Adipose Tissue Secretion and Expression of Adipocyte-Produced and Stromavascular Fraction-Produced Adipokines Vary during Multiple Phases of Weight-Reducing Dietary Intervention in Obese Women

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Context: Obesity is associated with altered plasma levels of adipokines involved in the development of insulin resistance and obesity-related metabolic disturbances.

Objective: The aim was to investigate diet-induced changes in adipokine production in sc abdominal adipose tissue (SAT) during a 6-month, multiphase, weight-reducing dietary intervention.

Design, Setting, Participants, and Interventions: Forty-eight obese women followed a dietary intervention consisting of a very low-calorie diet (VLCD) (1 month), followed by a weight-stabilization (WS) period, which consisted of a low-calorie diet (2 months), and a weight-maintenance diet (3 months).

Main Outcome Measures: Before and at the end of the VLCD and WS, samples of plasma and SAT were obtained. In a subgroup of 26 women, secretion of adipokines was determined in SAT explants, and in a subgroup of 22 women, SAT mRNA expression was measured.

Results: Body weight decreased and insulin sensitivity increased during the intervention. Plasma levels, SAT mRNA expression, and secretion rates of adipocyte-produced adipokines (leptin, serum amyloid A, and haptoglobin) decreased during the VLCD and increased during the WS period. Adipokines produced mainly from stroma-vascular cells (IL-6, IL-8, IL-10, IL-1Ra, TNFα, plasminogen activator inhibitor-1, and monocyte chemoattractant protein-1) increased or remained unchanged during VLCD and decreased to levels equal to or lower than prediet levels during the WS period. The diet-induced changes in homeostasis model assessment of insulin resistance correlated with changes in leptin plasma levels during VLCD, WS, and the entire dietary intervention period.

Conclusions: Diet-induced regulation of adipokine production in SAT differs according to their cellular origin (adipocytes vs. stroma-vascular cells) and diet phase (VLCD vs. WS). Insulin-sensitivity changes were associated only with those of plasma leptin. (J Clin Endocrinol Metab 97: E1176–E1181, 2012)
Obesity is associated with the development of metabolic and cardiovascular diseases. A number of bioactive molecules produced by adipose tissue (AT), such as cytokines, chemokines, and acute phase proteins, known as adipokines, have been suggested as a possible link between obesity and insulin resistance (1).

Different cell types in AT produce different adipokines. Adipokines produced mainly by adipocytes include leptin, adiponectin, haptoglobin, and serum amyloid A (SAA) (2, 3), whereas adipokines produced mainly by cells of the stroma-vascular fraction (SVF) include TNFα, IL-6, IL-10, IL-8, monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor 1 (PAI-1) (1, 4).

It has been hypothesized that the beneficial effects of lifestyle interventions in the treatment of obesity might be mediated by modification of the secretory profile and/or mRNA expression of adipokines in AT and reduction of systemic inflammation. However, previous studies have yielded inconsistent results (5).

In the present study, we investigated the mRNA expression and plasma levels of adipokines produced by adipocytes and SVF of AT at several time points during a 6-month, multiphase, weight-reducing dietary intervention (DI). In addition, we investigated the evolution of adipokine secretion from biopsy-derived explants of subcutaneous adipose tissue (SAT). The relationship between diet-induced changes of adipokines characteristics and improved insulin sensitivity was also explored.

**Subjects and Methods**

**Subjects**

Forty-eight obese, premenopausal, women (aged 35 ± 7 yr, range 22–51 yr) were recruited at the Third Faculty of Medicine, Charles University, Prague, Czech Republic. Exclusion criteria were a change in body weight greater than 3 kg within the 3 months preceding the study, diabetes, drug-treated obesity, pregnancy, participation in other trials, and alcohol or drug abuse. Volunteers gave written informed consents, and the study was approved by the Ethics Committee of the Third Faculty of Medicine.

