Adipokines oversecreted by omental adipose tissue in human obesity

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Submitted 26 February 2007; accepted in final form 13 June 2007

Maury E, Ehala-Aleksjev K, Guiot Y, Detry R, Vandenhooft A, Brichard SM. Adipokines oversecreted by omental adipose tissue in human obesity. Am J Physiol Endocrinol Metab 293: E656–E665, 2007.—Central-omentumal obesity plays a causative role in the pathogenesis of the metabolic syndrome. Adipokines are involved in the pathogenesis of this syndrome. However, adipokines secreted by omental adipose tissue (OAT) are still poorly characterized in human obesity. Therefore, we searched for novel adipokines abnormally secreted by OAT in obesity and examined their relationships with some features of metabolic syndrome and the respective contribution of adipocytes vs. stromal-vascular cells. OAT from obese and nonobese men was fractionated into adipocytes and SV cells, which were then cultured. Medium was screened by medium-scale protein arrays and ELISAs. Adipokine mRNA levels were measured by real-time RT-qPCR. We detected 16 cytokines secreted by each cellular fraction of lean and obese subjects. Of the 16 cytokines, six adipokines were newly identified as secretory products of OAT, which were dysregulated in obesity: three chemokines (growth-related oncogen factor, RANTES, macrophage inflammatory protein-1β), one interleukin (IL-7), one tissue inhibitor of metalloproteinases (TIMP-1), and one growth factor (thrombopoietin). Their secretion and expression were enhanced in obesity, with a relatively similar contribution of the two fractions. The higher proportion of macrophages and endothelial cells in obesity may contribute to this enhanced production as well as changes in intrinsic properties of hypertrophied adipocytes. Accordingly, mRNA concentrations of most of these adipokines increased during adipocyte differentiation. Eventually, expression of the investigated adipokines did correlate with several features of the metabolic syndrome. In conclusion, six adipokines were newly identified as oversecreted by OAT in obesity. These adipokines may link obesity to its cardiovascular or metabolic comorbidities.

OBESE PLAY A CAUSATIVE ROLE IN THE PATHOGENESIS OF THE METABOLIC SYNDROME THAT CLUSTERS SEVERAL ABNORMALITIES, INCLUDING INSULIN RESISTANCE, TYPE 2 DIABETES, DYSLIPIDEMIA, HYPERTENSION, AND CARDIOVASCULAR DISEASE (30).

Adipokines may be involved in this adverse health profile. Thus, adipose tissue secretes a variety of bioactive peptides that play important roles in insulin action, energy homeostasis, inflammation, and cell growth (35, 36). Some of these factors exert endocrine actions on distant organs, whereas others exert autocrine or paracrine effects on adipose tissue. Adipokines may locally regulate fat mass by modulating adipocyte size/number or angiogenesis (14). Increased fat mass leads to dysregulation of adipocyte functions, including oversecretion of deleterious adipokines and hyposecretion of beneficial ones, such as adiponectin (20, 36). This altered adipokine production may worsen the progression of obesity and promote the development of related circulatory and metabolic diseases (3, 36). Identifying new adipokines, the secretion of which is altered in obese subjects, is a crucial step for our understanding of the pathogenesis of this disorder and its comorbidities. So far, adipose tissue secretome analysis (2) has not been performed in obese individuals compared with lean ones.

Preferential accumulation of omental fat rather than subcutaneous fat appears to be a stronger risk factor for the adverse health profile linked to obesity (36). Yet adipokine secretion by omental fat is still poorly studied in humans. On the other hand, except for leptin and adiponectin, it is generally assumed that the bulk of adipokine secretion is due to nonfat cells issuing from the stromal-vascular (SV) fraction of adipose tissue and mostly to macrophages (8, 12). However, SV cells also contain endothelial cells and preadipocytes, which are known to release adipokines (32, 37). Moreover, the possibility of an active role of the other fraction containing mature adipocytes in obesity-related inflammation has recently been raised (34) but remains to be established. Thus, the respective contribution of the different cell types of adipose tissue to adipokine secretion needs to be reevaluated.

