNF only suppressed glucose uptake in insulin-stimulated subcutaneous tissue and this suppression was only observed in tissue from lean subjects.

These data support a relationship between the TNF system and body mass index (BMI), but not fat distribution, and suggest depot specificity of the TNF effect on glucose uptake. Furthermore, adipose tissue from obese subjects already appears insulin 'resistant' and this may be a result of the increased TNF levels.

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**Key words:** TNF $\alpha$ , TNF receptors, adipose tissue, human, insulin resistance, obesity.

Centre for Diabetes & Endocrine Research, University of Queensland, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Queensland, Australia 4102.

Manuela Good, PhD Student

Felicity M Newell, PhD Student

Larisa M Haupt, Research Fellow

Jonathan P Whitehead, Lions Senior Medical Research Fellow

Louise J Hutley, Queensland Government Smart State Fellow

Johannes B Prins, Professor of Endocrinology

**Correspondence to:** Dr Johannes Prins

Centre for Diabetes & Endocrine Research, University of Queensland, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Queensland, Australia 4102.

Tel: +61 7 3240 2690; Fax: +61 7 3240 2973

E-mail: jprins@cder.soms.uq.edu.au

## Introduction

The role of tumour necrosis factor (TNF) in the relationship between obesity and insulin resistance in humans is yet to be fully established.<sup>1</sup> In murine models of obesity, adipose tissue and circulating TNF levels are increased, and strategies that reduce circulating TNF levels rectify insulin resistance.<sup>2</sup> Substantial *in vitro* evidence indicates that TNF can influence insulin signal transduction and glucose uptake via a number of biochemical pathways.<sup>1,3-5</sup>

In humans, obesity is associated with increased amounts of adipose tissue and circulating TNF concentrations, and there is a fall in circulating TNF concentrations with weight loss.<sup>6</sup> Circulating TNF levels correlate with body mass index (BMI), visceral obesity and various markers of insulin resistance.<sup>7</sup> TNF has effects that impair preadipocyte differentiation through a number of mechanisms.<sup>8</sup> TNF stimulates leptin production,<sup>9</sup> which could regulate appetite and other metabolic parameters. TNF can regulate fat mass through its actions to impair lipogenesis,<sup>10</sup> induce lipolysis<sup>3</sup> and induce adipose cell apoptosis.<sup>11</sup> All these actions support the hypothesis that activation of the TNF system in obesity is a mechanism to limit ongoing weight gain, at the expense of inducing insulin resistance.<sup>12</sup> TNF also has direct effects that induce insulin resistance, including impairment of insulin receptor and insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation and activation of inhibitory serine/threonine IRS-1 phosphorylation.<sup>13</sup> TNF also inhibits AKT activity and impairs glucose transporter-4 (GLUT4) expression.<sup>14</sup>

These findings have led researchers to postulate that TNF is the molecular link between obesity and insulin resistance.<sup>15</sup> However, not all data are consistent with this postulation, especially in man. In contrast to the murine studies, neutralisation of circulating TNF has no effect on insulin sensitivity in obese, insulin-resistant humans.<sup>16</sup> Further, differing effects of TNF on glucose uptake and insulin action *in vivo* and *in vitro* have been reported. In most *in vitro* studies in human muscle and adipose tissue TNF enhances basal glucose uptake but impairs insulin-stimulated glucose uptake.

TNF is expressed as a 26-kDa cell surface transmembrane protein, which is cleaved to yield a 17-kDa soluble form of TNF.<sup>17</sup> It is likely that transmembrane and soluble TNF have differing actions. Expression of adipocyte transmembrane TNF is higher in obesity.<sup>18</sup> Both forms of TNF are

active and mediate their effects by activating two distinct membrane receptors, TNFR1 and TNFR2.<sup>17</sup> Both receptors are expressed in human adipose tissue but the relative roles of the TNF receptors in adipose tissue remain unclear. TNFR2 expression is greater in subcutaneous adipose tissue from obese females compared to that from lean controls.<sup>19</sup> Using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR), Hube *et al.* showed higher TNF, TNFR1 and TNFR2 expression in subcutaneous, compared with omental, adipose tissue.<sup>20</sup> TNF exerts its inhibitory effects on insulin signalling predominantly through stimulation of TNFR1.<sup>21</sup>

Within adipose tissue TNF is produced by adipocytes and stromal cells, particularly interstitial macrophages.<sup>6,22</sup> Recent data demonstrate that, on a per cell basis, adipose tissue-derived macrophages show greater expression of TNF than adipocytes.<sup>22</sup> However, we and others have demonstrated that in normal adipose tissue, the majority of TNF expression is due to adipocytes.<sup>6</sup> The relative proportion of adipocytes and macrophages in adipose tissue is likely to vary significantly and may have an influence on adipose TNF production.

