Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors

Tongjian You,1 Rongze Yang,3 Mary F. Lyles,1 Dawei Gong,3 and Barbara J. Nicklas1,2

1Section on Gerontology and Geriatric Medicine and 2Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, North Carolina; and 3Division of Diabetes, Metabolism and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland

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You, Tongjian, Rongze Yang, Mary F. Lyles, Dawei Gong, and Barbara J. Nicklas. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. Am J Physiol Endocrinol Metab 288: E741–E747, 2005. First published November 23, 2004; doi:10.1152/ajpendo.00419.2004.—Adipose tissue is a major source of inflammatory and thrombotic cytokines. This study investigated the relationship of abdominal subcutaneous adipose tissue cytokine gene expression to body composition, fat distribution, and metabolic risk during obesity. We determined body composition, abdominal fat distribution, plasma lipids, and abdominal subcutaneous fat gene expression of leptin, TNF-α, IL-6, PAI-1, and adiponectin in 20 obese, middle-aged women (BMI, 32.7 ± 0.8 kg/m²; age, 57 ± 1 yr). A subset of these women without diabetes (n = 15) also underwent an OGTT. In all women, visceral fat volume was negatively related to leptin (r = −0.46, P < 0.05) and to positively related to adiponectin (r = 0.38, P = 0.09) gene expression. Among the nondiabetic women, fasting insulin (r = 0.69, P < 0.01), 2-h insulin (r = 0.56, P < 0.05), and HOMA index (r = 0.59, P < 0.05) correlated positively with TNF-α gene expression; fasting insulin (r = 0.54, P < 0.05) was positively related to, and 2-h insulin (r = 0.49, P = 0.06) tended to be positively related to, IL-6 gene expression; and glucose area (r = −0.56, P < 0.05) was negatively related to, and insulin area (r = −0.49, P = 0.06) tended to be negatively related to, adiponectin gene expression. Also, adiponectin gene expression was significantly lower in women with vs. without the metabolic syndrome (adiponectin-β-actin ratio, 2.26 ± 0.46 vs. 3.31 ± 0.33, P < 0.05). We conclude that abdominal subcutaneous adipose tissue expression of inflammatory cytokines is a potential mechanism linking obesity with its metabolic comorbidi-

CARDIOVASCULAR DISEASE is the leading cause of death in the United States (2). Several cardiovascular risk factors, including abdominal obesity, dyslipidemia, hypertension, and insulin resistance, tend to cluster together in individuals (44). The National Cholesterol Education Program (NCEP)’s Adult Treatment Panel III (ATPIII) (9) recently defined the metabolic syndrome as presenting three or more of these five components: abdominal obesity, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), high blood pressure, and elevated fasting glucose.

The mechanisms underlying the metabolic syndrome are not well understood. Current evidence supports that abdominal, instead of total, obesity is a main determinant of the metabolic syndrome (5, 41). In this regard, older obese women may be at an increased risk for the metabolic syndrome, since total and central adiposity increases after the menopause transition (43). In addition, a proinflammatory state, as indicated by elevated circulating interleukin (IL)-6, tumor necrosis factor (TNF)-α, and the acute-phase reactant C-reactive protein, and a prothrombotic state, as indicated by increased plasminogen activator inhibitor type 1 (PAI-1), are usually present with the syndrome (9). Circulating levels of these cytokines have been linked with the individual risk components (6, 25) as well as the clustering of these risk factors (4, 45).

Adipose tissue is an important organ to produce various cytokines that are involved in inflammatory and thrombotic pathways, such as leptin, IL-6, TNF-α, adiponectin, and PAI-1 (17). Adipose gene expression of proinflammatory (TNF-α) and prothrombotic (PAI-1) cytokines is elevated with obesity (1, 13, 16), whereas the expression levels of adiponectin are lower in obese individuals (24). Therefore, these “adipokines” may be important factors linking central obesity to the other risk components of the metabolic syndrome.