The aim of the present study was to identify novel adipokines that potentially contribute to the pathogenesis of obesity and its comorbidities. To this end, we carried out cytokine protein profiling of adipokines secreted by omental adipose tissue (OAT) from lean and obese subjects and evaluated the respective contribution of adipocytes and SV cells. Data were confirmed by ELISA. We next examined whether the dysregulation of adipokine secretion in obesity resulted from impaired pretranslational mechanisms and was correlated with features of the metabolic syndrome. Last, we investigated whether expression and secretion of these adipokines changed during in vitro adipocyte differentiation.

SUBJECTS AND METHODS

Subjects. OAT was obtained from 10 obese and 10 nonobese men undergoing abdominal surgery after an overnight fast. Obesity was defined as a body mass index (BMI) of ≥30 kg/m². All cases were elective procedures to correct benign conditions (colon diverticulosis, evagination, and overweight status treated by vertical banded gastroplasty) or malignant disease (carcinoma of colon). Cancer cases had no evidence of disseminated disease, and the operation was curative. Localized colorectal cancer did not modify mRNA levels of the investigated adipokines (not shown). No patient exhibited overt bowel inflammatory disease, and none had undergone any significant weight change over the last 3 mo. Patients receiving hormones (e.g., insulin, glucocorticoids) or nonsteroidal anti-inflammatory drugs or taking any medications known to influence fat mass or metabolism...
(e.g., thiazolidinediones, systemic and nonspecific modulators of adrenergic receptors) were excluded. Two subjects in each group were receiving antihypertensive treatment (amlodipine, a calcium channel blocker).

For each patient, plasma was obtained by refrigerated centrifugation (4°C) before surgery and stored at −20°C. Measurements of glucose, insulin, C-reactive protein, and lipids were performed by routine procedures as described (11). The homeostasis model assessment-estimated insulin resistance index (HOMA-IR) was calculated as [(fasting glucose (mmol/l) × fasting insulin (mU/l))/22.5]. Plasma adiponectin and leptin levels were determined in cell samples (33).

For the last experiment, omental preadipocytes [obtained from SV cells treated with an erythrocyte lysing buffer (24)] were grown to confluence and then differentiated in vitro (19). Briefly, from day 0 (first day of differentiation) up to day 12, cells were fed with a chemically defined serum-free medium consisting of DMEM-F-12 (1:1) supplemented with 15 mM HEPES, 15 mM NaHCO₃, 3 mM glutamine, 1% (wt/vol) BSA without collagenase, for 30 min; as yet, it is required. Adipocytes (1 ml of packed cells) and SV cells (500 μg of tissue) were also cultured for 24 h, as described for cellular fractions. After culturing, cells or explants were harvested and frozen at −80°C. DNA concentrations were measured in cell samples (33).

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media were collected at the end of day 0 and day 12, and adipocytes were harvested at the indicated time points. The preadipocytes used in this experiment were obtained from obese subjects.

Cytokine arrays and ELISAs on adipocyte- or SV cell-conditioned media. Screening for cytokines secreted by cultured adipocytes or SV cells was performed by hybridizing medium with antibody-coated membranes according to the protocol supplied by the manufacturer [RayBio Human Cytokine Antibody Array C Series 1000, a kit combining membranes of Array VI and VII and allowing the simultaneous detection of 120 cytokines (cat. no. H0108010, for details see http://www.raybiotech.com/map/C_Series_1000.pdf; RayBiotech, Tebu-Bio, Boechout, Belgium)]. Briefly, 1.2 ml of medium was incubated with arrayed antibody supports for 1 h and 30 min at room temperature; membranes were then washed and incubated with the mix of biotin-conjugated antibodies for another 1 h and 30 min at room temperature. After washing, HRP-conjugated streptavidin was added to the membranes for 1 h at room temperature. The signal was developed with detection buffers, and membranes were exposed to autoradiographic films. Signal optical densities were within a linear range and quantified by scanning densitometry (Image Master TotalLab; Amersham Biosciences). Nonconditioned media (containing 10% FCS) were used as negative controls; the weak signals they generated after hybridization with the array membranes were considered as nonspecific and subtracted from those obtained in the presence of conditioned media. However, we cannot exclude that this background produced by FCS (possibly due to interspecies cross-reactivity of some antibodies) may have masked some low amounts of adipokines released by cultured cells (such as TNF-α). To compare the secretion between lean and obese individuals and between the two cellular fractions, signal densities were normalized to internal positive controls present on each membrane and then expressed as optical density per milligram DNA in each cellular fraction. Moreover, experiments on lean and obese subjects were always carried out simultaneously.