TNFR1 and TNFR2 are both cleaved by matrix metalloproteases to produce soluble receptors (sTNFR1 and 2). Circulating levels of these receptors are a recognised marker of 'activity' of the TNF system<sup>23</sup> and correlate with features of the metabolic syndrome.<sup>24</sup> TNF also has indirect effects that regulate whole body insulin sensitivity. TNF influences adipokine production, causing a decrease in adiponectin production and action, an increase in adipocyte interleukin (IL)-6 production and regulation of leptin production.<sup>25,26</sup> Peroxisome proliferator-activated receptor (PPAR) $\gamma$  activation by thiazolidinediones blocks many TNF actions and this may be an important component of their insulin-sensitising actions.<sup>27</sup>

In this study, we aimed to investigate expression of TNF and TNFR in human adipocytes relative to BMI, fat distribution and anatomical depot. Furthermore, we examined the effect of TNF on insulin-stimulated 2-deoxyglucose uptake in omental and subcutaneous adipose tissue explants to determine any depot- or BMI-specific effects of TNF on insulin action.

## Methods

### Subjects

The study protocol was reviewed and approved by the institutional ethical committees of the Princess Alexandra Hospital (PAH) and Wesley Hospital (Brisbane, Australia). Informed consent was received from all subjects before participation. Individuals with systemic illness or malignancy or those on medication known to influence glucose metabolism were excluded. To exclude diabetes mellitus, all subjects were interviewed at the time of gaining informed consent and a history of diabetes or uses of antidiabetic medications was sought. Hospital charts were reviewed and preoperative routine blood samples were assessed for presence of fasting hyperglycaemia.

Subjects fasted overnight before adipose tissue removal. Biopsies of adipose tissue were obtained from omental and

abdominal subcutaneous sites of patients undergoing elective abdominal surgery at the Wesley Hospital or PAH. Biopsies were obtained at the time of surgery and immediately transported to the laboratory in Dulbecco's Modification of Eagle's Medium (DMEM) (1,000 mg/L glucose) containing 2% Bovine Serum Albumin (BSA). Transport time was 30–40 minutes.

Two separate groups were used for expression studies and for 2-deoxyglucose uptake studies. Group 1 included six lean and 14 overweight to obese subjects (lean subjects average BMI =  $21.2 \pm 1.2$  kg/m<sup>2</sup> and average age  $67 \pm 3.8$  years; overweight to obese subjects average BMI =  $31.7 \pm 1.2$  kg/m<sup>2</sup> and average age  $57.9 \pm 3.5$  years). The subjects were further subdivided into two groups according to their body fat distribution as determined by their waist-to-hip ratio (WHR).

Twelve subjects with varying BMI were included in group 2. Group 2 included five lean and seven overweight to obese subjects (lean subjects average BMI =  $21.4 \pm 1.6$  kg/m<sup>2</sup> and average age =  $44.0 \pm 2.7$  years; overweight to obese group average BMI =  $32.5 \pm 3.2$  kg/m<sup>2</sup> and average age =  $44.8 \pm 6.2$  years). Lean subjects were defined as those with a BMI less than 25 kg/m<sup>2</sup>, overweight subjects were defined as those with a BMI between 25–30 kg/m<sup>2</sup> and obese subjects were defined as those with a BMI > 30 kg/m<sup>2</sup>. These definitions are in accordance with the current World Health Organization's classification of obesity. Central obesity was defined as a WHR above 0.8 in females and above 0.95 in males.

### Isolation of adipocytes and RNA

Adipocytes were isolated using collagenase digestion and centrifugation techniques, as previously reported.<sup>28,29</sup> Briefly, after removal of visible nerves, blood vessels and fibrous tissue, the adipose tissue biopsy was finely minced and incubated for one hour at 37°C in digest solution (25 mM HEPES, 5 mM glucose, 120 mM sodium chloride, 50 mM potassium chloride and 1 mM calcium chloride) containing 3 mg/mL type II collagenase and 1.5% bovine serum albumin. The ratio of digest solution to adipose tissue was 4:1. The resultant digest material was filtered through a 250  $\mu$ m mesh (Sigma) and adipocytes were separated from the stromovascular components by centrifugation at 250 g for five minutes at 4°C.

