Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo

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Mohamed-Ali, Vidya, Steven Goodrick, Karen Bulmer, Jeffrey M. P. Holly, John S. Yudkin, and Simon W. Coppack. Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E971–E975, 1999.—To investigate in vivo adipose tissue production of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and their soluble receptors: TNF receptor type I (sTNFR-I), TNF receptor type II (sTNFR-II), and IL-6 receptor (sIL-6R), we determined arteriovenous differences in their levels across abdominal subcutaneous adipose tissue in obese subjects. Subjects had a median (interquartile range) age of 44.5 (27–51.3) yr, body mass index (BMI) of 32.9 (26.0–46.6) kg/m², and %body fat of 42.5 (28.5–51.2) %. Although there was not a significant difference in the arteriovenous concentrations of TNF-α (P = 0.073) or sTNFR-II (P = 0.18), the levels of sTNFR-I (P = 0.002) were higher in the vein compared with artery, suggesting adipose tissue production of this soluble receptor. There was a significant arteriovenous difference in IL-6 (P < 0.001) but not in its soluble receptor (P = 0.18). There was no relationship between TNF-α levels and adiposity indexes (r_s = 0.12–0.22, P = not significant); however, levels of both its soluble receptor isomers correlated significantly with BMI and %body fat (sTNFR-I r_s = 0.42–0.72, P < 0.001; sTNFR-II r_s = 0.36–0.65, P < 0.05–<0.001). IL-6 levels correlated significantly with both BMI and %body fat (r_s = 0.51, P = 0.004, and r_s = 0.63, P < 0.001), but sIL-6R did not. In conclusion, 1) soluble TNFR-I is produced by adipose tissue, and concentrations of both soluble isomers correlate with the degree of adiposity, and 2) IL-6, but not its soluble receptor, is produced by adipose tissue and relates to adiposity.

TUMOR NECROSIS FACTOR-α (TNF-α) and interleukin-6 (IL-6) are proinflammatory cytokines with known potent effects in host defense (14). Both of these cytokines have been implicated in the regulation of lipid and glucose metabolism (5). Adipose tissue is a significant source of endogenous TNF-α, with its expression in fat tissue being elevated in obesity (11). Evidence for significant circulating levels of this cytokine is variable, and it is thought to operate mainly via autocrine/paracrine mechanisms in both adipose tissue and skeletal muscle (8, 9). IL-6 is also expressed in and released by adipose tissue, and its levels increase with obesity (15, 16).

Two structurally distinct TNF receptors, TNFR-I and TNFR-II, have been identified (4). The TNFR-I is thought to mediate most of the functions of TNF-α, whereas the actions of TNFR-II are as yet unclear and perhaps mainly cell specific. Both these receptors are expressed in human adipose tissue, and the soluble forms are present in the circulation (7). Although the nature of their physiological function is still unclear, at least in some studies in vitro, they inhibit the ligand-binding cell surface receptor, thereby acting as antagonists (23).

The biological activities of IL-6 are initiated by binding of the ligand to a single receptor. The IL-6 receptor comprises two chains, a ligand binding, predominantly extracytoplastic chain (IL-6R; gp80), and the signal-transducing gp130 chain (6, 12). The binding of IL-6 to IL-6R is predominantly an extracellular process. This complex, IL-6/IL-6R, can be formed with either soluble or membrane-bound IL-6R (22). The binding of IL-6 to IL-6R, in the presence of gp130, leads to the formation of high-affinity binding sites, gp130 dimerization, and signal transduction (24). The signal-transducing gp130 is abundantly expressed in most cell types, whereas IL-6R is expressed in a variety of cells in extremely low quantity (12). Unlike the case of TNF-α, in which the soluble receptors may function as inhibitors for the ligand (6, 23), in vivo, both recombinant and naturally produced sIL-6R appear to act as agonists in IL-6R-negative cells that express gp130 (6).

The source of these ligands and of their soluble receptors may be unclear. Both of these cytokines interact at several levels, often regulating similar metabolic processes. To test the hypothesis that the adipose tissue releases soluble TNF and IL-6 receptors, which may then act to localize and modulate effects of the cytokines, we investigated the in vivo release of these molecules by measuring the arteriovenous balance in their levels across an abdominal subcutaneous adipose tissue depot in nonobese, mainly obese, subjects.