Some cytokines identified by protein arrays were further quantified by specific ELISAs: human growth-related oncogen factor-α (GRO-α), interleukin-7 (IL-7), macrophage inflammatory protein-1β (MIP-1β), regulated upon activation normal T cells expressed and secreted (RANTES), tissue inhibitor of metalloproteinase-1 (TIMP-1), and thrombopoietin (TPO) [Quantikine or Quantikine HS (IL-7), R&D Systems, Minneapolis, MN]. Adiponectin and leptin were also measured in culture medium by RIA, as described above. For all these adipokines, there was a close correlation between absolute levels measured by ELISA/RIA and cytokine arrays (Pearson’s correlation coefficients \( r \) ranging between 0.75 and 0.92, \( P < 0.001 \)).

RNA extraction and real-time quantitative polymerase chain reaction. RNA from cells or tissue was extracted by using TriPure Isolation Reagent (Roche Diagnostics, Vilvoorde, Belgium). Two

Fig. 1. Macrophage infiltration and increased endothelial cell number in human omental adipose tissue (OAT) of obese subjects. The morphology of OAT of 1 representative lean subject [body mass index (BMI) 24 kg/m²; A–C] and 1 representative obese subject (BMI 43 kg/m²; D–F) is shown. Immunostaining for CD68 staining was used to detect macrophages (magnification \( \times 25 \); A, B, D, and E). The labeling around adipocytes (A and D) and within fibrous connective tissue septa (B and E) is more intense in obese than in lean subjects. Immunostaining of CD31 was used to detect endothelial cells (\( \times 25 \) magnification; C and F). This labeling observed in the intima of small vessels is again stronger in obese than in lean subjects.
micrograms of total RNA was reverse transcribed as described (10). Real-time quantitative polymerase chain reaction (RT-qPCR) primers were designed (Primer Express Software; Applied Biosystems) for CD31, CD68, EMR1, GRO [common to the 3 known isoforms (α, β, and γ)], IL-7, MIP-1β, RANTES, TIMP-1, TATA box-binding protein (TBP), and TPO (Table 1). Ten nanograms total RNA equivalents was amplified using iCycler iQ Real-Time PCR (Bio-Rad Laboratories, Brussels, Belgium), as described (10). Briefly, the threshold cycles ($C_T$) were measured in duplicate. $\Delta C_T$ values were calculated in every sample for each gene of interest as followed: $C_T$ gene of interest $- C_T$ reporter gene, with TBP as the reporter gene. Relative changes in the expression level of one specific gene ($\Delta \Delta C_T$) were calculated as $\Delta C_T$ of the test group minus $\Delta C_T$ of the reference group and then presented as $2^{-\Delta \Delta C_T}$. The PCR efficiency equaled 1 for each gene studied.

Presentation of the results and statistical analysis. Results are the mean ± SE for the indicated numbers of patients (i.e., nos. of independent cultures). Comparisons between two different groups (lean/obese or adipocytes/SV cells) were carried out using two-tailed unpaired Student’s t-test or Mann-Whitney test when appropriate. For adipocyte differentiation, comparisons between the different days were made by repeated ANOVA followed by the Newman-Keuls test. Correlation analyses were performed using Pearson’s test to examine the simple relationships of adipokine gene expression to selected variables. Multiple regression analyses were used to identify the independent determinants of adipokine gene expression and the percentage of the variance in the expression of a given adipokine that they explained ($R^2$). Predictor variables were selected from an initial set (including BMI, plasma leptin, adiponectin and triglycerides, HOMA-IR, and diastolic blood pressure) by the forward selection procedure (21). Statistical analyses were performed using GraphPad InStat version 3.00 (GraphPad Software, San Diego, CA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Characteristics of the subjects. As shown in Table 2, the obese men exhibited several clinical or laboratory features of the metabolic syndrome. Compared with age-matched controls, they were characterized by higher blood pressure, hyperinsulinemia and insulin resistance, abnormal lipid profile, increased plasma leptin, and decreased plasma adiponectin concentrations.