We have previously analysed the purity of our adipocyte population using biochemical and microscopic techniques and found that purity is consistently greater than 95% for adipocytes (data not shown). Contribution of stromal cells to the results is likely to be very low, therefore. Total ribonucleic acid (RNA) was prepared from isolated adipocytes using the method of Chomczynski and Sacchi.<sup>30</sup>

### Reverse transcriptase PCR

After RNA isolation, contaminating deoxyribonucleic acid (DNA) was removed by DNase digestion (Promega), the RNA was quantitated spectrophotometrically by absorbance at 260 nm on a UV-visible spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD, US), and RNA was stored at -70°C under ethanol until use. For RT-PCR,

50–100  $\mu\text{g}$  of RNA was transcribed to cDNA using random hexamers and the First Strand synthesis kit (Invitrogen Technologies, Invitrogen Australia Pty Limited, Mount Waverley, VIC, Australia) was used.

#### Real time PCR

Primer and labelled probe sets encoding human  $\beta$ -actin (VIC label; accession X01394) and TNF- $\alpha$  (FAM label; accession D12614) were Pre-developed Taqman Assay Reagents (PDAR; ABI Australia). Primers and probes encoding the human TNF-R1 (TNF receptor-1; FAM label; accession M58286) and TNF-R2 (TNF receptor-2; FAM label; accession M55994) were designed using the Primer Express program (ABI Australia) and guaranteed by the manufacturer.

TNF-R1 sequences were: forward primer 5'-CGC TAC CAA CGG TGG AAG TC-3'; reverse primer 5'-CAA GCT CCC CCT CTT TTT CA-3'; and probe 5'-6FAM-ACT CCA TTG TTT GTG GGA AAT CGA CAC C-TAMRA. TNF-R2 sequences were: forward primer 5'-GAG TGG TGA ACT GTG TCA TCA TGA-3', reverse primer 5'-GAG GCA CCT TGG CTT CTC TCT-3'; and probe 5'-6FAM-CAG GCA CAA GGG CTT CTT TTT CAC CTG-TAMRA-3'.

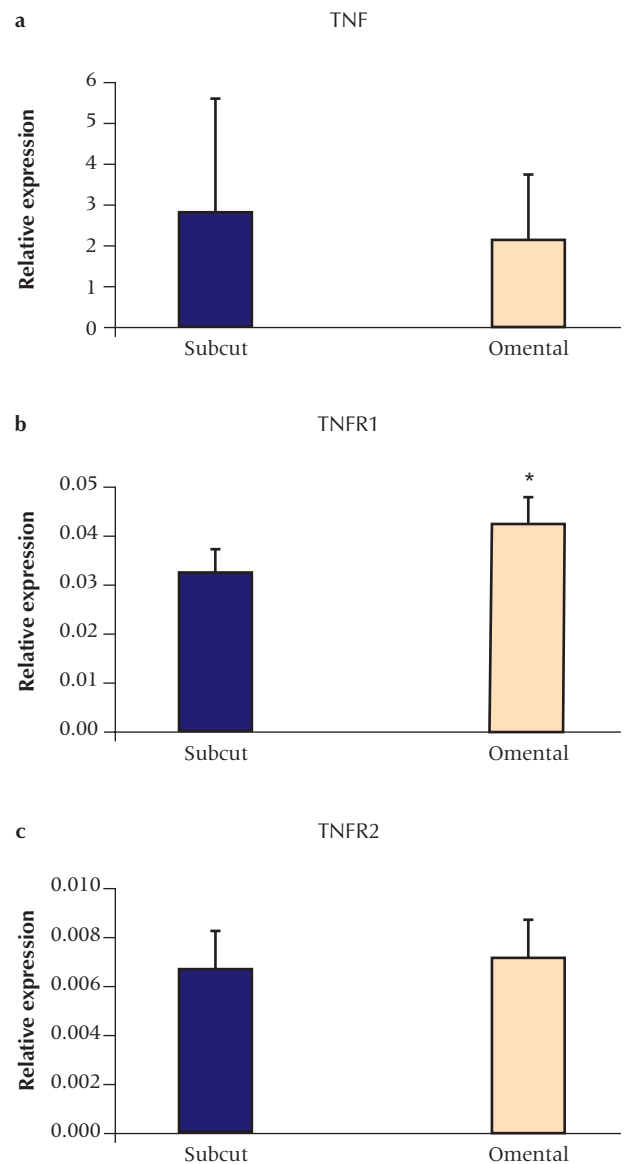
Real-time fluorescence was detected and analysed using the Applied Biosystems PRISM 7700 Sequence Detection System (Perkin-Elmer, US). PCR amplification was performed using a 96-well optical tray and caps with a 20  $\mu\text{l}$  final reaction volume. For each of the genes of interest, 50 ng of cDNA was added to a master mix containing the following: 1X Taqman universal PCR master mix, 300  $\mu\text{M}$  forward primer, 300  $\mu\text{M}$  reverse primer, 200  $\mu\text{M}$  probe and 1X  $\beta$ -actin PDAR. All reactions were performed in triplicate and repeated to ensure reproducibility. Following Taqman amplification and analysis, dCt values were calculated and were used for all future analyses.

