Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance

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Kern, Philip A., Subramanian Ranganathan, Chunling Li, Linda Wood, and Gouri Ranganathan. Adipose tissue tumor necrosis factor (TNF) and interleukin (IL)-6, which may cause obesity-related insulin resistance. We measured TNF and IL-6 expression in the adipose tissue of 50 lean and obese subjects without diabetes. Insulin sensitivity (SI) was determined by an intravenous glucose tolerance test with minimal-model analysis. When lean [body mass index (BMI) 25 kg/m²] and obese (BMI 30–40 kg/m²) subjects were compared, there was a 7.5-fold increase in TNF secretion (P < 0.05) from adipose tissue, and the TNF secretion was inversely related to SI (r = –0.42, P < 0.02). IL-6 was abundantly expressed by adipose tissue. In contrast to TNF, plasma (rather than adipose) IL-6 demonstrated the strongest relationship with obesity and insulin resistance. Plasma IL-6 was significantly higher in obese subjects and demonstrated a highly significant inverse relationship with SI (r = –0.71, P < 0.001). To separate the effects of BMI from SI, subjects who were discordant for SI were matched for BMI, age, and gender. By use of this approach, subjects with low SI demonstrated a 3.0-fold increased level of TNF secretion from adipose tissue and a 2.3-fold higher plasma IL-6 level (P < 0.05) compared with matched subjects with a high SI. Plasma IL-6 was significantly associated with plasma nonesterified fatty acid levels (r = 0.49, P < 0.002). Thus the local expression of TNF and plasma IL-6 are higher in subjects with obesity-related insulin resistance.

Type 2 diabetes

Obesity has become a national epidemic with enormous public health implications (25), and recent studies have demonstrated a further 6% increase in the incidence of obesity [body mass index (BMI) >30 kg/m²] over a 7-yr period (30). There is a strong correlation between obesity and insulin resistance in both diabetic and nondiabetic subjects (27), and the risk of diabetes increases 11-fold as the BMI increases from 20 to 30 (8). Although insulin resistance accompanies all patients who become obese, the degree of insulin resistance varies considerably, and the relationships between obesity, insulin resistance, and type 2 diabetes are not well understood.

Obesity represents an expansion of adipose tissue mass, and one explanation for obesity-related insulin resistance is the production of factors by adipose tissue that render some subjects more insulin resistant than others. Numerous adipocyte secretory products have recently been described that play a role in carbohydrate and lipid metabolism (14, 21, 23). One such adipocyte secretory product is tumor necrosis factor (TNF)-α. A new role for TNF was proposed in 1993 with the description of TNF expression by adipose tissue and the elevated expression of TNF in obese, insulin-resistant rodents and humans (17, 20, 24). Although it is unclear how adipose TNF expression may cause insulin resistance (36), TNF is known to impair insulin receptor signaling (18). TNF also inhibits lipoprotein lipase (LPL) and stimulates lipolysis in adipocytes (34), and the resulting increase in circulating nonesterified fatty acids (NEFA) would be expected to contribute to insulin resistance (7).

Another adipocyte secretory product that may be involved in insulin resistance is interleukin (IL)-6, which is a cytokine secreted by many cells, including adipocytes and adipocyte stromal cells (11, 15). Like TNF, IL-6 inhibits the expression of LPL, but, unlike TNF, IL-6 does not stimulate lipolysis (13, 16). IL-6 secretion is increased in the adipocytes of obese subjects (29) and may be important either as a circulating hormone or as a local regulator of insulin action.

Although many studies have examined the role of TNF in insulin resistance, relatively few of these have been in humans, and none has examined cytokine expression in detail along with the measurement of insulin resistance. In this study, we examined the expression of TNF and IL-6 in human adipose tissue from nondiabetic subjects with varying degrees of obesity and insulin resistance. We found that TNF secretion from human adipose tissue and circulating plasma IL-6 were both highly associated with obesity-associated insulin resistance.

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adipose tissue. This assay demonstrates intra-assay and interassay variation. This ELISA method was used to measure TNF in fasting plasma as well as TNF secretion by adipose tissue (see Relationship between TNF and obesity). TNF mRNA levels were measured by competitive RT-PCR, as described by us previously (24). IL-6 was measured in fasting plasma and secreted from adipose tissue using an ELISA assay (R&D Systems). This assay demonstrates intraand interassay variations of <5%.