Cellular composition of adipose tissue fractions in obesity. OAT was obtained from these subjects and fractionated into adipocytes and SV cells. Each fraction was relatively pure and almost devoid of any cellular contamination with the other fraction. Thus, mRNA abundance of the macrophage markers CD68 and EMR1 and of the endothelium marker CD31 was low in the adipocyte fraction (<7.5% of the amount in the SV fraction (see SUBJECTS AND METHODS)). There were no differences in mRNA levels of CD68, EMR1, and CD31 between the

Fig. 2. Cytokine antibody array membranes were used to detect adipokines secreted by each cellular fraction of omental adipose tissue. Raybiotech membranes were probed with 24 h conditioned medium. The relative cytokine level was determined by chemiluminescence detection and autoradiography. Among the 120 cytokines tested, 16 were detected into the medium. These cytokines were classified a posteriori into “adipokines already characterized in human obesity” (black) and “adipokines newly identified as oversecreted by OAT in human obesity” (red). This figure illustrates a representative array of adipocyte-conditioned medium from 1 obese subject. Note that IL-7 was detected only in stromal-vascular (SV) cell-conditioned medium. ApN, adiponectin; GRO, growth-related oncogen; bFGF, basic fibroblastic growth factor; MIP, macrophage inflammatory protein; TIMP-1, tissue inhibitor of metalloproteinase-1; TPO, thrombopoietin; RANTES, regulated upon activation normal T cells expressed and secreted; MCP-1, monocyte chemotactic protein-1.

Fig. 3. Secretion of adipokines already characterized in human obesity by adipocytes and SV cells isolated from OAT. Adipocytes and SV cells were prepared simultaneously from a fat sample of a given patient and then cultured independently for 24 h. Culture media were next incubated with cytokine array membranes (like those shown in Fig. 2). Adipokines levels were quantified by scanning densitometry of autoradiographic signals, normalized to internal positive controls, and expressed as optical density (OD) units/mg DNA in each cellular fraction. Values are means ± SE for 5 lean and 5 obese subjects. *P < 0.05 vs. lean; +P < 0.05 vs. adipocytes.
adipocyte fractions of lean and obese subjects (n = 10/group; not shown). Likewise, SV cells were almost devoid of adipocyte-specific markers (see SUBJECTS AND METHODS). However, CD68 and EMR1 mRNA abundance was higher in SV cells of obese than in those of lean subjects (2.6 ± 0.2 vs. 1.0 ± 0.1, n = 10/group, P < 0.001, and 2.5 ± 0.5 vs. 1.0 ± 0.1, P < 0.05, respectively). Similarly, CD31 mRNA of SV cells was fivefold more expressed in obese than in lean subjects (4.7 ± 1.0 vs. 1.1 ± 0.3, n = 10, P < 0.05). These data suggest either that there were more transcripts per SV cell in the obese subjects or that there were more macrophages and endothelial cells that express those transcripts.

To address this question, we performed immunohistochemistry and morphometric analysis on OAT. CD68 immunostaining of adipose tissue sections showed that infiltrating macrophages were more abundant around adipocytes and within fibrous connective tissue septa in obese than in lean subjects (Fig. 1, A, B, D, and E). Morphometry confirmed that the proportion of macrophages (Vv in % adipose tissue) was higher (P < 0.05) in obese (0.82 ± 0.16) than in lean subjects (0.16 ± 0.09). At first glance, light microscopic observation showed more capillaries and small size vessels in obese than in lean patients (Fig. 1, C and F). Immunostaining of CD31, which was detected only in the intima of small vessels and corresponded to endothelial cells, was also stronger in obese subjects. Accordingly, the proportion of endothelial cells (Vv in % adipose tissue) was also increased in obese compared with lean subjects (2.44 ± 0.86 vs. 0.22 ± 0.01%, P < 0.05). Taken together, these data indicate that the cellular composition of the SV fraction, but not of the adipocyte fraction, was altered in obesity.

