Secretion of Tumor Necrosis Factor-α Shows a Strong Relationship to Insulin-Stimulated Glucose Transport in Human Adipose Tissue

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Obesity is associated with a wide array of complications, such as insulin resistance and type 2 diabetes (1). The link between obesity and peripheral insulin resistance is not fully understood, although recent studies suggest that tumor necrosis factor (TNF) may play an important role (2).

Some animal models suggest that tumor necrosis factor (TNF)-α is a key component in obesity-linked insulin resistance because it inhibits insulin receptor signaling and glucose transport in insulin-sensitive tissues. However, in vivo data in humans have given conflicting results regarding the relationship between circulating TNF-α levels and insulin sensitivity. In the present study, the potential local role of TNF-α on insulin action in human subcutaneous adipose tissue was studied in 42 obese women (BMI 39 ± 10 kg/m²). We found a strong inverse correlation between adipose TNF-α secretion and maximum insulin-stimulated glucose transport in adipocytes that was independent of fat cell volume, age, and BMI (P < 0.001, r = 0.58). As much as one-third of the variation in insulin-stimulated glucose transport could be accounted for by variations in TNF-α secretion. There was no significant correlation (r = 0.11) between secretion of adipose plasminogen activator inhibitor 1 and glucose transport. Furthermore, subcutaneous adipose tissue of 4 obese women (BMI 40 ± 4) incubated with TNF-α for 24 h showed a one-third concentration-dependent inhibition of insulin-stimulated glucose transport (P < 0.01). In conclusion, adipose TNF-α may be an important specific and local factor in adipose tissue that influences the ability of insulin to stimulate glucose transport in human fat cells, at least in obese women. Diabetes 49:688–692, 2000

TNF-α is produced in several nonimmune cells, including adipocytes. In obesity, TNF-α expression in fat cells is increased in both humans (3,4) and experimental animals (5). The addition of neutralizing soluble TNF receptor immunoglobulin (TNFR-IgG) and the use of such proteins via gene transfer have been shown to increase insulin sensitivity in obese rodents (5,6). Obese mice with targeted null mutations in the genes encoding either TNF-α or its two receptors lacked insulin resistance (7). Furthermore, obese mice lacking TNF-α had increased insulin sensitivity and improved lipid metabolism in comparison to control animals (8). In contrast, others have found that p75 TNF receptor-deficient mice showed increased insulin sensitivity, whereas mice with combined p55 and p75 receptor deficiency did not (9).

In obese humans, it is unclear whether TNF-α mediates insulin resistance. TNF-α secretion is markedly increased in vitro from adipose tissue of obese subjects (3,4). However, TNF-α concentrations in the vein draining subcutaneous adipose tissue were shown to be similar to those in a peripheral vein, suggesting no or minimal mobilization of TNF-α from adipose tissue to the bloodstream (10). On the other hand, the secretion of the cytokine might have been prevented or too low for detection by the arteriovenous examination.

The relationship between circulating TNF-α and insulin sensitivity is unclear. Some studies show positive correlations with BMI and insulin sensitivity and show negative correlations with weight loss or other treatments, but the relationships are often weak, and frequently the variations of the TNF-α levels are low (11–18). No significant relationship between circulating TNF-α and insulin sensitivity was found in vivo in one study (19). Treatment of insulin-resistant subjects with TNF-α antibodies failed to improve insulin action in vivo (20). Thus, the role of TNF-α in obesity-mediated insulin resistance remains to be clearly defined in humans. However, the possibility exists that TNF-α may act in a paracrine fashion with respect to its effect on insulin sensitivity. It is interesting to note that TNF-α is also produced in human muscle and that the muscle production is increased in obesity (21).

If TNF-α produced in fat cells has clinical significance for insulin resistance in obesity, then there should be a quantitative relationship between adipose TNF-α production and systemic insulin action in obese subjects. We hypothesized that TNF-α modulates insulin action in a local fashion (autocrine/
paracrine). A local instead of distant effect might explain the failure of previous studies (8,10,19,20) to show a relationship between TNF-α and insulin action in vivo. To examine if adipose TNF-α modulates insulin action in fat cells, we determined the rate of TNF-α secretion from subcutaneous adipose tissue in relation to the effect of insulin on glucose transport in isolated subcutaneous fat cells from obese women. Adipocytes secrete a number of other proteins, such as plasminogen activator inhibitor 1 (PAI-1). The secretion rate of PAI-1 from human adipose tissue is increased in obesity (22). Therefore, we studied the secretion of TNF-α and PAI-1 in parallel to determine the specificity of TNF-α action. Finally, we examined if the in vitro addition of TNF-α to adipose tissue could influence insulin-stimulated glucose transport in adipocytes.

