Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue

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Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue. Am J Physiol Endocrinol Metab 286: E8–E13, 2004. First published September 16, 2003; 10.1152/ajpendo.00269.2003.—IL-8 is released from human adipose tissue. Circulating IL-8 is increased in obese compared with lean subjects and is associated with measures of insulin resistance, development of atherosclerosis, and cardiovascular disease. We studied (1) the production and release of IL-8 in vitro from paired samples of subcutaneous (SAT) and visceral (VAT) adipose tissue and (2) the production of IL-8 from whole adipose tissue, isolated adipocytes, and nonfat cells of adipose tissue. IL-8 release from VAT was fourfold higher than from SAT (P < 0.05), and IL-8 mRNA was twofold higher in VAT compared with SAT (P < 0.01). Dexamethasone (50 nM) attenuated IL-8 production by 50% (P < 0.05), and IL-1β (2 μg/l) increased IL-8 production up to 15-fold (P < 0.001). IL-8 release from whole SAT explants correlated with body mass index (BMI; r = 0.78; P < 0.001), as did IL-8 release from nonfat cells (r = 0.79; P < 0.001). However, no correlation was found between IL-8 release from the fraction of isolated adipocytes and BMI (r = 0.01).

In conclusion, we demonstrated an increased release of IL-8 from VAT compared with SAT. Furthermore, our data suggest that the observed elevation in circulating levels of IL-8 in obese subjects is due primarily to the release of IL-8 from nonfat cells from adipose tissue. The high levels of IL-8 release from human adipose tissue and accumulation of this tissue in obese subjects may account for some of the increase in circulating IL-8 observed in obesity.

interleukin-8; interleukin-6; human; obesity; atherosclerosis

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EXCESSIVE AMOUNTS OF ADIPOSE TISSUE are associated with the development of type 2 diabetes, premature atherosclerosis, and cardiovascular disease (22). The possible link between adiposity and the enhanced risk of developing health complications has not been fully elucidated. Human adipose tissue produces and releases a variety of substances, including several cytokines, e.g., adiponectin (19), leptin (20), tumor necrosis factor-α (15), interleukin-6 (IL-6) (12), and IL-8 (6), and it has been suggested that these adipose tissue-derived proteins (adipokines) may play a role in some of the obesity-related health complications (1).

It is increasingly apparent that accumulation of visceral adipose tissue (VAT), rather than excess body fat per se, is important for the adverse metabolic consequences associated with obesity (8). In VAT, catecholamines stimulate lipolysis more markedly than in subcutaneous adipose tissue (SAT), and the VAT depot has been demonstrated to be relatively resistant to the antilipolytic effects of insulin (25). These findings suggest that abdominal obesity has more deleterious health consequences than peripheral obesity and is especially associated with attenuated insulin sensitivity and development of type 2 diabetes and cardiovascular disease.

IL-8, a CXC chemokine, has been shown to be produced and released from human isolated adipocytes and whole adipose tissue cultures in a regulated manner (6). Besides its association with a number of different inflammatory processes, IL-8 has also been implicated in the pathogenesis of atherosclerosis (13, 21) and coronary heart disease (26). Plasma levels of IL-8 have been found to be significantly increased in patients with both type 1 and type 2 diabetes compared with healthy subjects (9, 29). Recently, Straczkowski et al. (27) reported that plasma levels of IL-8 in abdominally obese subjects were higher than in lean subjects. We (7) have previously reported that circulating IL-8 correlates with measures of adiposity and insulin sensitivity, suggesting an involvement of IL-8 in some of the obesity-related health complications.

The present studies were designed to determine whether there were differences between production and release of IL-8 in VAT compared with SAT. We also investigated whether IL-8 released by human adipose tissue explants comes predominantly from adipocytes or nonfat cells present in the adipose tissue. To compare our data with previous investigations of differential release of cytokines in the SAT and VAT depots, IL-6 release was investigated in parallel to IL-8.

MATERIALS AND METHODS

Subjects

For the study of depot-specific differences in IL-8 production (study 1), VAT and SAT were obtained from 10 normal-weight women [mean body mass index (BMI): 24 kg/m²; mean age: 45 yr] undergoing surgery due to gynecological diseases and from five morbidly obese women (mean BMI: 47 kg/m²; mean age: 38 yr) undergoing laparoscopic adjustable silicone gastric band surgery for the treatment of morbid obesity.

