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Subcutaneous Adipose Tissue Releases Interleukin-6, But Not Tumor Necrosis Factor-α, *in Vivo**

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ABSTRACT

We measured arterio-venous differences in concentrations of tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) across a sc adipose tissue bed in the postabsorptive state in 39 subjects [22 women and 17 men; median age, 36 yr (interquartile range, 26–48 yr); body mass index, 31.8 kg/m^2 (range, 22.3- 38.7 kg/m^2); percent body fat, 28.7% (range, 17.6–50.7%)]. A subgroup of 8 subjects had arterio-venous differences measured across forearm muscle. Thirty subjects were studied from late morning to early evening; 19 ate a high carbohydrate meal around 1300 h, and 11 continued to fast. We found a greater than 2-fold increase in IL-6 concentrations across the adipose tissue bed [arterial, 2.27 pg/mL (range, 1.42–3.53 pg/mL); venous, 6.71 pg/mL (range, 3.36–9.62 pg/mL); $P < 0.001$], but not across forearm muscle. Arterial plasma concentrations of IL-6 correlated significantly with body mass index (Spearman's r = 0.48; $P < 0.01$) and percent body fat (Spearman's r = 0.49; $P < 0.01$). Subcutaneous adipose tissue IL-6 production increased by the early evening (1800–1900 h) in both subjects who had extended their fasting and those who had eaten. Neither deep forearm nor sc adipose tissue consistently released TNFα [across adipose tissue: arterial, 1.83 pg/mL (range, 1.36–2.34 pg/mL); venous, 1.85 pg/mL (range, 1.44–2.53 pg/mL); P = NS: across forearm muscle: arterial, 1.22 pg/mL (range, 0.74–2.76 pg/mL); venous, 0.99 pg/mL (range, 0.69–1.70 pg/mL); P = NS]. Although both IL-6 and TNFα are expressed by adipose tissue, our results show that there are important differences in their systemic release. TNFα is not released by this sc depot. In contrast, IL-6 is released from the depot and is thereby able to signal systemically. (*J Clin Endocrinol Metab* **82:** 4196–4200, 1997)

OBESITY is associated with multiple metabolic and hormonal changes, including the development of insulin resistance, dyslipidemia (1), and increased adipose tissue release of leptin (2). Many of the metabolic consequences may be mediated by increased adipose tissue release of non-esterified fatty acids (3). However, adipose tissue expresses leptin (4) and other cytokine-like molecules (5–8), and several lines of evidence suggest a possible role for cytokines in regulating the size of the adipose tissue stores and other metabolic characteristics, such as systemic insulin resistance.

Tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) are cytokines with metabolic and/or weight-regulating effects. For both of these cytokines, messenger ribonucleic acid has been demonstrated in human adipose tissue, and both proteins are present in adipose tissue homogenates and culture media (5–8). TNFα induces insulin resistance by effects on phosphorylation of insulin receptors and the insulin receptor substrate-1 (9, 10). TNFα induces IL-6 messenger ribonucleic acid production (6). Both TNFα and IL-6 inhibit lipoprotein lipase (LPL) activity and decrease its production in murine adipocyte cell lines (11, 12) as well as increase lipolysis (13, 14). Such actions will help to limit obesity (15). TNFα and IL-6 cause weight loss in mice, which is inhibited by pretreatment with either anti-TNFα or anti-IL-6 monoclonal antibodies, respectively (16). It is, however, debatable as to whether cytokines expressed in adipose tissue can act as endocrine signals to bring about metabolic effects (17). To date, there are no data showing *in vivo* release of these molecules by human adipose tissue. We, therefore, undertook this study to test the hypothesis that adipose tissue releases IL-6 and TNFα, which could then act as endocrine mediators.

We used the Fick principle to determine release rates of TNFα and IL-6 from a sc adipose tissue depot, and in a subset of our subjects we also examined local release by forearm tissue. To determine whether cytokine release was modified acutely by food, the effects of food and fasting were also examined.