RESEARCH DESIGN AND METHODS

Subjects. There were 42 obese (BMI 43 ± 5.6 kg/m²) but otherwise apparently healthy women free of regular medication included in the first study of the relation between TNF-α and insulin action. All subjects were premenopausal and in a completely sedentary or took part in athletic performances. The women were 39 ± 10 years of age and were investigated in the morning after an overnight fast. First, using a commercially available radioimmunoassay kit (Pharmacia, Uppsala, Sweden), a venous blood sample was obtained for the determination of plasma glucose (by the routine chemistry laboratory at Huddinge Hospital) and insulin concentrations. Thereafter, subcutaneous adipose tissue was obtained from the paraumbilical region by needle aspiration under local anaesthesia using 5–10 ml of 0.5% lidocaine. It has previously been demonstrated that this form of local anaesthesia does not influence adipocyte metabolism (23). In a second study, the in vitro effects of TNF-α were examined in 4 women (age 40 ± 4 years). Abdominal subcutaneous adipose tissue was obtained from 3 obese women undergoing gastric restrictive surgery for weight loss and 1 woman undergoing a hysterectomy (BMI 37 ± 5.6 kg/m²). They were otherwise healthy and free of regular medication. The adipose tissue specimens were performed on non-operated subjects undergoing elective surgery for nonmalignant disorders. The women in the second study fasted overnight until the adipose specimen was removed from the surgical incision made at the beginning of surgery. Premedication and general anaesthesia were conducted as described before (24). The study was approved by the local committee on ethics at the Karolinska Institute, and informed consent was given by each subject.

Subcutaneous adipose tissue was used for the clinical characteristics of the 42 obese women included in study 1. Most subjects were hyperinsulinemic despite normal plasma glucose values, indicative of insulin resistance. One woman had a diabetic fasting plasma glucose value but no clinical symptoms of diabetes. Adipose tissue preparation. One portion of the adipose tissue was used for preparation and isolation of fat cells according to Rodbell (25). In brief, adipocytes were separated from stromal cells by tissue in a shaking bath at 37°C for 60 min with gentle shaking (120 rpm) in 5 ml Krebs-Ringer bicarbonate buffer (pH 7.4) with 40 g/1% purified bovine serum albumin. Adipocyte suspensions were then rinsed 3 times in collagenase-free buffer using nylon filters. Fat cell sizes were measured by direct microscopy, and the mean adipocyte diameter was calculated from measures of 100 cells. Because adipocytes have 95% lipid content and are spherical in shape, their volume and weight can be estimated from their diameter (26).

The other isolated cells were used to study glucose transport. The remaining adipose tissue pieces were used for secretion studies. TNF-α secretion. TNF-α secretion from adipose tissue was determined exactly as previously described (3). In brief, the tissue was cut into small pieces (10–25 mg) and then incubated at 37°C (3.0 ml medium/300 mg tissue) in a medium consisting of sterile KRP buffer (pH 7.4), endotoxin-free bovine serum albumin (4 g/100 ml), and glucose (1 mg/ml), with air as the gas phase. After a 2-h incubation, a 1-ml aliquot of the medium was removed and stored at –70°C for subsequent analysis of TNF-α, which was done at regular intervals using a sensitive enzyme-linked immunosorbent assay (HSTA 50; R&D Systems, London). The interassay coefficient of variation was 7%, and the intra-assay coefficient of variation was 22%. TNF-α secretion rates were expressed as percent of lipid or per fat cell, as has been described for PAI-1 secretion (22).