**Dietary intervention and clinical investigation**

The DI lasted 6 months. During the first phase (1 month), participants followed a very-low caloric diet (VLCD) of 800 kcal/d (liquid formula diet; Redita, Promil, Czech Republic). The subsequent weight-stabilization (WS) period consisted of a low-calorie diet (2 months; 600 kcal/d less than the estimated energy requirement) and a weight-maintenance diet (3 months).

Patient examinations including anthropometry, blood sampling, and needle biopsy of SAT [performed under local anesthesia in the abdominal region as previously reported (6)] were done in a fasting state before the start of the DI, after VLCD and again after WS. For the AT investigation, the participants were alternately divided into two groups. In group 1 (n = 22), AT was frozen at −80 C and stored until mRNA expression analysis. In group 2 (n = 26), AT was used immediately to assess secretion of adipokines in the biopsy samples (explants). Insulin resistance was assessed using homeostasis model assessment of insulin resistance (HOMA-IR) [(fasting blood glucose [millimoles per liter] × fasting insulin [millimicrons per liter])/22.5].

**In vitro adipose tissue secretion**

Secretion from SAT explants was investigated as described previously (7, 8). Briefly, 400 mg of AT explants was incubated in 4 ml of Krebs/Ringer phosphate buffer (pH 7.4) supplemented with 40 g/liter of BSA and 1 g/liter of glucose for 4 h at 37 C. Then 200 μl aliquots of medium were stored at −80 C until analysis. The explants were used for total DNA content measurement (MasterPure DNA purification kit; Epicenter Biotechnologies, Madison, WI).

**Media and plasma analysis**

Nonesterified fatty acid (NEFA) levels were determined using an enzymatic procedure (Wako, Richmond, VA). Plasma levels of glucose, insulin, and C-reactive protein (CRP) were determined using standard methods. Plasma and conditioned media levels of adipokines were determined using multiplex human cytokine and adipocyte Milliplex panels (Millipore-Merck, Bedford, MA). IL-1Ra was measured with ELISA (R&D Systems, Minneapolis, MN).

**RNA analysis**

Total RNA was isolated as previously described (6). mRNA expression of 13 genes (ADIPOQ, LEP, IL1B, IL6, IL8, TNF, CCL2, SERPIN1, HGF, IL-1RN, IL10, SAA1, haptoglobin) were assessed using quantitative PCR with a sequence detection system (ABI PRISM 7900; Applied Biosystems, Foster City, CA) and custom TaqMan low-density arrays with TaqMan gene expression assays (Applied Biosystems) (Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). Ribosomal 18S RNA was used as an endogenous control. Results are expressed as 2^(-ΔΔCt) values.

**Statistical analysis**

Data were log transformed and analyzed using SPSS version 13.0 software (SPSS Inc., Chicago, IL). ANOVA with repetitive measures was used for analysis of diet-induced evolution of clinical data, mRNA, media, and plasma levels. Correlations of diet-induced relative changes between adipokines and clinical characteristics during VLCD, WS, and whole DI were performed using the Pearson’s parametric test.

**Results**

**Clinical characteristics**

Clinical data of all participants before DI (baseline) and during DI are shown in Table 1. When compared with baseline, body weight decreased by 7.7% during VLCD and by 11.1% after WS. Body mass index (BMI), fat mass (FM), and waist circumference showed a similar pattern of change. HOMA-IR decreased during VLCD, and the reduction was still present at the end of the WS phase.
Adipokines in AT during Dietary Intervention