Subjects and Methods

Subjects and cannulas

Arterio-venous differences were measured in 39 healthy Caucasian subjects (22 women and 17 men) studied in the postabsorptive state (after fasting overnight). This group had a median age of 36 yr (interquartile range, 26–48 yr), a body mass index (BMI) of 31.8 kg/m^2 (range, 22.3–38.7 kg/m^2), a percent body fat of 28.7% (range, 17.63–50.7%), and a total fat mass of 27.8 kg (range, 12.3–56.5 kg). Cannulas were inserted, using local anesthesia, into a radial artery and a superficial epigastric vein draining the sc abdominal adipose tissue (18, 19). A deep antecubital vein (18–21) was also cannulated in 8 of the leaner male subjects [age, 26 yr (range, 22–34 yr); BMI, 23.8 kg/m^2 (range, 21.8–25.6 kg/m^2)]; these subjects were chosen with the expectation that their deep forearm tissue would contain less adipose tissue (19). The oxygen saturation in the deep forearm venous samples was less than 60%, implying that deep venous blood was being sampled (20). All lines were kept patent by a

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slow infusion of isotonic saline. Blood samples were taken simultaneously from the different sites. Previous work has shown that venous blood from superficial epigastric veins approximates the effluent from an adipose tissue bed, and arterio-venous differences across abdominal adipose tissue yield results in good agreement with those of microdialysis studies (22). Deep antecubital vein samples, taken 2 min after inflation of a wrist cuff, approximate the venous effluent of the skeletal muscle.

Body composition was measured by electrical bioimpedance (Biostat, Douglas, UK) and dual photon absorptiometry (Lunar Instruments, Madison, WI) in those subjects in whom forearm cannulas were inserted. The waist/hip ratio was measured using a steel tape.

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

Blood sampling

Postabsorptive samples were obtained (in duplicate) between 1100–1400 h, corresponding to a overnight fast of 13–15 h. Nineteen subjects [10 women and 9 men; BMI, 33.2 kg/m^2 (range, 25.0–46.0 kg/m^2); age, 46 yr (range, 27–52 yr)] then ate a high carbohydrate meal (energy content, 50% of estimated 24-h basal metabolic rate of the subject; 70% energy from carbohydrate, 20% from fat, and 10% from protein). Postprandial samples were taken 1, 3, and 5 h after eating. Eleven female subjects [BMI, 34.6 kg/m^2 (range, 20.6–36.7 kg/m^2); age, 34 yr (range, 24–40 yr)] continued their fast for a similar period. In both fed and prolonged fasted groups, final samples were taken between 1800–1900 h.

To investigate whether drawing samples from indwelling cannulas *per se* affected cytokine concentrations, samples were drawn by routine venepuncture as well as via a cannula. In 11 different healthy Caucasian volunteer subjects [6 women and 5 men; age, 31 yr (range, 24–36 yr); BMI, 22.4 kg/m^2 (range, 21.5–24.1 kg/m^2)], samples were taken by routine venepuncture and from a cannula left in the contralateral arm for at least 15 min before sampling.

Blood flow measurements and assays

Abdominal sc adipose tissue blood flow was measured using the ^{133}Xe washout technique, based on the principle that the disappearance of ^{133}Xe radioactivity is proportional to adipose tissue blood flow (23). Blood flow measurements were made twice during the postabsorptive baseline. At the time of each subsequent blood sample, an additional blood flow measurement was made. ^{133}Xe washout was measured using a Mediscint system (24) (Oakfield Instruments, Witney, UK) during the period of baseline sampling and during the postprandial or prolonged fast. Adipose tissue blood flow was calculated as previously described (2, 19), using a partition coefficient of 10 mL/g for all subjects. Forearm blood flow was measured by venous occlusion plethysmography (19, 21) (Hokansen system, P.M.S. Instruments, Maidenhead, UK). Plasma flow was calculated from blood flow and hematocrit.