EXPERIMENTAL SUBJECTS

Arteriovenous differences for TNF-α and IL-6 were determined after an overnight fast in 60 Caucasian subjects having a range of adiposity. In a representative subset of 30 subjects (20 females and 10 males), their respective soluble receptors were also assayed. The subset had a median (interquartile range) age of 44.5 (27.0–51.3) yr, body mass
index (BMI) of 32.9 (26.0–46.6) kg/m², and a median percent body fat of 42.5 (28.5–51.2) %.

All subjects gave written informed consent to these studies, which had previously been approved by the local ethics committee.

MATERIALS AND METHODS

Cannulas were inserted, using local anesthesia, into a radial artery and a superficial epigastric vein draining the subcutaneous adipose tissue (3, 13). Lines were kept patent by a slow infusion of isotonic saline. Blood samples were taken simultaneously from the two sites. Previous work has shown that venous blood from superficial epigastric veins approximates the effluent from an adipose tissue bed, and arteriovenous differences across abdominal adipose tissue yield result in good agreement with those of microdialysis studies (19).

Body composition was measured by electrical bioimpedance (Bodystat, Douglas, Isle of Mann, UK).

Blood flow measurements and assays. Abdominal subcutaneous adipose tissue blood flow was measured using the 133Xe washout technique on the basis of the principle that the disappearance of 133Xe radioactivity is proportional to adipose tissue blood flow (13). Blood flow measurements were made twice during the postabsorptive blood sampling, and 133Xe washout was measured using a Mediscint system (Oakfield Instruments, Witney, UK). Adipose tissue blood flow was calculated as previously described, by use of a partition coefficient of 10 ml/g for all subjects (13, 21).

Plasma glucose levels were determined with glucose oxidase reagent (Beckman, Brea, CA), and specific insulin was assayed with a two-site ELISA (Dako Diagnostics, Ely, UK). TNF-α and IL-6 were measured using the high-sensitivity two-site ELISA from R & D Systems (Oxford, UK). The limit of detection of the human TNF-α assay was 0.10 pg/ml, with intra- and interassay coefficients of variation (CV) of 6.9 and 8.4%. For human IL-6, the limit of detection was 0.09 pg/ml, and intra- and interassay CVs were 5.3 and 9.2%. The assays for human sIL-6R, sTNFR-I, and sTNFR-II were all sensitive to 3 pg/ml, with intra- and interassay CVs <5% (R & D Systems).

All samples from one individual were always run on the same plate.

Calculations and statistics. The local cytokine and soluble receptor production by subcutaneous adipose tissue was calculated by the Fick principle, i.e., the product of the arteriovenous difference and local plasma flow. All data are presented as the median and interquartile range. Comparisons between sites were made using Wilcoxon’s paired tests, and Spearman’s rank correlations were used to determine the relationships between variables.

RESULTS

Arterial and abdominal venous cytokine and soluble receptor concentrations. The 60 Caucasian subjects had normal glucose and insulin concentrations [5.0 (4.8–5.3) mmol/l and 57.9 (33.4–82.0) pmol/l, respectively]. In these subjects, fasting abdominal vein levels of IL-6, but not those of TNF-α, were significantly higher than in the artery (P < 0.001 and P = 0.073, respectively), suggesting release of IL-6 but not TNF-α (Fig. 1, A and B).

Table 1. Arterial and venous concentrations of cytokine soluble receptors

<table>
<thead>
<tr>
<th></th>
<th>Arterial</th>
<th>Venous</th>
<th>P</th>
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<tbody>
<tr>
<td>sTNFR-I</td>
<td>971 (872–1,323) pg/ml</td>
<td>1,091 (894–1,495) pg/ml</td>
<td>0.002</td>
</tr>
<tr>
<td>sTNFR-II</td>
<td>2,174 (1,830–2,618) pg/ml</td>
<td>2,246 (1,885–2,928) pg/ml</td>
<td>0.18</td>
</tr>
<tr>
<td>sIL-6R</td>
<td>36.8 (30.1–43.2) ng/ml</td>
<td>36.8 (31.9–43.5) ng/ml</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Data are shown as medians of 30 subjects, with interquartile ranges in parentheses. sTNFR-I and sTNFR-II, soluble tumor necrosis factor receptor types I and II, respectively; sIL-6R, soluble interleukin-6 receptor.