For the study of IL-8 production in different cell types from the adipose tissue we investigated (study 2), SAT was obtained from eight obese women (mean BMI: 32 kg/m²; mean age: 41 yr) under-
going open abdominal surgery (abdominoplasty) and nine morbidly obese subjects (8 women and 1 man) with a mean BMI of 45 kg/m² and a mean age of 41 yr undergoing laparoscopic gastric bypass with Rouxenyo gastroenterostomy surgery. All subjects were fasted over-
night before surgery but had not been on any type of dietary restriction before surgery. No cancer patients were included in the study, and none of the subjects had any metabolic disorders or received any medication known to influence adipose tissue metabolism. However, one patient in study 2 had nonmedically treated type 2 diabetes. The studies had been approved by the local ethics committee (Aarhus County no. 1999/4510) or the University of Tennessee Institutional Review Board, and all subjects gave informed consent.

Adipose Tissue Incubations

Study 1. Paired samples of whole adipose tissue from the SAT and VAT depots were minced into fragments of ~10 mg and preincubated for 24 h for stabilization. Then the tissue was incubated for 72 h with either the proinflammatory cytokine IL-1β (2 μg/l) or the anti-
inflammatory corticosteroid dexamethasone (50 nM), as previously described (6). The incubation time and concentrations of IL-1β and dexamethasone were chosen as these concentrations are suggested to elicit maximal biological effects, as previously found (6, 12).

Study 2. When the cell type from the adipose tissue involved in the production of IL-8 was determined, three fractions were compared: whole adipose tissue explants, isolated adipocytes, and nonfat cells from the adipose tissue. Separation of the adipose tissue was performed in a stepwise manner as previously described (10). In brief, adipose tissue was minced into small pieces (~<20–30 mg) and incubated for 30 min and centrifuged for 30 s at 400 g to remove erythrocytes and other pieces of tissue containing insufficient adipocytes to float. From this preparation, adipose tissue explants (100 mg/ml) were incubated for 48 h, and IL-8 was measured in the incubation medium. In parallel, 1 g of whole adipose tissue was digested with collagenase (2 mg) in 3 ml of buffer for 2 h. Isolated adipocytes and nonfat cells were separated from undigested tissue by filtration through a 200-μm nylon mesh cloth at the end of the digestion period. Nonfat cells were separated from the adipocytes by centrifugation for 1 min at 400 g. Isolated adipocytes and nonfat cells from the adipose tissue (in separate tubes) were resuspended in fresh buffer and centrifuged again at 400 g. Both fractions were then resuspended in 5 ml of medium and, like adipose tissue explants, incubated for 48 h.

Preadipocyte Cultures

Isolation and culture of adipose tissue-derived stromal cells (prea-
dipocytes) were performed as previously described (14, 23). In brief, adipose tissue was rinsed and digested using 1 g/ml collagenase in PBS (pH 7.4) containing 20 mg/ml BSA for 50 min under intermittent shaking. Mature adipocytes were removed while the preadipocyte fraction was incubated with an erythrocyte-lysing buffer [155 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA-Na (pH 7.3)] for 10 min. After centrifugation, the pellet was resuspended in growth medium, filtered, transferred to a sterile tissue culture flask, and maintained in an incubator at 37°C, 5% CO₂. Preadipocytes were inoculated in DMEM-Ham’s F-12 medium (1:1 vol/vol) supplemented with 10% fetal calf serum, 15 mM NaHCO₃, 15 mM HEPES, and antibiotics. After cell attachment for 16–20 h, the medium was removed by aspiration, and the cells were repeatedly washed (this is defined as day 0). Then, cells were cultured in growth medium (serum-free DMEM-
Ham’s F-12 medium supplemented with 33 μM biotin, 17 μM pantothenate, and 15 mM HEPES, and to induce adipocyte differentiation, 100 nM cortisol, 100 nM insulin, 200 μM triiodothyronine and, for the first 3 days, 0.2 mM isobutylmethylxanthine). The medium was changed every 2–3 days.

Determination of Protein Levels

IL-8 and IL-6 protein levels in the culture medium were measured using a human enzyme-linked immunosorbent assay (ELISA). The amount of protein in the medium from paired SAT and VAT incuba-
tion was normalized for cellular DNA content as previously described (17).