The plasma glucose concentration was determined with glucose oxidase reagent (Beckman, Brea, CA). Insulin was assayed specifically using a commercial kit (Dako Diagnostics, Ely, UK). TNFα and IL-6 were measured using enzyme-linked immunosorbent assays that are specific for the total amounts (*i.e.* bound and unbound) of cytokines (R&D Systems, Oxford, UK). The TNFα assay had a limit of detection of 0.10 pg/mL, an intraassay coefficient of variation (CV) of 6.9%, and an interassay CV of 8.4%. The IL-6 assay had a limit of detection of 0.09 pg/mL, an intraassay CV of 5.3%, and an interassay CV of 9.2%. All samples from one individual were always run in the same batch.

Calculations and statistics

The local cytokine production by sc adipose tissue was calculated by the Fick principle (21), *i.e.* the product of the arterio-venous difference and local plasma flow. To estimate by extrapolation the contribution of adipose tissue to systemic turnover of IL-6, the local secretion rate was multiplied by the total body fat mass, as reflected by body composition measurement (19, 21). These calculations assume adipose tissue homogeneity. Although there is evidence for adipose tissue heterogeneity in general (9), there are no specific data relating to whether such heterogeneity applies to IL-6 and TNFα, so such extrapolations should be interpreted with caution.

All data are presented as the median (with the interquartile range in *parentheses*), except in the figures, where means and SEMs are used for simplicity. Comparisons between sites and of trends with time were made using Wilcoxon's paired tests and ANOVA, respectively (on data normalized by logarithmic transformation as necessary). To determine the relationships between variables, Spearman's correlation coefficients (r_s) were used.

Results

Arterial and abdominal venous cytokine concentrations

In 39 healthy Caucasian subjects with normal plasma concentrations of glucose [4.9 mmol/L (range, 4.6–5.2 mmol/L)] and insulin [59.3 pmol/L (range, 30.2–81.2 pmol/L)], postabsorptive concentrations of IL-6 were significantly higher ($P < 0.001$) in abdominal venous samples [6.71 pg/mL (range, 3.36–9.62 pg/mL)] than in arterial samples [2.27 pg/mL (range, 1.42–3.53 pg/mL); Fig. 1]. Conversely, arterial and abdominal venous concentrations of TNFα were similar [1.83 pg/mL (range, 1.36–2.34 pg/mL) and 1.85 pg/mL (range, 1.44–2.53 pg/mL), respectively].

Significant correlations were found between indexes of obesity (BMI and body fat mass) and arterial IL-6 concentrations, but not with TNFα concentrations (Table 1). Although glucose and insulin concentrations tended to be higher in the obese subjects, these variables did not correlate significantly with IL-6 concentrations (data not shown).

Adipose tissue cytokine production rates

Subcutaneous abdominal adipose tissue IL-6 release *in vivo* was determined from net arterio-venous balance and local plasma flow and was 3.84 pg/100 g adipose tissue/min (range, 1.86–11.6 pg/100 g adipose tissue/min) for the whole group. Local adipose tissue IL-6 production was greater in subjects with a higher waist/hip ratio (correlation of postabsorptive local IL-6 production rate per 100 g sc adipose tissue with waist/hip ratio; $r_s = 0.56$; $P < 0.01$).