**TABLE 1. Clinical parameters of 48 obese women before and at the end of different phases of multiphase dietary intervention**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before diet</th>
<th>After VLCD</th>
<th>After WS</th>
</tr>
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<tbody>
<tr>
<td>Weight (kg)</td>
<td>95.9 ± 12.5</td>
<td>88.5 ± 12.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.3 ± 12.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>34.8 ± 3.8</td>
<td>32.1 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.9 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>40.2 ± 8.8</td>
<td>34.7 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.4 ± 8.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>56.3 ± 5.8</td>
<td>54.1 ± 6.2</td>
<td>53.7 ± 6.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>103.7 ± 9.5</td>
<td>97.1 ± 9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.5 ± 9.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>5.24 ± 0.52</td>
<td>4.97 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.02 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (mU/liter)</td>
<td>12.4 ± 6.7</td>
<td>7.2 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.7 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycerol (μmol/liter)</td>
<td>170 ± 81</td>
<td>128 ± 48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128 ± 64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEFA (μmol/liter)</td>
<td>740 ± 277</td>
<td>924 ± 347&lt;sup&gt;b&lt;/sup&gt;</td>
<td>634 ± 228&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.5 ± 0.7</td>
<td>1.1 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.0 ± 0.8</td>
<td>4.0 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL (mmol/liter)</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>hs-CRP (mg/liter)</td>
<td>5.5 ± 0.9</td>
<td>2.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.9 ± 1.7</td>
<td>1.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
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Values are means ± SD. HDL, High-density lipoprotein; hs-CRP, high-sensitivity CRP.

<sup>a</sup> P < 0.01 compared with baseline values (paired t test).
<sup>b</sup> P < 0.001 compared with baseline values (paired t test).
<sup>c</sup> P < 0.05 compared with baseline values (paired t test).

**mRNA expression of adipokines**

**Adipocyte-produced adipokines (Fig. 1A and Supplemental Table 2)**

mRNA expression of SAA and haptoglobin decreased after VLCD and increased thereafter, ultimately returning to baseline. Leptin mRNA levels decreased during VLCD and remained lower than prediet values at the end of WS. Adiponectin mRNA expression was unchanged during the DI.

**SVF-produced adipokines (Fig. 1B and Supplemental Table 2)**

mRNA levels of IL-1Ra and hepatocyte growth factor (HGF) increased during VLCD and returned to baseline by the end of WS. IL-10 expression was up-regulated during VLCD but decreased below baseline values after WS. IL-6, PAI-1, and MCP-1 mRNA levels were unchanged during VLCD but decreased below baseline levels during WS. mRNA levels of IL-1β, IL-8, and TNFα mRNA expression were unchanged from baseline throughout the DI.

**Secretion rate of adipokines**

**Adipocyte-produced adipokines (Fig. 1A and Supplemental Table 2)**

Secretion rate of leptin, SAA, and haptoglobin decreased during VLCD and remained lower than prediet values at the end of WS. Secretion rates of adiponectin were unchanged during DI.

**SVF-produced adipokines (Fig. 1B and Supplemental Table 2)**

IL-1Ra, IL-10, IL-8, and TNFα secretion rates increased during VLCD with a subsequent decrease to prediet values by the end of WS. MCP-1 secretion rate was unchanged during VLCD but was lower than baseline by the end of WS. HGF and PAI-1 secretion rates did not change during the DI. The IL-1β concentrations in the media were under the limit of detection.

**Plasma levels of adipokines**

**Adipocyte-produced adipokines (Fig. 1A and Supplemental Table 2)**

Plasma levels of leptin, SAA, and haptoglobin decreased after VLCD and remained significantly lower at the end of WS phase compared with prediet conditions. Plasma adiponectin levels were unchanged during DI.

**SVF-produced adipokines (Fig. 1B and Supplemental Table 2)**

Plasma levels of IL-1β, IL-6, IL-8, IL-10, and HGF were unchanged during the DI. IL-1Ra and TNFα concentrations increased after VLCD but decreased under baseline by the end of WS. MCP-1 concentrations were unchanged after VLCD but decreased during WS to values that were below baseline values. PAI-1 levels declined during VLCD and remained that way after WS.