#### Glucose uptake

Actrapid, recombinant human insulin, was purchased from Novo Nordisk Pharmaceutical (North Rocks, NSW, Australia), and [ $^3\text{H}$ ]-2-deoxyglucose was obtained from Amersham Pharmacia Biotech (Baulkham Hills, NSW, Australia). [ $^{14}\text{C}$ ]-Inulin was purchased from Dupont NEN (North Sydney, NSW, Australia). Dulbecco's Modification of Eagles Medium (DMEM) (1,000 mg/L glucose), penicillin, streptomycin, glutamine and unlabelled 2-deoxy-D-glucose were supplied by ICN Biomedicals (Seven Hills, NSW, Australia). The scintillation fluid used was Insta-gel Plus and was purchased from Packard Services (Mt Waverley, Victoria, Australia). All other chemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

As previously described,<sup>31</sup> whole tissue adipose explants (approximately 10 mg), excluding visible connective tissue and blood vessels, were removed from the biopsy material and pre-incubated for 24 hours at 37°C with 5 nM TNF placed in DMEM (1,000 mg/L glucose) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100  $\mu\text{g}/\text{ml}$  streptomycin, 10% fetal calf serum and 2% BSA and incubated for 30 minutes at 37°C under 5%  $\text{CO}_2$ . Explants were serum starved for one hour then incubated in 0.5 ml KRH buffer

**Figure 1. Comparison of TNF, TNFR1 and TNFR2 expression in subcutaneous (subcut) and omental adipocytes**



Columns represent mean + SEM of all subjects (n=20).

\*p=0.007 vs. subcutaneous adipocytes using one-way analysis of variance (ANOVA)

with either 0 or 100 nM insulin for 15 minutes at 37°C under 5%  $\text{CO}_2$ . 50  $\mu\text{M}$  2-deoxyglucose, 0.04  $\mu\text{M}$  [ $^3\text{H}$ ]-2-deoxyglucose (0.66  $\mu\text{Ci}/\text{ml}$ ), and 42  $\mu\text{M}$  [ $^{14}\text{C}$ ]-Inulin, (0.22  $\mu\text{Ci}/\text{ml}$ ) were then added to the explants and they were incubated for a further 20 minutes at 37°C.

Following incubation, explants were washed in ice-cold KRH buffer to stop vesicular transport and washed a further four times in the same buffer in order to remove unbound label. Explants were blotted and then weighed using Mettler scales.  $^3\text{H}$  and  $^{14}\text{C}$  radioactivity were determined by scintillation counting using a Minaxa Scintillation counter (Packard). Basal glucose uptake was taken as 2-deoxyglucose uptake in

**Table 1. Physical characteristics of lean, overweight and obese subjects**

Group 1	N (m/f)	Age (years)	BMI (kg/m <sup>2</sup> )	WHR
Lean	6(4/2)	67.0±3.8	21.2±1.2	0.91±0.04
Overweight/ obese	14(4/10)	57.9±3.5	31.7±1.2*	0.88±0.02

Values are expressed as mean ± SEM. Student t-test: \*p<0.0001 vs. lean.

**Key:** BMI = body mass index; WHR = waist-to-hip ratio

the absence of insulin. Experiments were carried out in triplicate. Data were expressed as DPM/mg wet weight. The small amount of extracellular 2-deoxyglucose remaining was corrected from the [<sup>14</sup>C]-Inulin remaining in the sample. The reliability coefficient of the assay was 0.96 (n=8).