Tissue culture experiments. Under sterile conditions, adipose tissue specimens were cut into fragments of ~5–10 mg and divided for incubation in three plastic tubes (1 g adipose tissue/5 ml medium). Care was taken to avoid connective tissue and blood vessels. Four experiments were performed. The 3 plastic tubes each contained 5 ml serum-free medium 199 with Earl's salt (Life Technologies, Paisley, Scotland) and 30 g purified bovine albumin (Sigma, St. Louis, MO). 10 mmol/L HEPES (Life Technologies), and 100 μg/ml penicillin-streptomycin. pH was adjusted to 7.4. One of the tubes also contained 10 ng/ml TNF-α (human recombinant; Sigma) and another tube 100 ng/ml TNF-α. The adipose tissue specimens were incubated for 24 h at 37°C in 90%/humidity and 5.0%/CO₂ in an incubator. After the incubation, adipocytes were isolated and glucose transport was measured in the absence or presence of 1 mmol/l insulin as described below. During the lipogenesis experiment, TNF-α was present in the incubation medium in the same concentration used during the 24-h incubation.

PAI-1 secretion. PAI-1 secretion was analyzed in the same sample used for the TNF-α measurements. Thus, PAI-1 and TNF-α were measured under identical incubation conditions. PAI-1 was analyzed exactly as previously described (22) using an immunoassay kit (TinElize PAI-1; Biopool International, Umeå, Sweden). This assay was performed on 40 subjects.

Glucose transport. Direct determination of glucose transport can be made using radiolabeled glucose analogs that do not metabolize, such as 3-O-methylglucose (27). We used an indirect method that measures the incorporation of very small amounts of [3-3H]glucose into adipocyte lipids (28). This method needs much less tissue than the direct method. Using human fat cells, it has been described that the latter method is comparable with the 3-O-methylglucose method (29,30).

The method is described in detail elsewhere (29). In brief, isolated fat cells were incubated at a concentration of 2%/vol/vol in KRP buffer (pH 7.4) containing albumin (40 mg/ml), [3-3H]glucose (5 × 10⁸ dpm/ml), unlabeled glucose (1 μmol/l), and human insulin in different concentrations (0–70 nmol/l). The incubations were conducted for 2 h at 37°C with air as the gas phase (n = 2 for each type of incubation in study 1, and n = 8 for each type of incubation in study 2). Incubations were stopped by rapidly chilling the incubation vessels to 4°C. Thereafter, the incorporation of radiolabeled glucose into adipocyte lipids was determined exactly as previously described (29). The incorporation of radiolabeled glucose into lipids (i.e., lipogenesis) reflects glucose transport because at micromolar glucose concentrations, glucose transport is the rate-limiting step for lipogenesis in human fat cells, as discussed previously (29). Lipogenesis was expressed as the amount of glucose incorporated either per lipid weight of fat cells or per fat cell number, as described (29).

Analysis of concentration response curves. The concentrations of insulin used in the study were half-maximum effective insulin concentration (EC₅₀) were determined by linear regression analyses of log-log transformations of the concentration response curves and were expressed as log mole per liter (31). Changes in these values reflect events at or near the receptor, as discussed in detail by Kenakin (32). Insulin responsiveness was calculated as lipogenesis at the maximum effective hormone concentration minus the basal value. A maximum insulin effect was reached in each experiment. Changes in insulin responsiveness reflect distal events in insulin receptor signaling, whereas changes in insulin sensitivity mirror events at or near the receptor (32,33).

Statistical analysis. Values are given as the mean ± SE. Analysis of variance or covariance, Student’s paired or unpaired t test, and either single or multiple regression analyses were used for statistical comparisons. Calculations were made using the Statview software program (Statview; Abacus Concepts, Berkeley, CA). Lipogenesis values were logarithmically transformed to normalize the data.

RESULTS

Glucose transport and TNF-α secretion. Glucose transport (measured indirectly as lipogenesis) was stimulated by insulin in a concentration-dependent manner (values not shown). Half-maximum effect occurred at 2.5 ± 0.4 pmol/l of insulin. The maximum effective insulin concentrations (1–10 nmol/l) caused an approximately 2-fold stimulation of basal lipogenesis. The mean basal value was 0.15 nanomoles of glucose per 2 h per gram of lipid (range 0.02–0.92), whereas mean maximal insulin stimulated value was 0.32 nanomoles of glucose per 2 h per gram of lipid (range 0.06–1.3).