Previous work has shown that glucose tolerance and insulin sensitivity are reduced in individuals with elevated circulating levels of inflammatory and thrombotic biomarkers (21, 29, 31, 38, 39). In addition, a few studies also show that glucose tolerance and insulin sensitivity are negatively related to adipose tissue expression of inflammatory and thrombotic genes (12, 13, 18, 19, 25). However, adipose tissue gene expression of various inflammatory (leptin, TNF-α, IL-6, adiponectin) and thrombotic (PAI-1) cytokines has not been investigated in a single study design with regard to the relationship to glucose tolerance, hyperinsulinemia, and other metabolic risk factors, especially in obese postmenopausal women. Moreover, abdominal adipose gene expression of these key cytokines may distinguish obese individuals with and without the metabolic syndrome.

Thus the purpose of this study was to investigate 1) whether body composition, abdominal fat distribution, lipids, glucose tolerance, and hyperinsulinemia are related to adipose tissue gene expression of leptin, TNF-α, IL-6, PAI-1, and adiponectin in obese women and 2) whether abdominal adipose tissue gene expression of these proteins differs between women with and without the metabolic syndrome.

SUBJECTS AND METHODS

Subjects. All 20 women enrolled in the study met the following inclusion/exclusion criteria: 1) overweight or obese (body mass index

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(BMI = 25–40 kg/m², and waist circumference >88 cm), 2) older (age = 50–70 yr, and ≥1 yr without menses), 3) nonsmoking, 4) not on hormone replacement therapy, 5) sedentary (<15 min of exercise, 2×/wk) in the past 6 mo, and 6) weight stable (<5% weight change) for at least 6 mo before enrollment. All women provided informed consent to participate in the study according to the guidelines of the Wake Forest University Institutional Review Board for Human Research. Initial evaluations included a medical history review, physical examination, fasting blood profile (lipoprotein lipids and glucose), and 12-lead resting electrocardiogram.

Participants with evidence of untreated hypertension (blood pressure >160/90 mmHg), hypertriglyceridemia (triglycerides >400 mg/dl), insulin-dependent diabetes, active cancer, liver, renal or hematologic disease, or other medical disorders were excluded. Among the eight medicated women, five women were under antihypertensive therapy [Attace (ramipril), Hctz (hydrochlorothiazide), or Norvasc (amlodipine besylate)], two women were on cholesterol-lowering medication [Zocor (simvastatin)], and four women were on antidiabetic medications [Glucophage (metformin) or Glucotol (glipizide)]. Of these women, one was under all three therapies and another one was on both antihypertensive and antidiabetic medications.

Study design. After fulfillment of recruitment inclusion/exclusion criteria, all women were told to maintain their current dietary habits and sedentary lifestyle before and during research testing. The first day of testing consisted of the anthropometric measurements and dual-energy X-ray absorptiometry (DEXA), followed by measurement of maximal aerobic capacity (V₀₂ max). To eliminate potential effects of physical activity on metabolic variables and adipose tissue gene expression, blood draw and fat biopsies were performed at least 5 days after the V₀₂ max test. On the second testing day, a fasting blood sample was drawn for the repeated determination of lipoprotein lipids in all women, and an oral glucose tolerance test (OGTT) was performed in the nondiabetic subjects (screening fasting glucose <50–70 mg/dl and not on antidiabetic therapy). On the third testing day, the abdominal adipose tissue biopsy and the CT scan were performed.

Body composition and fat distribution. Height and weight were measured to calculate BMI (kg/m²), waist (minimal circumference), and waist-to-hip ratio. Total body fat was determined by DEXA (Hologic Delphi QDR, Bedford, MA). Visceral and subcutaneous adipose tissue volumes around the abdomen were measured by a four-slice multidetector computed tomography system (GE Medical Systems, Milwaukee, WI).

Maximal aerobic capacity. V₀₂ max was measured on a motor-driven treadmill (Medical Graphics, Minneapolis, MN) during a progressive exercise test to voluntary exhaustion, as previously described (32). A valid V₀₂ max was obtained when at least two of these three criteria were met: 1) plateau in oxygen consumption (<200 ml/min change) with increasing work rate, 2) maximal heart rate >90% of age-predicted maximal heart rate (220 beats/min – age), and 3) respiratory exchange ratio of at least 1.10.