#### Presentation of results and statistical analyses

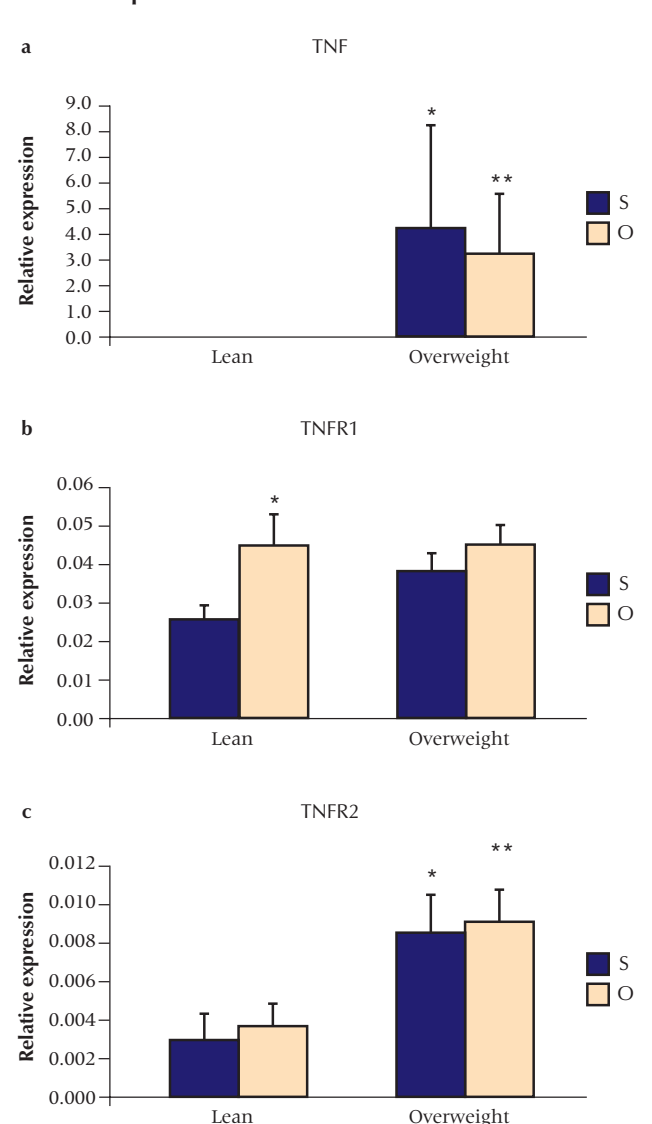
Results are expressed as means +/- standard error of the mean (SEM). As a result of natural inter-individual variations in human studies, the standard errors of the mean are relatively high. Student *t*-tests were used to test whether age, BMI and WHR differed between groups. One-way analysis of variance (ANOVA) followed by *post-hoc* analysis when total ANOVA indicated a significant difference were used in the comparison of TNF, TNFR1 and TNFR2 expression data. Pearson's correlation coefficients were used to quantify the relations between variables. Glucose uptake data were analysed using the Repeat Measures Analysis of Variance. All calculations were made using SPSS version 10.0. A *p* value < 0.05 was considered statistically significant.

Due to a relative lack of knowledge of the degree of variation in the parameters examined in this study, classical power calculations were not feasible. Sample size therefore was determined based on previous adipose and muscle studies undertaken by us and others that consistently yield results that are interpretable, reproducible and have statistical significance using samples of 5–10.<sup>11,28,29,31,32</sup> In our direct experience of adipose tissue study, if a significant observation is not obtained with 5–6 subjects, study of larger numbers of subjects rarely provides any further data. Thus, 20 subjects were employed in this study.

## Results

### Depot-specific expression of TNF, TNFR1 and TNFR2 in adipose tissue

Expression of TNF, TNFR1 and TNFR2 was compared in paired samples of omental and subcutaneous adipose tissue. TNFR1 was expressed at a significantly greater level in omental adipocytes compared to subcutaneous adipocytes (figure 1, *p*=0.007). Expression of TNF and TNFR2 did not differ from one depot to the other (figure 1). There was a strong correlation between TNF and TNFR2 expression in both omental and subcutaneous adipose tissue (*r*=0.638, *p*=0.002 omental; *r*=0.701, *p*=0.001 subcutaneous). However, there was

**Figure 2. Comparison of TNF, TNFR1 and TNFR2 expression in lean vs. overweight/obese subjects in subcutaneous and omental adipose tissue**

Columns represent mean + SEM of six lean and 14 overweight/obese subjects. (a) \**p*=0.014, \*\**p*=0.049 vs. lean. (b) †*p*=0.012 omental vs. subcutaneous adipose tissue. (c) \**p*=0.022; \*\**p*=0.046 vs. lean. Data were analysed using one-way ANOVA

**Key:** s = subcutaneous; o = omental

no significant correlation between TNF and TNFR1 expression in either depot (data not shown).

### Influence of BMI and WHR on adipose tissue expression of TNF, TNFR1 and TNFR2

The lean and overweight/obese subjects in group 1 were matched for age and WHR (table 1). Expression of TNF and TNFR2 was significantly higher in overweight/obese subjects than in lean subjects (figure 2): TNF *p*=0.014 subcutaneous and *p*=0.049 omental; TNFR2 *p*=0.022 subcutaneous and *p*=0.046 omental. No depot-specific expression of TNF or

**Table 2. Physical characteristics of subjects with central and peripheral obesity**

Group 1	N (m/f)	Age (years)	BMI (kg/m <sup>2</sup> )	WHR
Central obesity	8(3/5)	58.6±3.8	31.3±1.4	0.94±0.02
Peripheral obesity	6(2/4)	57.3±5.2	31.2±2.1	0.81±0.03*

Values are expressed as mean ± SEM. Student t-test: \*p<0.0001 vs. central obesity.