**Statistics.** All data are expressed as means ± SE. To analyze data between groups, a one-way ANOVA was performed, and secondary analysis was performed with the Student’s t-test with Bonferroni correction. Analysis of trends was performed using linear regression after log transformation. The Wilcoxon matched-pair sign-rank test was used for the paired data in Table 2.
RESULTS

Relationship between TNF and obesity. To better define the effects of obesity on TNF expression, we measured TNF mRNA in the adipose tissue from each subject, along with plasma TNF and TNF secretion from the adipose tissue. Subjects were divided into four BMI groups representing lean (BMI <25), overweight (BMI 25–30), moderately obese (BMI 30–40), and obese subjects (BMI >40). Figure 2A shows TNF mRNA levels from subjects with increasing BMI. There was considerable variability among the obese subject groups with respect to TNF mRNA levels, such that the differences between normal lean subjects (BMI <25 kg/m²) and obese subjects were not statistically significant (NS; Fig. 2A). TNF protein was also measured in these subjects; however, as shown in Fig. 2B, there was no relationship between plasma TNF and BMI. However, TNF secretion from the adipose tissue was higher in obese subjects. Mean TNF secretion was 0.16 ± 0.06 pg/μg DNA in lean subjects (BMI <25 kg/m²), and 1.21 ± 0.36 pg/μg DNA in subjects with a BMI between 30 and 40 kg/m² (P < 0.05). Subjects with a BMI >45 kg/m² demonstrated slightly lower TNF secretion (0.90 ± 0.21 pg/μg DNA), but this was not significantly decreased compared with subjects with a BMI of 30–40 kg/m². This effect of BMI on TNF secretion was still present when women and Caucasians were each considered separately and when subjects with impaired glucose tolerance were eliminated. TNF secretion from adipose tissue was also low in subjects with low body fat. TNF secretion in subjects with <30%, 30–45%, and >45% body fat was 0.16 ± 0.07 (n = 10), 0.76 ± 0.16 (n = 14), and 1.1 ± 0.28 pg/μg DNA (n = 18, P < 0.05 vs. <30% group).

TNF expression and insulin sensitivity. As expected, there was a significant relationship between obesity and insulin sensitivity. As described previously by others (22), the relationship between BMI and SI is curvilinear and best represented by a log/log transformation, and in our subjects, BMI and SI were significantly related (r = −0.65, P < 0.001). Because SI varies considerably among nonobese subjects with normal glucose tolerance, we did not divide SI into subgroups but instead examined TNF expression over the spectrum of SI. There was no significant relationship between either plasma TNF or TNF mRNA levels and SI (data not shown). However, there was a significant decrease in TNF secretion with increasing SI (Fig. 3), such that most of the insulin-sensitive subjects (SI >5) had lower levels of TNF secretion, and most of the insulin-resistant subjects (SI <2) had the highest levels of TNF secretion.

IL-6 expression with obesity and insulin resistance. The adipose tissue fragments secreted relatively high levels of IL-6. When IL-6 expression was examined in the same BMI groups, as described in the preceding section for TNF, there was a tendency for an increase in IL-6 secretion from adipose tissue with increasing BMI and increasing body fat (Fig. 4B); however, these changes were not statistically significant. Plasma IL-6, however, was strongly associated with increasing obesity (Fig. 4A). In lean subjects (BMI <25), plasma IL-6 was 0.73 ± 0.23 pg/ml and increased about fourfold to 2.86 ± 0.61 pg/ml in the most obese subjects (BMI >40, as shown in Table 1).
In a similar manner, plasma IL-6 was lower in subjects with low percent body fat. Plasma IL-6 was 0.84 ± 0.19 pg/ml (n = 10) in subjects with <30% body fat and was 2.05 ± 0.38 (n = 14) and 2.58 ± 0.44 (n = 18) pg/ml in subjects with 30–45 and >45% fat, respectively (P < 0.05). The relationship between SI and plasma IL-6 was examined in the same manner as described for TNF. In contrast to TNF, adipose-secreted IL-6 demonstrated no significant relationship with SI (r = 0.04, P = NS). However, there was a highly significant relationship (r = −0.71, n = 38, P < 0.001) between plasma IL-6 and SI, as shown in Fig. 5. Plasma IL-6 was 3.0 ± 0.53 pg/ml in the most insulin-resistant subjects (SI <2) and was 0.82 ± 0.19 pg/ml in the most insulin-sensitive subjects (SI >5, P < 0.05).

One mechanism by which TNF may cause insulin resistance is through an increase in adipocyte lipolysis, leading to a rise in plasma NEFA. Hence, the relationship between cytokine expression and plasma NEFA was examined. The only significant relationship with plasma NEFA levels was with plasma IL-6 and adipose TNF secretion. As shown in Fig. 6, there were significant increases in plasma NEFA levels in subjects with higher levels of plasma IL-6 (r = 0.54, P < 0.001). There was also a significant association between plasma NEFA and TNF secretion (r = 0.35, n = 37, P < 0.05), although this association was less robust than the association with IL-6.