Table 2. Molar ratios of soluble receptors to ligands

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
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<tbody>
<tr>
<td>TNFR-I/TNF-α</td>
<td>344 (409–327)</td>
<td>364 (505–339)</td>
</tr>
<tr>
<td>TNFR-II/TNF-α</td>
<td>771 (859–646)</td>
<td>749 (1,065–664)</td>
</tr>
<tr>
<td>sIL-6R/sIL-6</td>
<td>7.4×10⁶ (9.7×10⁶–3.2×10⁶)</td>
<td>2.1×10⁶ (4.3×10⁶–0.2×10⁶)</td>
</tr>
</tbody>
</table>

Data are shown as medians, with interquartile ranges in parentheses. Molar ratios of sTNFR-I and sTNFR-II to tumor necrosis factor-α (TNF-α) and of sIL-6R to IL-6 were calculated by assuming the following molecular masses: 17 kDa for TNF-α (19), 30 kDa for sTNFR-I and sTNFR-II (19), 26 kDa for IL-6 (20), and 50 kDa for sIL-6R (21).
Table 3. Correlations of measures of adiposity with basal cytokine concentrations

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>%Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r_s )</td>
<td>( P )</td>
</tr>
<tr>
<td>TNF-( \alpha )</td>
<td>0.20–0.18</td>
<td>0.13–0.15</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.31–0.33</td>
<td>0.02–0.009</td>
</tr>
</tbody>
</table>

Data are shown as Spearman’s rank correlations.

In the subset of 30 subjects, levels of sTNFR-II were higher than those of sTNFR-I, as reported previously (9). Of the two TNF soluble receptors studied, venous levels of sTNFR-I were significantly higher than in the artery (\( P = 0.002 \)), demonstrating adipose tissue production of this soluble receptor, sIL-6R levels were similar in the vein and the artery (Table 1).

Thus these data show adipose tissue release of IL-6 but not of its soluble receptor and of soluble TNF receptor but not of its ligand.

Regulation of cytokines and their soluble receptors. Levels of IL-6, both arterial and venous, were unrelated to those of sIL-6R (\( r_s = 0.03–0.17; P = 0.86–0.39 \)). No relationship was apparent between TNF-\( \alpha \) and sTNFR-II (\( r_s = -0.05–0.14; P = 0.81–0.47 \)) or between TNF-\( \alpha \) and sTNFR-I (\( r_s = 0.17–0.34; P = 0.35–0.06 \)).

There was a strong, positive correlation between the two soluble TNF receptors (\( r_s = 0.49–0.71; P = 0.005–P < 0.001 \)).

The molar ratios of sTNFR-I and sTNFR-II to TNF-\( \alpha \) and of sIL-6R to IL-6 were calculated with the assumption of the following molecular masses: 17 kDa for TNF-\( \alpha \) (2), 30 kDa for sTNFR-I and sTNFR-II (2), 26 kDa for IL-6 (10), and 50 kDa for sIL-6R (18). These data show that both TNF and IL-6 soluble receptor concentrations are far in excess of their respective ligands (Table 2).

Obesity, and cytokines and their soluble receptors. Significant correlations were found between indexes of obesity (BMI and %body fat) and concentrations of IL-6 (\( r_s = 0.31–0.50; P = 0.02–P < 0.001 \); Table 3), sTNFR-I (\( r_s = 0.62–0.72; P < 0.001 \)), and sTNFR-II (\( r_s = 0.39–0.65; P = 0.03–P < 0.001 \)) (Figs. 2 and 3) but not with those of sIL-6R (\( r_s = 0.19–0.25; P = 0.32–0.18 \)) or TNF-\( \alpha \) (\( r_s = 0.15–0.22; P = 0.27–0.09 \)). There were no significant relationships between the molar ratios of the sTNFRs to TNF-\( \alpha \) and measures of obesity; however, significant negative correlations were apparent between the sIL-6R-to-IL-6 ratio and BMI and percent body fat (BMI and sIL-6R-to-IL-6 ratio, arterial \( r_s = -0.43; P = 0.02 \); venous \( r_s = -0.38; P = 0.04 \); percent body fat and sIL-6R-to-IL-6 ratio, arterial \( r_s = -0.51; P = 0.006 \); venous \( r_s = -0.38; P = 0.05 \)).