**Key:** BMI = body mass index; WHR = waist-to-hip ratio

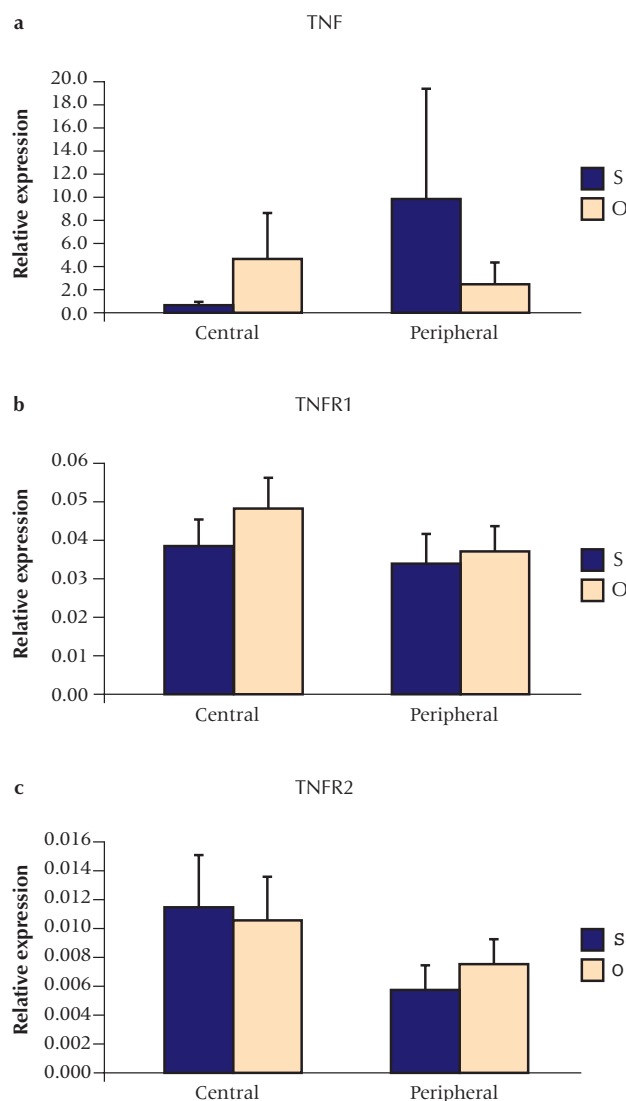
**Table 3. Physical characteristics of lean and overweight/obese subjects**

Group 2	N (m/f)	Age (years)	BMI (kg/m <sup>2</sup> )	WHR
Total	12(2/10)	43.3±3.1	27.8±2.4	0.84±0.02
Lean	5(1/4)	44.0±2.7	21.4±1.6	0.80±0.02
Overweight/obese	7(1/6)	44.8±6.2	32.5±3.2*	0.90±0.04

Values are expressed as mean ± SEM. Student t-test: \*p<0.05 vs. lean.

**Key:** BMI = body mass index; WHR = waist-to-hip ratio

**Figure 3. Comparison of TNF, TNFR1 and TNFR2 expression in subjects with central (n=8) vs. peripheral adiposity (n=6)**



Columns represent mean + SEM. Data were analysed using one-way ANOVA

**Key:** s = subcutaneous; o = omental

TNFR1 was observed in either group. In contrast, TNFR1 expression did not differ between lean and overweight/obese subjects (figure 2). TNFR1 showed significant depot-specific expression (omental greater than subcutaneous) in lean subjects (figure 2, p=0.012).

The overweight and obese subjects were divided into those with central versus peripheral body fat distribution based on their WHR and were matched for age and BMI (table 2). There were no significant differences in expression levels of TNF, TNFR1 or TNFR2 between the two groups in either omental or subcutaneous adipose tissue (figure 3).