Determination of mRNA Levels

RNA was isolated using TRIzol reagent. For the real-time reverse transcriptase PCR (RT-PCR), cDNA was made with random hexamer primers as described (GeneAmp PCR kit; PerkinElmer Cetus, Nor-
walk, CT). PCR-mastermix containing the specific primers Hot Star Taq DNA polymerase and SYBR-green was then added. IL-8 sense primer TTGGCAGCCTTCTGATTTC and antisense primer AACT-
TCTCCACAACCCCTC-G spanned a product of 291 base pairs. IL-6 sense primer AAATGCCAGCCTGCTGACGAG and antisense primer ACAAACAATCT-GAGGTGCCCATGCTAC spanned a product of 150 base pairs. Adiponectin sense primer CATGACCAG-
GAAACCAGCAGT and antisense primer TGAATGCAGCGG-TAT spanned a product of 301 base pairs. As previously described (5), real-time quantification was performed with a SYBR-green real-
time RT-PCR assay with an iCycler PCR machine from Bio-Rad (Bio-Rad Laboratories, Hercules, CA). Relative gene expression of β-actin to IL-8, IL-6, or adiponectin was calculated as described in the User Bulletin No. 2, 1997 from PerkinElmer. Samples were amplified in duplicate.

Materials

For the DNA normalization, the fluorescent dye 3-methyl-2-[(1-[3-
(trimethylamino)propyl]-4(1H)-pyridiniumide][methyl]benzoxazo-
lium diiodide was purchased from Wako Bioproducts (Wako Chem-
icals, Neuss, Germany); materials for real-time RT-PCR were ob-
tained as described in Ref. 5; materials for adipose tissue incubations and ELISA measurements were obtained as described in Refs. 6 and 10. All other materials were obtained from Sigma Chemical (St. Louis, MO).

Statistical Analysis

The values are presented as means ± SE. The SPSS statistical packet (SPSS v. 8.0; SPSS, Chicago, IL) was used for the calculations. The data were found to be normally distributed, and a paired t-test was used for comparison between adipokine protein levels and mRNA levels in the VAT and SAT samples obtained from the same individ-

Fig. 1. IL-8 and IL-6 protein levels in subcutaneous (SAT) and visceral adipose tissue (VAT). Comparison of IL-8 and IL-6 (inset) protein release in adipose tissue fragments obtained from the SAT or VAT depot from lean [mean body mass index (BMI): 24 kg/m², n = 6] and obese subjects (mean BMI: 47 kg/m², n = 5). Adipose tissue was incubated for 72 h. Data represent means ± SE. *P < 0.05 (SAT vs. VAT) and #P < 0.05 (lean vs. obese).
Data represent means ± SE; n = 5 in each experiment. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control.

**RESULTS**

**Regional Difference in IL-8 in Whole Adipose Tissue Explants**

In lean subjects (BMI: 24 kg/m²), IL-8 release to the medium was increased fourfold from VAT compared with SAT (0.46 ± 0.07 vs. 1.74 ± 0.39 μg protein/μg DNA, P < 0.05; Fig. 1). A similar result was obtained in morbidly obese subjects (BMI: 47 kg/m²), where IL-8 release was increased threefold from VAT compared with SAT (0.87 ± 0.22 vs. 2.19 ± 0.58 μg protein/μg DNA, P < 0.05; Fig. 1). When obese and lean subjects were compared, IL-8 production in SAT was higher in obese subjects but to a nonsignificant degree (0.46 ± 0.07 in lean vs. 0.87 ± 0.22 μg protein/μg DNA in obese subjects; P = 0.09; Fig. 1).

The mRNA levels of IL-8 in incubated adipose tissue explants were twofold higher in VAT compared with SAT from lean (4.86 ± 0.85 vs. 8.86 ± 1.52 arbitrary units, P < 0.01) and obese subjects (4.71 ± 1.21 vs. 8.32 ± 1.69 arbitrary units, P < 0.05), respectively (data not shown). In incubated adipose tissue, no difference in mRNA levels was observed between lean and obese subjects (data not shown). However, in fresh, nonincubated (immediately frozen) adipose tissue obtained from the subcutaneous depot, mRNA levels were threefold higher in adipose tissue from obese compared with lean subjects (0.16 ± 0.04 vs. 0.06 ± 6 × 10⁻³ arbitrary units; n = 6, P < 0.05).

**Regional Differences in IL-6**

When assessed in parallel with IL-8, IL-6 protein levels displayed rather similar results. As for IL-8, IL-6 release was twofold higher in VAT compared with SAT explants from lean (P < 0.05) and obese subjects (P < 0.05), respectively (Fig. 1, inset). Compared with lean subjects, IL-6 release from obese subjects was up to fourfold higher in the SAT (0.03 ± 0.004 vs. 0.14 ± 0.05 μg protein/μg DNA, P < 0.05) and VAT depots (0.09 ± 0.02 vs. 0.28 ± 0.08 μg protein/μg DNA, P < 0.05), respectively (Fig. 1, inset).