Cytokines and insulin resistance independent of obesity. Insulin resistance is exacerbated by obesity, leading to a significant relationship between SI and BMI. Therefore, we examined the relationship between adipose cytokine expression and SI without the confounding effects of BMI. To factor out obesity, we identified subjects who were of the same BMI but who were discordant for SI. We compared the cytokine expression of subjects with insulin resistance (SI <2.0) with that of subjects with less insulin resistance (SI >3.0) who were matched for BMI (±5 kg/m²), age (±10 yr), and gender. Using these criteria, we were able to match nine subjects with SI <2.0 with nine subjects with SI >3.0. As shown in Table 2, these subjects were well matched for age and BMI, and there were significant differences in SI by virtue of subject selection. No differences were noted between plasma TNF or adipose...
IL-6 expression. However, the insulin-resistant subjects had significantly higher levels of plasma IL-6 as well as significantly higher levels of adipose TNF secretion ($P < 0.05$). In these matched subjects, TNF secretion and plasma IL-6 were two- to threefold higher in the insulin-resistant subjects.

Previous studies have demonstrated that IL-6 and TNF interact with each other in both 3T3-L1 adipocytes and mice (3, 16). We examined TNF and IL-6 expression from each subject’s adipose tissue to determine whether there was any relationship between IL-6 and TNF expression. As shown in Fig. 7, there was a strong linear relationship between the secretions of IL-6 and TNF from the adipose tissue ($r = 0.81$, $P < 0.0001$). On the other hand, there was no significant relationship between plasma IL-6 and plasma TNF (data not shown).

**DISCUSSION**

Since the initial description of TNF expression by adipose tissue, several lines of evidence have suggested that TNF overproduction by adipose tissue may be involved in the pathogenesis of the insulin resistance of obesity. TNF mRNA levels were high in obese, insulin-resistant rodents, and the infusion of a soluble TNF binding protein into insulin-resistant rats improved insulin sensitivity and improved the defect in insulin receptor and insulin receptor substrate-1 autophosphorylation in fat and muscle (18, 20). Recent studies using genetic manipulations resulting in knockout or depletion of TNF or TNF receptor have confirmed the importance of TNF in rodent insulin resistance (9, 19, 40), although one such study (37) found no role for TNF or the TNF receptor in insulin resistance.

Relatively few studies have examined the relationship between TNF and insulin resistance in humans. Studies by us (24) and others (1, 17) demonstrated elevated levels of adipose TNF mRNA and protein in obese subjects and a decrease in TNF with weight loss. No study has examined the relationship between $S_I$ and TNF, although one study noted a significant correlation between TNF mRNA levels and fasting insulin (17), and several studies observed a decrease in TNF after weight loss (12, 17, 24). High TNF secretion from human adipose tissue was associated with decreased $[^3H]$glucose incorporation into lipids (26).

It is not clear whether TNF functions locally or circulates in a sufficiently high concentration to influence distant targets. Plasma TNF has been measured, and several studies have observed increased plasma TNF levels in obese subjects and in subjects with hyperinsulinemia or insulin resistance (10, 42, 43). Plasma TNF was elevated in male diabetic subjects compared with male controls, but no such relationship was observed in women (35). In an attempt to bind plasma TNF and reverse insulin resistance in humans, diabetic or insulin-resistant subjects have been given an injection of anti-TNF binding protein. In both studies, there was no improvement in insulin resistance (31, 33).

The role of IL-6 in insulin resistance has been much less studied. IL-6 is secreted by many cells, including adipocytes and adipose stromal cells (11, 15) and is increased after a meal (32). Like TNF, IL-6 inhibits the expression of LPL, but unlike TNF, IL-6 does not stimulate lipolysis (13, 16). Linking IL-6 to insulin resistance are studies demonstrating increased IL-6 secretion in the adipocytes of subjects with obesity (29) and diabetes (2).

In the studies described herein, we measured TNF and IL-6 gene expression at several levels from the adipose tissue of lean and obese subjects and related this expression to $S_I$, a reliable measure of insulin sensitivity. Both IL-6 and TNF were expressed and secreted by human adipose tissue, although IL-6 levels were much higher in both adipose tissue and plasma. The most consistent relationship between cytokine expression and obesity-related insulin resistance involved increased TNF secretion from adipose tissue and increased plasma IL-6 levels. Elevated TNF and IL-6 expression was found in subjects who were only moderately obese (BMI >30) and increased progressively with decreasing $S_I$. The relationship between...