*Effect of TNF on basal and insulin-stimulated glucose uptake*

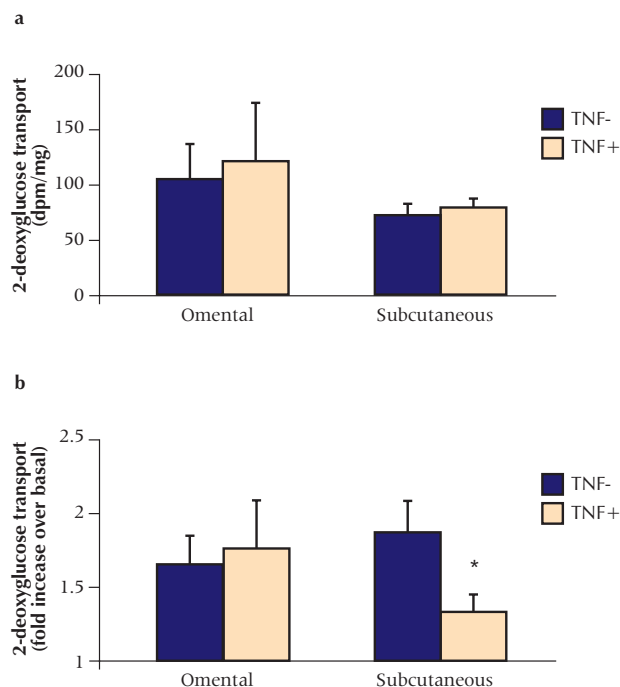
A second group of subjects were used in this part of the study, as shown in table 3. The subject groups were matched for age and WHR. TNF had no effect on basal glucose uptake in either depot (figure 4). TNF exposure decreased insulin-stimulated glucose uptake in subcutaneous, but not omental, adipose tissue, thereby reducing insulin's effects by 67% (figure 4, p=0.019). When lean and overweight/obese subjects were analysed separately, insulin significantly increased 2-deoxyglucose uptake in both depots in lean subjects (figure 5, p=0.005 omental and p=0.003 subcutaneous). In contrast, insulin had no significant effect on glucose uptake in the overweight/obese (figure 5).

The inhibitory effect of TNF on insulin-stimulated glucose uptake in subcutaneous adipose tissue appeared to be related to the BMI of the subjects, with greater inhibition of insulin-stimulated glucose uptake in lean subjects compared to overweight/obese subjects (r=0.570, p=0.067). However, this did not reach statistical significance in our study group.

**Discussion**

In our study TNFR1, but not TNF or TNFR2, was found to have site-specific differences in adipocyte gene expression, with significantly higher expression in omental adipocytes. This depot-specific expression was more marked in lean subjects. The lack of depot-specific expression of TNF that

**Figure 4.** (a) Comparison of basal 2-deoxyglucose uptake (dpm/mg wet weight adipose tissue) in subcutaneous and omental adipose tissue incubated with or without TNF for 24 hours. (b) Comparison of insulin-stimulated 2-deoxyglucose uptake (shown as fold increase over basal) in subcutaneous and omental adipose tissue incubated with or without TNF for 24 hours

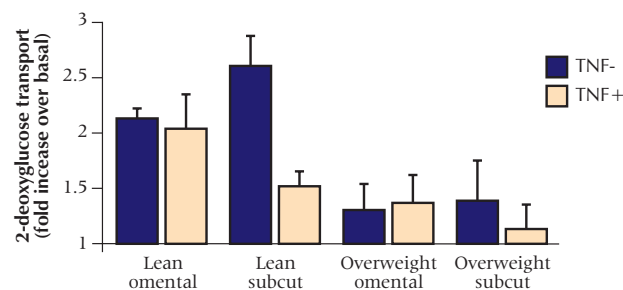


\* $p = 0.019$  vs. control without TNF. Columns represent mean values and SEM for all subjects ( $n=12$ ). Data were analysed using the repeat measures analysis of variance

we observed is consistent with previous reports.<sup>33-35</sup> In contrast, Hube *et al.*<sup>20</sup> found higher expression of TNF, TNFR1 and TNFR2 in the subcutaneous depot. Possible reasons for the conflicting results between the Hube study and ours include differences in BMI (the obese group in the Hube study had a much higher average BMI [ $48.2 \pm 8.4 \text{ kg/m}^2$ ] than the obese group in our study [ $31.7 \pm 1.2 \text{ kg/m}^2$ ]); the use of semi-quantitative mRNA analysis in the Hube study; and the fact that Hube *et al.* also used whole adipose tissue, rather than isolated adipocytes, in their analysis. Hube *et al.* may therefore have measured a greater contribution of production of TNF and its receptors by stromal cells in addition to adipocytes. Indeed, recent studies have indicated a greater proportion of interstitial macrophages in the adipose tissue of obese subjects.<sup>36</sup> Additionally, the proportion of stromal cells may differ between the depots, leading to the observed site-specific differences in TNF receptor expression.