Concentrations of IL-6 in both arterial and abdominal venous samples taken during the afternoon and evening (until 1800–1900 h) were consistently higher ($P < 0.01$, by ANOVA)

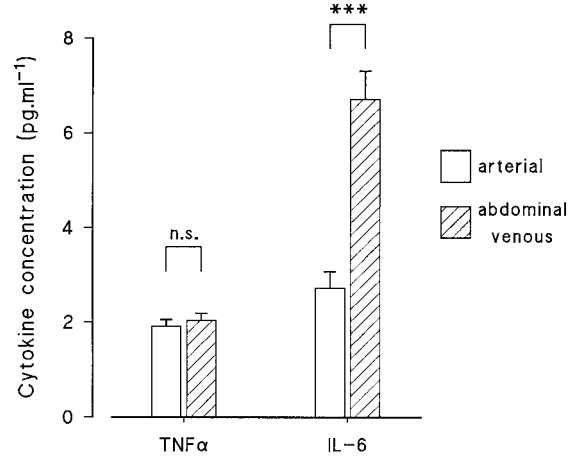

FIG. 1. Postabsorptive arterial and superficial abdominal venous concentrations of TNFα and IL-6 in 39 subjects. ***, Significant difference ($P < 0.001$).

than those taken earlier in the day (1100–1400 h) regardless of whether subjects had eaten. Blood flows were similar in both groups and did not change significantly with time, but arterio-venous differences widened in the afternoon and evening in both groups (see Fig. 2). The local adipose tissue IL-6 production rate increased significantly ($P < 0.05$ for both groups) compared to values seen around 1100–1400 h [by 160% (range, 120–200%) in the fed group and by 140% (range, 110–190%) in the fasted group]. A secondary reanalysis comparing six women from the fasted group with six age- and body composition-matched women from the fed group similarly showed local IL-6 production increasing significantly in both groups with time, but no significant effect of eating.

There were no significant differences in the concentration of TNFα between the arterial and abdominal samples even after prolonged fasting or after eating (data not shown). Although in a few subjects, venous concentrations of TNFα were higher than those in arterial blood, the reverse was true for a similar number. There was no consistent trend to the TNFα arterio-venous difference, with obese individuals no more likely to show net release than lean subjects.

TABLE 1. Relationship of measures of obesity to circulating concentrations of tumor necrosis factor-α and interleukin-6 in 39 subjects

	Arterial tumor necrosis factor-α	Arterial interleukin-6
Body mass index	0.16	0.48[a]
% Body fat	0.25	0.49[a]
Total body fat	0.18	0.55[a]

Spearman rank correlations are shown between the variables studied. Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. Percent body fat was measured by electrical bioimpedance (Biostat, Douglas, UK), and total body fat was determined as the product of body weight (kilograms) and percent body fat.

[a] $P < 0.01$.

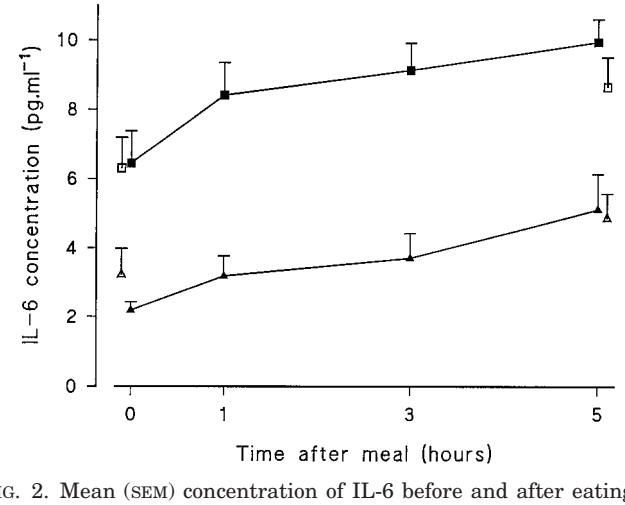

FIG. 2. Mean (SEM) concentration of IL-6 before and after eating a meal. *Solid symbols* are data from 19 subjects who ate a high carbohydrate meal at time zero (around 1300 h). *Open symbols* are data from 11 subjects who continued to fast. *Triangles* are concentrations in arterial samples. *Squares* are concentrations in superficial abdominal veins.