Lipoprotein lipids. Blood samples were collected in EDTA-treated vacutainers via venipuncture in the early morning (0700–0900) after a 12-h fast. The blood samples were centrifuged at 4°C for 20 min, and plasma was separated and stored at −70°C until analysis. Plasma triglycerides (TG), total cholesterol (TC), HDL-C, and low-density lipoprotein cholesterol (LDL-C) were measured by standardized hospital laboratory procedures.

OGTT. After an overnight fast, a 20-gauge polyethylene catheter was placed in an antecubital vein for blood sampling. Blood samples were drawn at different time points before (−10 and 0 min) and after (30, 60, 90, and 120 min) a 75-g glucose ingestion for the determination of plasma glucose and insulin. Plasma glucose was measured with the glucose hexokinase method (Bayer Diagnostics, Tarrytown, NY). Plasma insulin was determined by a chemiluminescent immunoassay, using an IMMULITE analyzer (Diagnostics Products, Los Angeles, CA). The estimate of insulin sensitivity by homeostasis model assessment (HOMA) score was calculated with the formula reported by Matthews et al. (28).

Cytokine gene expression, obesity, and metabolic risk factors

Adipose tissue cytokine gene expression. Abdominal subcutaneous adipose tissue was taken by aspiration with a 16-gauge needle under local anesthesia (2% xylocaine) after an overnight fast. The samples were put in warm saline and transported immediately to the laboratory where they were washed twice with saline to eliminate blood and other connective tissue. Immediately after the washing, ∼0.5 g of tissue was snap frozen in liquid nitrogen and then stored at −80°C for later isolation of total RNA for gene expression of cytokines.

Total RNA was isolated from frozen adipose tissue samples with the RNeasy lipid tissue kit (Qiagen, Valencia, CA). This technique integrates phenol-guanidine lysis and silica gel-membrane purification of total RNA. The isolated total RNA was quantified by measurement of absorbency at 260 and 280 nm, and its integrity was verified using agarose gels (1%) stained with ethidium bromide. Total RNA samples were stored at −80°C until measurement of gene expression.

Cytokine mRNA expression was measured by real-time RT-PCR. First, 1 µg of total RNA was used for the reverse transcription reaction to synthesize the first-strand cDNA, using the random hexamer primers and following the instructions of the Advantage RT-PCR Kit (Clontech, Palo Alto, CA). Real-time quantification of cytokine to β-actin mRNA was performed, using ABI Taqman PCR kits (with commercially available assay-on-demand primers for leptin, TNF-α, IL-6, PAI-1, adiponectin, and β-actin) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). Leptin, TNF-α, IL-6, PAI-1, adiponectin, and β-actin mRNA were amplified in different wells and in duplicates, and the increase in fluorescence was measured in real time. Data were obtained as threshold cycle (Ct) values. Relative gene expression was calculated using the formula (1/2)^r*Ct cytokine – Ct β-actin.

Statistical analysis. Statistical analyses were performed using JMP version 4.0 for Windows (SAS Institute, Cary, NC). Adipose tissue cytokine gene expression data were not normally distributed, so the logarithm of each cytokine expression was used for group comparisons. Group differences were determined by Student’s t-test. Spearman’s correlation coefficients were calculated for relationships between cytokine expression levels and individual metabolic variables. All data are presented as means ± SE, and the level of significance was set at P < 0.05 for all analyses.

RESULTS

Sample characteristics. Descriptive characteristics, body composition, abdominal fat distribution, and aerobic fitness in all 20 women are shown in Table 1. Fasting plasma lipoprotein lipid, glucose, insulin, and other glucose tolerance measures are shown in Table 2. All 20 women had plasma lipoprotein lipids and fasting glucose data, since the screening and testing data were combined. Fifteen women had data for fasting insulin and other OGTT variables. The descriptive data for abdominal adipose tissue gene expression levels are shown in Table 3 for all 20 women.