**Associations**

**Anthropometric indices**

The changes in BMI and FM during the entire DI correlated positively with the diet-induced changes in plasma leptin, CRP, IL-6, PAI-1, and SAA and with the secretion rate (r = 0.563, P < 0.05) and mRNA levels (r = 0.563, P < 0.05) of leptin (Supplemental Table 3).

**Homeostasis model assessment of insulin resistance**

The diet-induced changes in HOMA-IR correlated positively with changes in plasma leptin during VLCD and WS as well as during the entire DI, whereas the correlations with changes in the leptin secretion rate and plasma SAA were found during DI only (r = 0.563, P < 0.05 and r = 0.563, P < 0.05, respectively). Importantly, these correlations persisted after adjustment to the diet-induced FM changes.

When stratifying groups of subjects according to HOMA-IR decreases during the entire DI, the subgroup with smaller HOMA-IR decreases presented lower relative diet-induced decreases in plasma leptin and SAA (Sup-
Discussion

Our results show that a 6-month, multiphase DI modifies SAT mRNA expression, secretion rate and plasma levels of adipokines. The pattern of the diet-induced changes differed with respect to the phase of DI and to the cellular origin of the respective adipokine. During initial VLCD, mRNA expression, secretion rate, and plasma levels of adipocyte-produced adipokines decreased, except for adiponectin. During the subsequent WS phase, an increase toward prediet levels was observed. For adipokines produced predominantly from SVF, mRNA expression, and secretion rate increased or remained unchanged during VLCD but decreased during the WS phase.

The decrease in the mRNA and plasma levels of adipocyte-produced adipokines (leptin, SAA, haptoglobin) is in agreement with other studies using low-calorie diets (7, 9–11). The secretion rate of leptin was found to decrease during the DI in a single study (7).

Variable diet-induced responses in mRNA expression of SVF-produced cytokines have been observed in SAT (5, 12–14). Plasma levels of IL-6 and TNFα have been investigated in a number of studies, which either reported no change (5, 9, 15) or a decrease (16–18) after diet-induced weight loss. Similarly, decreases or no changes in IL-6, IL-8, and TNFα secretion rates from SAT have been found (7, 13).

In a recent study, we demonstrated that the regulation of mRNA expression of many genes is dependent on the severity and duration of calorie restriction and that the...
genes expressed predominantly in adipocytes show a clearly distinct pattern of regulation from those expressed predominantly in macrophages (6). mRNA expression responses in the present study are in agreement with the latter study. Importantly, in the present study, we also focused on secretory characteristics of adipokines and observed that secretion rates paralleled changes in mRNA levels.

The up-regulation of proinflammatory cytokines in SAT during VLCD could be associated with increased lipolysis as released fatty acids activate proinflammatory pathways through Toll-like receptor 4 signaling (19). This concept is supported by a study of Kosteli et al. (20), in which an association between macrophage recruitment in AT and circulating concentrations of NEFA or AT lipolysis during caloric restriction was found in mice. Similarly, we found a correlation between DI-induced changes in plasma NEFA with plasma MCP-1 and leptin changes (data not shown). These results suggest the overall stimulation of the immune response after VLCD as a putative stress reaction to a marked calorie restriction. It also supports the potential interplay between immune cells and adipocytes that may associate fatty acid metabolism and inflammation.

In this study we did not find an association between an improvement in insulin sensitivity and decreases in proinflammatory adipokines during the respective dietary phases. Leptin was the only adipokine whose diet-induced changes in plasma positively correlated with the changes in HOMA-IR during VLCD and WS as well as during the entire DI, even when adjusted for fat mass.

In conclusion, we demonstrate that the regulation of mRNA expression and secretion of adipokines from SAT clearly differs between periods of severe calorie restriction (VLCD) and periods of moderate energy restrictions or energy balance (WS), and importantly, it differs with respect to the main cellular origin of the adipokines (adipocytes vs. SVF fraction). We observed that diet-induced changes of insulin sensitivity were related to the changes in plasma levels of leptin.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

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