Forearm studies

No significant arterio-venous differences of either TNFα or IL-6 were seen across the forearm in eight subjects [arterial TNFα, 1.22 pg/mL (range, 0.74–2.76 pg/mL); forearm venous TNFα, 0.99 pg/mL (range, 0.69–1.70 pg/mL); arterial IL-6, 1.87 pg/mL (range, 1.57–2.31 pg/mL); forearm venous IL-6, 2.34 pg/mL (range, 1.28–4.09 pg/mL)].

Levels of TNFα and IL-6 did not differ significantly in 11 paired samples obtained by venepuncture and through an indwelling cannula, showing the lack of influence of sampling through an indwelling cannula on these concentrations (data not shown).

Extrapolations of adipose tissue IL-6 production rates

Assuming that the whole body adipose tissue mass (WBFM), as determined by body composition measurements, released IL-6 at the same rate as the sc depot studied here, we estimated by extrapolation the WBFM IL-6 production rate [1.41 ng/min (range, 0.22–2.67 ng/min]. The WBFM IL-6 production rate so calculated correlated with the circulating IL-6 concentration ($r_s = 0.63$; $P < 0.001$), waist/hip ratio ($r_s = 0.56$; $P < 0.01$), BMI ($r_s = 0.41$; $P < 0.01$), and percent body fat ($r_s = 0.46$; $P < 0.01$; Fig. 3).

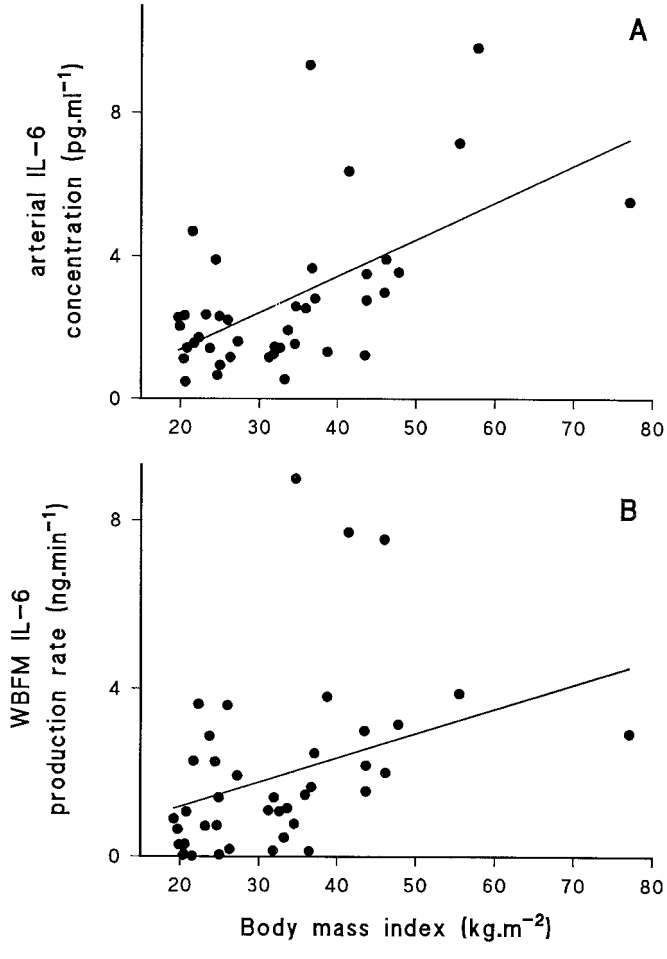

FIG. 3. Correlations of the arterial concentration (A) and the WBFM production rate (B) of IL-6 to BMI.

Discussion

These data have shown, for the first time, *in vivo* release of IL-6 by human sc adipose tissue and that such release is greater in the evening compared to around noon. Indeed, it can be calculated, using the estimate of IL-6 half-life of 3 min (25), that a WBFM with the same production rate as the sc adipose tissue studied here would produce 15–25% of the systemic IL-6 around noon and 25–35% in the evening.