The correlation between TNF-α secretion and lipogenesis is shown in Table 2. TNF-α secretion showed no relation to

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Characteristics of the study group</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>39 (21–64)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>43 (31–60)</td>
</tr>
<tr>
<td>Fasting plasma insulin (μU/ml)</td>
<td>21 (7–51)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>6.1 (4.8–7.5)</td>
</tr>
</tbody>
</table>

Data are means (range).
TABLE 2
Relationship between TNF-α and PAI-1 secretion from adipose tissue and glucose transport

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Insulin</th>
<th>EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>0.14 (0.36)</td>
<td>0.58 (0.0001)</td>
<td>0.24 (0.15)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.12 (0.45)</td>
<td>0.11 (0.51)</td>
<td>0.07 (0.67)</td>
</tr>
</tbody>
</table>

Data are r (P). Relationships were determined by simple regression analysis. Basal, no hormone present; insulin, glucose transport at maximum effective concentration minus basal values (responsiveness).

basal lipogenesis but showed a strong relation to maximum insulin-stimulated lipogenesis (responsiveness). Up to one-third of the variation in insulin responsiveness could be explained by variations in TNF-α secretion (adjusted r² = 0.34). Insulin sensitivity, measured as EC50, was not significantly related to TNF-α secretion. The individual values for TNF-α secretion and insulin responsiveness are shown in Fig. 1. From this figure, it can be seen that two subjects had a very rapid rate of TNF-α secretion. However, the relationship between TNF-α secretion and insulin responsiveness was statistically significant, even when these two values were omitted in the statistical analysis (r = 0.49).

Because the between-assay coefficient of variation for the TNF-α measures was ~20%, we also separately analyzed data from assays where TNF-α measures were made at the same time using a single kit. Three such sets of determinations were performed (n = 17, 14, and 11), and the relationship between TNF-α secretion and insulin responsiveness was statistically significant for each (r = 0.47, 0.49, and 0.67, respectively; P < 0.01). Furthermore, the assays did not significantly interact with the relationship between insulin responsiveness and TNF-α secretion (F = 0.74; P = 0.48, analysis of covariance).

To examine whether TNF-α was an independent regressor for insulin-induced responsiveness of glucose transport, we performed a multiple regression analysis (Table 3). The analysis showed that only TNF-α secretion and fat cell volume contributed significantly to the variation in responsiveness after adjusting for differences in age and BMI. TNF-α secretion and fat cell volume together explained ~50% of the variation in insulin responsiveness (adjusted r²).

Similar result patterns were obtained for the relationship between TNF-α secretion and lipogenesis when data were expressed either per gram of lipid or per fat cell number (not shown).

To directly examine whether TNF-α influences glucose transport in adipose tissue, in vitro experiments were performed (Fig. 2). Tissue fragments from 4 obese women were incubated in a sterile and serum-free medium for 24 h with 0, 10, or 100 ng/ml of human recombinant TNF-α. When used in these concentrations, TNF-α has previously been shown to have lipolytic effects on adipocyte cell lines (34). Isolated adipocytes were prepared thereafter and investigated as above in the absence or presence of a maximum effective (10 nmol/l) concentration of insulin. There was no significant effect of the cytokine on basal lipogenesis. However, insulin-induced (insulin minus basal) lipogenesis was significantly (P = 0.008 by analysis of variance) and concentration-dependently inhibited by TNF-α. Because n = 8 in each type of adipocyte incubation (basal or insulin), it was also possible to statistically validate the results within a certain individual. This examination revealed that TNF-α significantly inhibited insulin-stimulated lipogenesis within each subject as well (data not shown).

TABLE 3
Multiple regression analysis of 4 parameters versus insulin responsiveness of glucose transport

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BMI</th>
<th>Fat cell volume</th>
<th>TNF-α secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.06 (0.67)</td>
<td>0.09 (0.52)</td>
<td>-0.36 (0.014)</td>
<td>-0.46 (0.002)</td>
</tr>
</tbody>
</table>

Data are partial r (P). Responsiveness was calculated as described in the text. See footnote to Table 2 for details.
Glucose transport and PAI-1 secretion. As also shown in Table 2 and Fig. 3, there were no significant correlations between PAI-1 secretion and any of the parameters related to lipogenesis. In addition, there was no correlation between the secretion rates of TNF-α and PAI-1 (r = 0.02; figure not shown).