We used overweight and obese subjects as a combined group in our study, as previously we have found that insulin resistance in adipose tissue occurs at a lower BMI than the value previously expected.<sup>31</sup> In our previous study, subjects with a BMI between 25 to 30  $\text{kg/m}^2$  were as insulin-resistant

**Figure 5.** Comparison of insulin-stimulated 2-deoxyglucose uptake (shown as fold increase over basal) in subcutaneous and omental adipose tissue in lean ( $n=5$ ) and overweight/obese ( $n=7$ ) subjects



Columns represent mean values and SEM. Data were analysed using the repeat measures analysis of variance  
Key: subcut= subcutaneous

in their adipose tissue as subjects with a BMI greater than 30. Our results show an up-regulation of TNF and TNFR2 with overweight and obesity. This is in agreement with a study performed by Hotamisligil *et al.*, which showed that TNFR2, but not TNFR1, was increased in the adipose tissue in obese subjects.<sup>19</sup>

To the best of our knowledge, no previous study has examined the effect of body fat distribution on expression of TNF and its receptors in adipose tissue. This is the case despite the observations that circulating TNF concentrations show a strong correlation with visceral adiposity, and that TNF has been demonstrated to have depot-specific effects on adipose cells.<sup>32,37</sup> Our study showed no significant association between WHR and TNF, TNFR1 or TNFR2 expression in adipose tissue.

In addition to studying gene expression of TNF and its receptors, we also analysed the effect of TNF on glucose uptake in adipose tissue, relative to BMI and anatomical depot. After 24 hours' incubation, TNF did not significantly affect basal glucose uptake in either omental or subcutaneous adipose tissue in our study. This is consistent with the study of Hauner *et al.*, who examined glucose uptake in newly differentiated human preadipocytes isolated from mammary adipose tissue of normal weight subjects.<sup>3</sup> Our results showed no effect of TNF on insulin-stimulated glucose uptake in the omental tissue; however, in the subcutaneous tissue TNF inhibited the insulin-stimulated component of glucose uptake by almost 70%. This represents the first report of depot-specific effects of TNF on glucose uptake and insulin action.

In addition to the depot-specific effect of TNF on insulin-stimulated glucose uptake in adipose tissue, the effect also appears to be inversely related to BMI. The data in figure 5 confirm those of our previous report,<sup>31</sup> demonstrating *in vitro* insulin-stimulated glucose uptake in adipose tissue from lean, but not overweight/obese, subjects. The insulin resistance that already exists in the adipose tissue of the obese group may explain the lack of TNF effect on insulin-stimulated glucose uptake in these subjects. It is possible that this

insulin resistance may reflect the high TNF levels found *in vivo* in obese subjects.<sup>38,39</sup> In combination with our findings of increased adipocyte TNF expression in obese subjects (figure 2), it may also suggest that TNF may be exerting an autocrine or paracrine effect to reduce insulin action in adipose tissue of obese subjects.<sup>40</sup>

The lack of TNF suppression of insulin-stimulated glucose uptake in omental tissue, even from lean individuals, is of interest. Available evidence from murine and human studies suggests that TNF effects that inhibit insulin action are mediated by TNFR1.<sup>14</sup> Thus, on the basis of our demonstration that TNFR1 expression is significantly greater in the omental depot, it might have been expected that TNF inhibition of insulin action would be greater in that depot. Our demonstration of TNF inhibition of insulin action in subcutaneous tissue is, therefore, unexpected. One possible explanation may be that depot-specific expression of sTNFR acts locally to modulate TNF action.

In conclusion, our results indicate that the expression of TNF and TNFR2 is associated with BMI, but not WHR. Therefore, increased expression of TNF and its receptors in adipose tissue does not appear to be associated with central adiposity, in which the risk of developing type 2 diabetes and insulin resistance is increased. TNFR1 expression is independent of both parameters, but shows depot-related differences in lean subjects. There are no differences between the depots in expression of TNF, TNFR1 and TNFR2 in those with central or peripheral obesity, that might explain the observed reduction in sensitivity to insulin in omental adipose tissue.

Furthermore, there were site-specific differences in the action of TNF in inducing insulin resistance. Insulin-stimulated 2-deoxyglucose uptake in omental tissue was not affected by TNF, whereas subcutaneous adipose tissue displayed inhibition of insulin-stimulated 2-deoxyglucose uptake after exposure to TNF. This occurred predominantly in lean subjects. The adipose tissue of obese and overweight subjects is already insulin-resistant, and this may explain the lack of effect of TNF in the adipose tissue of these individuals.

### Acknowledgements

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### Conflict of interest

None declared.

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## Clinical guide for the healthcare professional

### High Density Cholesterol: The New Target

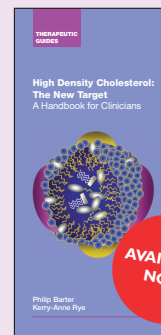
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