<table>
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<th></th>
<th>$S_I$</th>
<th>BMI</th>
<th>Age, yr</th>
<th>Plasma TNF, pg/ml</th>
<th>Plasma IL-6, pg/ml</th>
<th>Adipose TNF Secretion, pg/μg DNA</th>
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<tbody>
<tr>
<td>Insulin sensitive</td>
<td>4.6</td>
<td>34.9</td>
<td>40.3</td>
<td>1.72 ± 0.26</td>
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<tr>
<td>Insulin resistant</td>
<td>1.5</td>
<td>34.8</td>
<td>39.1</td>
<td>1.87 ± 0.31</td>
<td>3.21 ± 0.79*</td>
<td>1.42 ± 0.47*</td>
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Values are means ± SE. $S_I$, insulin sensitivity; TNF, tumor necrosis factor; IL-6, interleukin-6. *$P < 0.05$ vs. insulin sensitive.

![Fig. 7. Relationship between IL-6 and TNF secretion from adipose tissue. IL-6 and TNF (pg/μg DNA) were measured in the medium from adipose tissue pieces cultured for 2 h, and the level of medium IL-6 was plotted against the level of TNF.](https://example.com/fig7.jpg)
plasma IL-6 and $S_1$ was very strong, with a highly significant inverse correlation and a fivefold difference between the most insulin-resistant and most insulin-sensitive subjects. Thus both TNF and IL-6 were associated with both obesity and insulin resistance; however, it was the adipose-secreted form of TNF and the plasma level of IL-6 that displayed the strongest relationships.

The subjects in this study were heterogeneous with regard to degree of obesity, gender, and race, and it is possible that a study using a more focused group of subjects would yield different results. However, we observed no consistent effect of gender or race on cytokine expression in these subjects. This study also relied on plasma cytokine levels and cytokine secretion from adipose tissue, and these measurements may not be reflective of cytokine biological effects at the tissue level.

Because obesity and insulin resistance are related to each other, we wished to determine whether TNF and IL-6 expression were related to insulin resistance independently of obesity. As described in Table 2, we paired insulin-resistant subjects with more-insulin-sensitive subjects and matched them for BMI and age. By use of this analysis, high levels of TNF secretion and plasma IL-6 were both significantly associated with insulin resistance. Thus the expression of these cytokines was associated with insulin resistance independently of obesity.

There are differences in the expression of TNF and IL-6 that may be important in understanding their functions. IL-6 was secreted at high levels from adipose tissue, and there was a significant arteriovenous difference in IL-6 across the adipose tissue bed, whereas there was no arteriovenous difference with TNF (29). We found no relationship between plasma TNF and obesity or insulin resistance, although other studies have noted increased plasma TNF with obesity (2, 42, 43). IL-6 and TNF may interact with each other, as suggested by the strong correlation between TNF secretion and IL-6 secretion in this study and by previous studies that demonstrated increased IL-6 expression in response to TNF (3, 16). Together, these data suggest that TNF functions locally at the level of the adipocyte in a paracrine fashion, perhaps stimulating the secretion of NEFA, IL-6, or other circulating substances. On the other hand, plasma IL-6 circulates at high levels and may be more important systemically and perhaps represents a hormonal factor that induces muscle insulin resistance.

It is noteworthy that two studies have tried, and failed, to reverse insulin resistance with an injection of anti-TNF binding proteins (31, 33). On the basis of the studies described herein, we can speculate on several possible reasons for the failure of anti-TNF therapy in humans. If TNF functions in a paracrine or autocrine fashion in adipose tissue, then the anti-TNF binding proteins may not reach the microcirculation in sufficient concentration to prevent TNF-mediated effects. In addition, our data raise the possibility that IL-6 is the major circulating component of obesity-related insulin resistance.

The development of insulin resistance with increasing adiposity suggests that an adipocyte product may be important in insulin resistance. Both TNF and IL-6 are adipocyte products that are overexpressed in obese insulin-resistant subjects, and we have shown that the secretion of these cytokines is interrelated. Some of these cytokines may function systemically, others may function locally, and still others may function to increase the secretion or synthesis of other adipocytokine factors or to act as an adjuvant to the actions of other insulin resistance factors. One such insulin resistance factor is NEFA, which are closely associated with insulin resistance (28, 39). TNF stimulates lipolysis in adipocytes (34); hence, it is possible that TNF functions at the level of the adipocyte to stimulate lipolysis. Although IL-6 is not known to stimulate lipolysis (13, 16), we found a significant relationship between plasma IL-6 and plasma NEFA levels, whereas the relationship between TNF expression and plasma NEFA was much less robust.

These studies provide the first comprehensive analysis of IL-6 expression in obese, insulin-resistant humans and add to the data on TNF expression. Together, these studies suggest that obesity-related insulin resistance represents a complex syndrome, mediated by a number of adipocyte secretory products, which ultimately lead to defects in insulin action in other target organs.

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