DISCUSSION

In this study, we investigated for the first time the relationship between TNF-α production and glucose transport in human adipose tissue. The results suggest that TNF-α produced locally in adipose tissue of obese women may play an important role in modulating the action of insulin on glucose transport in adipocytes. Adipose TNF-α secretion did not relate to basal (no insulin present) glucose transport but correlated strongly with the maximal capacity of insulin to stimulate glucose transport. As much as one-third of the variation in insulin responsiveness could be explained by variations in TNF-α production (adjusted r² = 0.34), suggesting that, although important, TNF-α is not the only factor that influences insulin action in fat cells. We found that fat cell size was also related to maximum insulin-stimulated glucose transport. TNF-α and fat cell size together explained half of the variance in glucose transport. The other half remains at present unaccounted for.

We measured TNF-α secretion from whole fat tissue. It was not possible to include experiments with TNF-α secretion from isolated fat cells because of the limited amount of adipose tissue available. Isolated human adipocytes have been shown to secrete TNF-α (3). Therefore, it remains to be established to what extent TNF-α is an autocrine or paracrine factor in the modulation of insulin action in human adipose tissue. However, such a separation may not be important from a clinical perspective, since fat cells are likely to respond in a similar fashion to TNF-α from the fat cells themselves or other cells in the adipose tissue. It should also be noted that TNF-α secretion from isolated fat cells might be artificially altered by the collagenase isolation procedure.

Variations in insulin sensitivity reflect events at the insulin receptor itself or in its close vicinity (32). In contrast, variations in maximal insulin effect (responsiveness) mirror events at a postreceptor level (32,33). We found only a weak nonsignificant correlation between TNF-α secretion and insulin sensitivity (EC₅₀), whereas TNF-α secretion and insulin responsiveness showed a strong correlation. These data indicate that TNF-α primarily modulates insulin action on glucose metabolism in human fat cells at some point distal to the receptor. TNF-α may directly inhibit GLUT4, the major glucose transport protein in fat cells (35). However, a number of post–insulin receptor events in hormone-stimulated glucose transport could be influenced by TNF-α. Unfortunately, it was not possible for us to determine the exact site of TNF-α action, since the necessary methods (polymerase chain reaction, Western blot, enzymatic assays) would have required too large amounts of adipose tissue.

Adipose tissue secretes a number of proteins besides TNF-α (36), including PAI-1. The observed lack of correlation between insulin action and PAI-1 secretion strongly suggests that the effects seen with TNF-α are specific. Moreover, there was no relationship between the adipose secretion rates of PAI-1 and TNF-α, suggesting that the production of these two proteins is regulated in a different manner in human adipose tissue.

This study was performed on subcutaneous adipose tissue from obese women, and it remains to be determined whether results apply also for men and for nonobese subjects. Also, we do not know how the results relate to other fat depots. Unfortunately, it is not possible to obtain visceral fat in this type of clinical investigation, although it should be remembered that subcutaneous adipose tissue is the major fat depot, constituting ~80% of all adipose tissue.

To further study the importance of TNF-α, in vitro experiments on subcutaneous adipose tissue obtained from obese women were performed. These data show that incubation of tissue under tissue culture conditions for 24 h with TNF-α caused a concentration-dependent inhibition of maximum insulin-stimulated glucose transport in isolated fat cells but that the cytokine had no effect on basal glucose transport. The TNF-α effect was statistically significant within as well as between subjects. These data are in complete harmony with the results from the clinical study (i.e., the first study). We realize that some caution should be exercised when extrapolating in vitro data to the situation in vivo. Nevertheless, when the present in vitro results are considered together with the in vivo results, we propose that TNF-α is important for the local modulation of insulin action on glucose transport in human adipose tissue, at least in obese women.

In conclusion, TNF-α may be an important local factor that influences the ability of insulin to stimulate glucose transport in human fat cells.

ACKNOWLEDGMENTS

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REFERENCES


FIG. 3. Relationship between PAI-1 secretion and insulin-stimulated glucose transport. See legend to Fig. 1 for further details.