Identification of adipokines secreted by each subcellular fraction. To identify adipokines that are secreted by each subcellular fraction of OAT from lean and obese subjects, we cultured independently for 24 h adipocytes and SV cells, and then screened conditioned media by cytokine protein arrays.

![Fig. 4. Secretion of adipokines newly identified as oversecreted by OAT in human obesity by adipocytes (A) and SV cells (B) isolated from OAT of lean and obese subjects. Adipocytes and SV cells were cultured independently for 24 h. Adipokines were first identified by cytokine protein arrays (like that shown in Fig. 2). Afterward, adipokine concentrations in culture medium were quantified by specific ELISAs and expressed in pg/μg DNA in each cellular fraction. Values are means ± SE for 5 lean and 5 obese subjects. *P < 0.05 vs. lean; +P < 0.05 vs. adipocytes. GF, growth factor.](http://ajpendo.physiology.org/).
Among the 120 cytokines tested, 16 were secreted into the medium (Fig. 2). These cytokines were divided into two categories: “already characterized adipokines” and “OAT adipokines newly identified as dysregulated in human obesity”. Adipokines of these two categories are indicated in black and red, respectively (Fig. 2).

Adipokines already characterized in human obesity. The first category includes adipokines whose expression or secretion by adipose tissue has already been compared between lean and obese subjects and between the different cellular fractions. These studies have been carried out mostly on subcutaneous adipose tissue (7, 27, 28, 34).

On the basis of our own screening, we classified in the first category several interleukins (IL-1β, IL-1ra, IL-6, IL-8, and IL-10), two chemokines [monocyte chemoattractant protein-1 (MCP-1) and MIP-1α], the basic fibroblastic growth factor (bFGF), and two adipocyte-specific hormones, adiponectin and leptin (Figs. 2 and 3). We confirmed the increased expression/secretion of several adipokines, already described in the subcutaneous depot of obese subjects (7, 23, 27, 28). Thus, IL-1β, IL-1ra, IL-6, IL-8, IL-10, MCP-1, and leptin were increased in at least one subcellular fraction of the omental adipose depot from obese subjects (Fig. 3). Conversely, adiponectin was downregulated in obesity as expected (Fig. 3A) (43).

As our data were normalized per milligram DNA, we compared on a per cell basis adipokine secretion by adipocytes and SV cells (Fig. 3). We confirmed that adiponectin and leptin were specifically secreted by isolated adipocytes. We further showed that secretion of IL-6, IL-8, and MCP-1 was higher in SV cells than in adipocytes, in agreement with other results obtained in subcutaneous adipose tissue (7). Conversely, the secretion of the anti-inflammatory cytokine IL-10 was higher in adipocytes, in line with another report (27).

On the whole, our data were rather similar to those previously described, which indirectly validates the results we obtained by cytokine arrays. We further validated adiponectin and leptin secretion by RIA. Adiponectin secreted by adipocytes of obese subjects was lower than that of lean subjects.
Adipokines newly identified as oversecreted by OAT in human obesity. Thanks to cytokine protein arrays, we also identified GRO and its α-isofrom RANTES, MIP-1β, TIMP-1, TPO, and IL-7 as novel secretory products of human OAT that are overproduced in obesity (Fig. 2 and data not shown). These data were confirmed by using specific ELISAs for each adipokine (Fig. 4). The increased secretion in obesity occurred in both adipocytes and SV cells. This rise amounted up to eight-fold for some adipokines. Except for IL-7 that was undetectable in adipocyte-conditioned medium, there were few differences in secretion between the two cellular fractions (Fig. 4).

The highest secretion values were observed for TIMP-1 (~300 pg/μg DNA), whose concentrations were in a range similar to those of adiponectin (~400 pg/μg DNA). Likewise, with the exception of IL-7, the secretion ranges of the other “new” adipokines (~0.2–50 pg/μg DNA) were similar to (or even higher than) those of leptin.