**Regulation of IL-8 Protein and mRNA Levels**

In both SAT and VAT incubated for 72 h, IL-1β (2 μg/l) increased IL-8 release and IL-8 mRNA levels up to 10-fold (P < 0.001) and 15-fold (P < 0.001), respectively (Fig. 2). IL-1β displayed no depot difference in its stimulatory effects (Fig. 1). No difference was observed between the stimulatory effects of IL-1β in adipose tissue from lean compared with obese subjects (data not shown). Dexamethasone (50 nM) decreased release and mRNA levels of IL-8 by up to 70% (P < 0.05) and 65% (P < 0.01), respectively, in the two depots (Fig. 2). No difference was observed between the inhibitory effects of dexamethasone in the two depots (Fig. 2).

**IL-8 Release from Whole Adipose Tissue Explants, Isolated Adipocytes, and Nonfat Cells**

IL-8 release from whole adipose tissue explants, isolated adipocytes, and nonfat cells obtained from SAT from subjects with a mean BMI of 32 compared with subjects with a mean BMI of 45.
BMI of 45 is shown in Fig. 3. The release of IL-8 by isolated adipocytes was 3.5% of that by whole adipose tissue at a BMI of 45 and 13% at a BMI of 32, respectively (Fig. 3). The release of IL-8 by isolated adipocytes as a percentage of that by nonfat cells was 6% at a BMI of 45 and 21% at a BMI of 32 (Fig. 3).

**Relationship Between IL-8 Release and BMI**

IL-8 release by whole adipose tissue explants of SAT was significantly correlated to BMI ($r_p = 0.78$ and $P < 0.001$). However, the correlation was due primarily to IL-8 release from the fraction of nonfat cells of adipose tissue ($r_p = 0.79$ and $P < 0.001$) and not from isolated mature adipocytes, since no relationship ($r_p = 0.01$ and $P = 0.97$) between IL-8 release by isolated adipocytes and BMI was found (Fig. 4).

**Adipokine mRNA Levels in Preadipocytes During Differentiation**

After collagenase treatment of whole adipose tissue, the isolated stromal-vascular cell fraction contains different cell types, such as preadipocytes and endothelial cells as well as some immune cells (e.g., monocytes and macrophages). Therefore, we investigated IL-8 and IL-6 mRNA levels in preadipocytes during differentiation (up to day 18). Because adiponectin is known to be produced exclusively by the adipocytes (19), mRNA levels of this protein were determined in parallel. By a logarithmic scale, Fig. 5 illustrates that IL-8 and IL-6 mRNA levels were higher right after preparation of preadipocyte cultures and that this finding was reversed during preadipocyte differentiation (Fig. 5). The mRNA levels of IL-8 were found to be $>10$-fold higher than mRNA levels of IL-6 during preadipocyte differentiation. As opposed to the two cytokines, adiponectin mRNA levels increased during preadipocyte differentiation from nearly zero to a level comparable to that in mature, isolated adipocytes [in arbitrary units (AU), from day 0: $5.8 \times 10^{-3} \pm 1.6 \times 10^{-3}$ AU; to day 18: $2.4 \pm 1.2$ AU vs. isolated adipocytes: $4.1 \pm 1.2$ AU]. The mRNA levels of IL-8 and IL-6 in mature, isolated adipocytes were 250-fold ($4 \times 10^{-3} \pm 1 \times 10^{-3}$ vs. $0.99 \pm 0.41$ arbitrary units) and 150-fold ($8 \times 10^{-4} \pm 5 \times 10^{-4}$ vs. $0.12 \pm 0.04$ arbitrary units) higher than in preadipocytes at day 18, respectively (Fig. 5).

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![Figure 4](http://www.ajpendo.org) (Correlation between IL-8 release and BMI. Explants, mature isolated adipocytes, or nonfat cells (combined data for undigested adipose tissue matrix and isolated SV cells) obtained from SAT of 17 subjects were incubated for 48 h. Each point is from a single individual.)