Adipose tissue IL-6 and sTNFR-I production rates. Net subcutaneous abdominal adipose tissue IL-6 and sTNFR-I release in vivo was determined from net arteriovenous balance and local plasma flow and was 2.72 (0.68–5.19) pg·100 g adipose tissue·min\(^{-1}\) and 1,454.0 (777.09–1,941.03) pg·100 g adipose tissue·min\(^{-1}\), respectively, for the whole group.

There was a significant relationship between production of sTNFR-I and venous TNF-\( \alpha \) (\( r_s = 0.40; P = 0.03 \)).

DISCUSSION

Previous studies have shown a relationship between the expression of TNF-\( \alpha \) in fat tissue and measures of obesity, as well as a reduction in this expression on weight loss (20). The relationship between circulating TNF-\( \alpha \) and obesity and insulin resistance is less clear. The reason for this discrepancy may be that TNF-\( \alpha \) functions mainly via paracrine/autocrine mechanisms. More recently, it has been shown that both TNF-\( \alpha \) and TNF-II are expressed in human adipose tissue and that levels of soluble TNFR-II in the systemic circula-
tion correlate positively with obesity (7). This study suggests that the source of the soluble TNF receptors may, at least in part, be adipose tissue. Our results show, for the first time, that there is in vivo release of sTNFR-I by human subcutaneous adipose tissue. The significant correlation between net adipose tissue release of sTNFR-I and circulating TNF-α concentrations suggests that this production may be regulated by the ligand itself. Alternatively, sTNFR-I production by adipose tissue could increase the circulatory half-life of TNF-α and, hence, its circulating concentration.

Therefore, these results support the suggestion put forward by Hotamisligil et al. (7) of adipose tissue-derived soluble TNF receptors in obesity. Furthermore, we also show a strong correlation between the two soluble receptors, perhaps suggesting coregulation of receptors, with the levels of both soluble receptors being strongly related to measures of obesity. The relationship between sTNFR-II and obesity, even though we had no clear evidence for net production of this molecule by the adipose tissue, may suggest an indirect effect via sTNFR-I or TNF-α. Alternatively, although the production rate for sTNFR-II may be very similar to that of sTNFR-I, because its absolute concentration is much higher, the arteriovenous difference is perhaps more difficult to detect. We also confirm higher circulating concentration of sTNFR-II, perhaps due to slower clearance of this molecule, compared with that of sTNFR-I.

We have previously reported in vivo production of IL-6 by adipose tissue, and we confirm this result on a larger number of subjects in this study (15). Unlike the inhibitory effects of soluble receptors on the activity of TNF-α, most of the data show that the sIL-6R facilitates and enhances IL-6 activity (6, 17). We show that sIL-6R does not appear to be released by this adipose tissue depot and does not show a relationship between obesity and its levels. The physiological significance of these soluble receptors is unclear. The adipose tissue release of the inhibitory sTNFRs may localize the effects of TNF-α within the tissue, enabling it to function as an autocrine/paracrine factor. Furthermore, it has also been shown that IL-6 inhibits TNF-α expression in primary rat astrocytes, although this effect has not been reported in adipocytes (1). Thus, in adipose tissue, TNF-α may induce the release of IL-6, which may then act as the endocrine signal emanating from adipose tissue, but with TNF-α playing an autocrine/paracrine role.

It is interesting to note the vast excess of the soluble receptors for both the cytokines compared with ligand. The assays employed in this study are ELISAs and determine all the immunologically active protein; thus, this therefore raises the question as to whether all the peptides measured are also functionally active. Furthermore, because most of the evidence suggests that sTNFRs are inhibitory but that sIL-6R enhances the ligand activity, the excess circulating binding proteins would have very different physiological implications.

Our findings support the concept that IL-6 is an endocrine factor released from adipose tissue, whereas the same tissue is an active producer of sTNFR-I, the prime function of which is to ensure that TNF-α acts principally as an autocrine/paracrine factor in this tissue. In conclusion, these novel results suggest a mechanism for the regulation of IL-6 and TNF-α by abdominal subcutaneous adipose tissue.

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