In agreement with secretion levels, mRNA levels of these newly identified adipokines were higher in obese than in lean subjects in both adipocytes and SV cells (Fig. 5). mRNA abundance of a given adipokine was approximately similar in adipocytes and SV cells apart from IL-7, whose expression was lower in adipocytes than in SV cells for each group of subjects (P < 0.001 or less; data not shown).

To ascertain that collagenase digestion had no effect on adipokine expression, we compared mRNA levels of these newly identified adipokines in tissue fragments (explants) cultured for 24 h and in adipocytes and SV cells isolated from the same subjects and cultured for the same time period. We did not find any significant differences in mRNA abundance between cultured tissue and the respective cultured cellular fractions for each adipokine (data not shown). Next, we ruled out that culturing per se could influence our data. To this end, we measured adipokine expression in fresh OAT of lean and obese subjects (Table 3). We found qualitatively similar results to those previously described: an increase of adipokine gene expression in obesity (Table 3).

Hence, OAT releases six adipokines newly identified as dysregulated in human obesity. The two subcellular fractions of OAT contribute approximately equally to their secretion.

### Table 3. Adipokine gene expression in fresh omental adipose tissue from lean and obese subjects

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokine C-X-C</td>
<td>1.00±0.43</td>
<td>3.93±1.97*</td>
</tr>
<tr>
<td>Chemokine C-C RANTES</td>
<td>1.00±0.28</td>
<td>2.51±0.43†</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>1.00±0.35</td>
<td>3.70±0.30†</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>1.00±0.32</td>
<td>3.83±0.73†</td>
</tr>
<tr>
<td>Growth factor</td>
<td>1.00±0.14</td>
<td>2.50±1.11*</td>
</tr>
<tr>
<td>IL-7</td>
<td>1.00±0.17</td>
<td>1.70±0.31*</td>
</tr>
</tbody>
</table>

Results are shown as means ± SE for 10 lean and 10 obese subjects. Adipokine mRNA levels were quantified by RT-qPCR, normalized to the levels of TBP, and presented as relative expression compared with values of lean subjects. *P < 0.05; †P < 0.01 vs. lean.

(410 ± 77 vs. 724 ± 185 pg/μg DNA, P < 0.05), whereas a reverse pattern was observed for leptin (4.0 ± 1.0 vs. 0.2 ± 1.1 pg/μg DNA) for obese vs. lean, P < 0.05). Again, both adipokines were undetectable in SV cell-conditioned media by this method.
in vitro adipocyte differentiation fits with its mRNA abundance, which was much lower in isolated mature adipocytes than in SV cells (see above). These observations, together with the fact that IL-7 secretion by SV cells was already very low, may perhaps explain why IL-7 was undetectable in adipocyte-conditioned medium from isolated mature cells. In the present experiment, for each adipokine, 24-h secretion levels showed a qualitatively similar evolution to mRNAs when day 0 and day 12 were compared (Fig. 6, insets).

**DISCUSSION**

Six adipokines were newly identified as oversecreted by OAT in obese subjects. Thus, our data strengthen the concept that white adipose tissue is a source of proinflammatory molecules and obesity a low-grade inflammatory disease (35). The secretion levels of these newly identified adipokines, except for IL-7, were in similar ranges to the already characterized ones. This finding suggests that the secretion of these new adipokines may be physiologically relevant, an idea further supported by pieces of evidence for their involvement in the pathogenesis of the metabolic syndrome (see below). We further showed that their enhanced secretion in obesity involved pretranslational mechanisms. In agreement with this finding, mRNA levels of RANTES in subcutaneous adipose tissue of 13 subjects were very recently found to be positively correlated with the BMI (46).

Usually, nonfat cells or SV cells are considered to be the main source of proinflammatory adipokine release by adipose tissue (7, 8, 15). Most of these studies were performed on the subcutaneous adipose depot. Here, we showed that, except for IL-7, adipocytes and SV cells of OAT contribute roughly similarly to the secretion/expression of the five other adipokines. In consideration of its higher secretion by SV cells, IL-7 behaves more like some other ILs [such as IL-6 and IL-8 (7, 15, and our own array data)]. The significant contribution of adipocytes to the other adipokines is supported by the increased production of these factors during in vitro differentiation. Microarray gene profiling of adipocytes or SV cells from obese vs. nonobese Pima Indians also pointed out an active role of mature adipocytes in obesity-related inflammation (34, 37).