There was no net secretion of TNFα by this adipose tissue depot, suggesting that TNFα from sc adipose tissue does not influence LPL action (11), lipolysis (13), or insulin signaling (9, 10) through endocrine mechanisms (6, 26). Arterio-venous difference studies cannot exclude the possibility that TNFα acts as an autocrine or paracrine mediator of insulin resistance (9, 10). Like IL-6, TNFα also has a rapid turnover in plasma (27), and one would expect to see an arterio-venous difference across a tissue actively releasing significant amounts of the cytokine. Systemic levels of TNFα correlate with insulin resistance in subjects with cancer and chronic sepsis (28). TNFα antibodies reduce insulin resistance in rodents, but in a recent study, an infusion of anti-TNFα antibodies to subjects with noninsulin-dependent diabetes mellitus did not change the insulin sensitivity of either glucose or fat metabolism (28).

Adipose tissue heterogeneity is well recognized (4, 29) and allows the possibility that other adipose tissue beds may secrete TNFα or that various disease states may induce such release. Our results do not exclude the possibility that different adipose tissue depots release different combinations of cytokines; thus, sc tissue may preferentially release leptin (4) and IL-6, whereas visceral may mainly release TNFα.

Our results show that IL-6 is released by adipose tissue. Adipose tissue release has been shown *in vivo* for only a few proteins, such as LPL (30), adipsin (31), and leptin (2). IL-6 is thus only the second protein "hormone" reported to be released by human sc adipose tissue.

The release of IL-6 into the systemic circulation and the fact that this release is greater in obese subjects support a possible novel role for IL-6 as a systemic regulator of body weight (an adipostat) and a regulator of lipid metabolism. Our results for IL-6 show similarities with findings for leptin (2, 32), with a clear net release of both molecules that increases with adiposity. To act as an adipostat, a molecule should be released by adipose tissue and be capable of bringing about metabolic changes so as to restore energy balance, either via the hypothalamus or by effects on other tissues. Evidence suggests that IL-6 satisfies these criteria. Firstly, IL-6 is expressed by 3T3 L1 cells, pericardial fat pads, and mammary adipose tissue (6, 8, 33). We now show IL-6 release by adipose tissue, although the cell type of origin cannot be determined by tissue arterio-venous balance studies. Secondly, IL-6 reduces LPL activity both *in vitro* and *in vivo* (11, 12), which may down-regulate adipose tissue triglyceride deposition and promote futile cycling. Thirdly, IL-6 stimulates thermogenesis and satiety via the synthesis of PGs and corticotropin-releasing factor (34), perhaps contributing to the control of obesity. IL-6 receptors are present in the hypothalamus (35), which also supports the proposal that this cytokine has direct central actions. Fourthly, IL-6 modulates the action of aromatase, a key regulatory enzyme for estrogen metabolism (8); estrogens have long been recognized as influencing satiety and adipose tissue distribution. Finally, there are several potential mechanisms for the interaction between leptin and cytokine signals. TNFα modulates the synthesis of both leptin (36, 37) and IL-6 (6, 8). The leptin receptor shares homology with the gp130 signal-transducing component of the IL-6 receptor (38). This might allow IL-6 to modulate the actions of leptin, a molecule that is produced by adipocytes (39), binds to hypothalamic receptors, and regulates energy balance by causing changes in food intake, physical activity, and thermogenesis (40).

We conclude that adipose tissue releases large amounts of IL-6 *in vivo*. IL-6 appears able to mediate several weight-regulating processes. IL-6 adipose tissue production and systemic concentrations increase with adiposity. Adipose tissue production of IL-6 is greater in the evening than around noon regardless of whether subjects eat a high carbohydrate meal [in contrast to leptin (32)]. It is attractive to speculate that IL-6 and leptin could act synergistically to maintain adipose tissue energy equilibrium. Thus, IL-6, previously considered a proinflammatory cytokine with the ability to induce the full acute phase response, may also have a housekeeping role in lipid metabolism.

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