![Figure 5](http://www.ajpendo.org) (IL-8 and IL-6 mRNA levels during preadipocyte differentiation. mRNA levels of IL-8 and IL-6 were compared in preadipocyte cultures during differentiation, as described in MATERIALS AND METHODS. For comparison, mRNA levels in mature, isolated adipocytes were included. Data are presented on a log scale and represent means ± SE; n = 6.)
DISCUSSION

The present study demonstrates for the first time that IL-8 protein release and IL-8 mRNA levels are higher in human VAT compared with SAT. In accord with previous findings (6), both mRNA levels and release of IL-8 were found to be regulated by the proinflammatory cytokine IL-1β (stimulated) and the corticosteroid dexamethasone (attenuated), however, without any regional differences. IL-8 release from isolated adipocytes was ∼3.5 and 6% of that released by whole adipose tissue and the fraction of nonfat cells, respectively. In addition, we found that this high release of IL-8 from the fraction of nonfat cells did not seem to be explained by IL-8 production by preadipocytes. Interestingly, we found that IL-8 release from incubated explants of SAT cultures was correlated to BMI but that this correlation was due primarily to IL-8 release from nonfat cells in the adipose tissue rather than from isolated mature adipocytes. The correlation between adipose tissue-derived IL-8 and BMI was found in both obese (BMI 32 kg/m²) and morbidly obese (BMI 47 kg/m²) subjects, suggesting that a similar positive association may be found in lean subjects. In parallel with these novel findings on IL-8, we observed a higher IL-6 release from the VAT depot compared with the SAT depot, which is in accord with previous findings by Fried et al. (12).

The chemokine IL-8 may be involved in the pathogenesis of atherosclerosis and cardiovascular disease, since in vivo studies have found higher plasma levels of IL-8 in patients with unstable coronary heart disease (2, 26). In vitro studies have demonstrated that several inflammatory cells involved in the pathogenesis of atherosclerosis possess IL-8 receptors (28), and IL-8 has been suggested to induce chemotaxis, which may be involved in the formation of atherosclerotic plaque (16, 21). Large amounts of IL-8 are released by the cells of the immune system (e.g., monocytes and macrophages) (3), and our results show that IL-8 is released from both isolated adipocytes and nonfat cells from the adipose tissue but more so from the latter fraction. In the present study, IL-8 mRNA and IL-8 release were significantly higher in VAT compared with SAT. These findings are in line with the hypothesis that adipose tissue-derived IL-8 may be involved in health complications associated with excess accumulation of intra-abdominal fat (e.g., atherosclerosis and cardiovascular disease). Recent studies have also found that IL-8 levels in the circulation correlated with measures of adiposity (e.g., BMI and fat mass), indicating a possible relationship between this adipose tissue-derived chemokine and the obese state (9, 27). In support of this, we found in the present study that adipose tissue-derived IL-8 was correlated with BMI. However, during preadipocyte differentiation, IL-8 mRNA levels were found to be very low compared with the mRNA levels in mature, isolated adipocytes. These data suggest that inflammatory cells and/or endothelial cells within the adipose tissue matrix are responsible for the correlation between IL-8 and BMI.

Straczkowski et al. (27) found that plasma levels of IL-8 were higher in obese subjects compared with lean subjects. This finding could be related to the fact that obesity per se is known to be associated with a chronic state of low-grade inflammation exemplified by the increment in circulating levels of markers of inflammation such as C-reactive protein, fibrinogen, and IL-6 (4, 11). Increased fat accumulation, especially in the visceral depot, has been demonstrated to be highly associated with a decrement in insulin sensitivity (18) as well as an increment in development of cardiovascular disease (8). Increasing evidence suggests that adipokines may participate in the pathogenesis of obesity-related health complications (24). However, whether IL-8 released from the adipose tissue may have endocrine effects on other tissues awaits investigations on whether there is a net release of IL-8 from the adipose tissue to the circulation. Furthermore, it will be of importance to identify specific cell types in the adipose tissue that may be involved in the increased IL-8 production and release. Finally, it is unknown whether collagenase treatment of adipose tissue to obtain isolated adipocytes may affect adipose tissue-derived cytokines in an unphysiological manner, for example, by depletion of these cytokines in the isolated adipocytes.

In conclusion, we found IL-8 production and release to be higher in VAT compared with SAT and IL-8 release to be significantly correlated with BMI. Even though the incremental release of IL-8 seems to be due primarily to release of IL-8 from nonfat cells of the adipose tissue, the high levels of IL-8 released from whole adipose tissue and the enhanced accumulation of this tissue in obese subjects suggest that adipose tissue-derived IL-8 may account for some of the increase in circulating IL-8 levels observed in obesity.

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