Overproduction of adipokines by adipocytes or SV cells in obesity may reflect either altered properties of the cells or altered cellular composition within a given fraction. Because of the...
relative purity of the adipocyte fraction, we assume that the overproduction of adipokines by this fraction was due mainly to altered properties of obese adipocytes. Changes in intrinsic properties of hypertrophied adipocytes are in agreement with the increase in constitutive activity of the proinflammatory transcription factor NF-κB and the upregulation of adipokine expression during fat cell differentiation (Ref. 4 and our own results). Moreover, adipocyte size has recently been shown to be an important determinant of dysregulated adipokine expression and secretion, with the hypertrophic adipocytes shifting their immune balance toward the production of proinflammatory molecules (26, 42). In agreement with these last two reports, the expression of our six adipokines in the adipocyte fraction was positively correlated with the BMI of the subjects enrolled in this study. Alternatively, adipocyte inflammation may be caused by adipokines secreted by nonfat cells, but this phenomenon is exacerbated when adipocytes are hypertrophied (44).

Overproduction of adipokines by the SV fraction may rather reflect changes in cellular composition, as both macrophage and endothelial cell numbers were increased in fat tissue of obese subjects and both cell types are fair sources of proinflammatory cytokines (32, 37). Human obesity has already been associated with increased accumulation of macrophages in adipose tissue (8, 12). We further showed that more capillaries and small size vessels, together with a higher proportion of endothelial cells in OAT, accompanied obesity. Only one study performed in obese mice reported a higher density of microvessels in adipose tissue (6). To our knowledge, microvessel density has not yet been examined in human obesity. This novel finding sheds light on the relationships between inflammation, angiogenesis, and adiposity. Inflammation may lead to angiogenesis, and chemokines may be viewed as regulatory links between these two processes (39). Moreover, adipose tissue growth depends on its ability to recruit new capillaries (40). Eventually, some adipokines, like TIMP-1, a predictor of human adiposity (31), may increase the rate of adipocyte differentiation in vivo and in vitro, thereby promoting adipose tissue expansion (1).

Exacerbated release of adipokines may also have repercussions on distant target tissues and play a role in the development of obesity-linked disorders such as cardiovascular disease and type 2 diabetes. First, cardiovascular disease: this suggestion is supported by the inverse relationships between the expression of most investigated adipokines and plasma levels of the antiatherogenic factor adiponectin and by the positive relationships between some of these adipokines and well-known cardiovascular risk factors (blood pressure, plasma lipids). Among the newly identified chemokines, GRO (especially GRO-α), MIP-1β, and RANTES have already been described as involved in atherosclerosis mainly by modulating the inflammatory recruitment of monocytes/macrophages (5, 38). High systemic TPO levels could also contribute to cardiovascular disease by increasing both platelet counts and size (41). Eventually, IL-7 and TIMP-1 have been implicated in unstable angina and carotid plaque formation, respectively (9, 47). Second, type 2 diabetes: again, the negative correlations between adipokine expression and circulating adiponectin, which is also an important insulin sensitizer, support this idea as well as the positive correlations between some adipokines and the degree of insulin resistance. In agreement with our data, GRO, MIP-1β, and TIMP-1 have been implicated in skeletal muscle insulin resistance in vitro (13). Likewise, RANTES, whose systemic concentration was higher in individuals with impaired glucose tolerance or type 2 diabetes, may be implicated in type 2 diabetes development (25).

In conclusion, we have identified six adipokines overexpressed by OAT in human obesity. These adipokines may connect obesity to cardiovascular and metabolic disorders.

ACKNOWLEDGMENTS

We thank Dr. M.-L. Delporte and L. Noël for helpful assistance.

GRANTS

This work was supported by grants from the Foundation of Scientific and Medical Research (3.4580.05) and Grant ARC 05/10-328 from the General Division of Scientific Research. E. Maury has a fellowship from the Fonds pour la formation à la Recherche dans l’Industrie et dans l’Agriculture (Belgium).

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