Correlations between gene expression levels. TNF-α gene expression correlated positively with expression of leptin (r = 0.48, P < 0.05), IL-6 (r = 0.81, P < 0.001), and PAI-1 (r = 0.50, P < 0.05). IL-6 expression correlated positively with PAI-1 expression (r = 0.71, P < 0.001). In addition, leptin expression tended to be positively related to adiponectin expression (r = 0.43, P = 0.06). There were no other significant correlations between adipose cytokine gene expression levels.
Table 1. Descriptive characteristics, body composition, and aerobic fitness

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SE or Percentage</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>57±1</td>
<td>50–69</td>
</tr>
<tr>
<td>Years postmenopause</td>
<td>10±2</td>
<td>2–30</td>
</tr>
<tr>
<td>% African-American subjects</td>
<td>35%</td>
<td>NA</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>87±2.2</td>
<td>72.7–105.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>32.7±0.8</td>
<td>26.4–39.9</td>
</tr>
<tr>
<td>Waist, cm*</td>
<td>97.9±1.8</td>
<td>88.6–112.7</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>37.7±1.5</td>
<td>29.1–53.7</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>48.8±1.1</td>
<td>41.7–58.6</td>
</tr>
<tr>
<td>% Body fat</td>
<td>42±2.8</td>
<td>34.5–49.8</td>
</tr>
<tr>
<td>Subcutaneous fat volume, cm³</td>
<td>2.059±0.133</td>
<td>1.074–3.574</td>
</tr>
<tr>
<td>Visceral fat volume, cm³</td>
<td>6.016±0.371</td>
<td>4.099–9.825</td>
</tr>
<tr>
<td>SBP, mmHg*</td>
<td>129±3</td>
<td>98–151</td>
</tr>
<tr>
<td>DBP, mmHg*</td>
<td>75±2</td>
<td>56–90</td>
</tr>
<tr>
<td>VO₂max, ml·min⁻¹·kg⁻¹⁻¹</td>
<td>20.9±0.6</td>
<td>15.6–25.5</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE, percentages, or ranges as shown; n = 20 for all variables. SBP, systolic blood pressure; DBP, diastolic blood pressure; VO₂max, maximal aerobic capacity; NA, not applicable. *Metabolic syndrome components defined by National Cholesterol Education Program (NCEP)’s Adult Treatment Panel III (ATPIII).

Table 2. Plasma metabolic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, mg/dl*</td>
<td>112.6±7.5</td>
<td>54.0–176.0</td>
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<tr>
<td>TC, mg/dl</td>
<td>201.8±6.3</td>
<td>153.0–254.5</td>
</tr>
<tr>
<td>HDL-C, mg/dl*</td>
<td>53.7±2.1</td>
<td>34.0–69.5</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>124.5±6.0</td>
<td>78.0–173.0</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>98.4±4.6</td>
<td>76.8–146.0</td>
</tr>
<tr>
<td>2-h Glucose, mg/dl</td>
<td>127.1±9.6</td>
<td>87.0–205.0</td>
</tr>
<tr>
<td>Glucose area, mg·dl⁻¹·h⁻¹</td>
<td>16.754±1.150</td>
<td>12.390–26.640</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>59.3±6.8</td>
<td>22.2–108.3</td>
</tr>
<tr>
<td>2-h Insulin, pmol/l</td>
<td>462.7±78.4</td>
<td>138.9–1,132</td>
</tr>
<tr>
<td>Insulin area pmol·h⁻¹·l⁻¹</td>
<td>51.249±7.792</td>
<td>18.356–122,135</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.95±0.25</td>
<td>0.73–3.72</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE or ranges as shown; n = 20 for lipids and fasting glucose, and n = 15 for the other variables. TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA, homeostasis model assessment. *Metabolic syndrome components defined by NCEP-ATPIII.

Table 3. Abdominal subcutaneous adipose tissue gene expression

<table>
<thead>
<tr>
<th>Gene mRNA ratio</th>
<th>Mean ± SE</th>
<th>Range</th>
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<tbody>
<tr>
<td>Leptin-β-actin mRNA ratio</td>
<td>0.34±0.03</td>
<td>0.18–0.72</td>
</tr>
<tr>
<td>TNF-α-β-actin mRNA ratio</td>
<td>0.0013±0.0002</td>
<td>0.000003–0.00269</td>
</tr>
<tr>
<td>IL-6-β-actin mRNA ratio</td>
<td>0.0015±0.0003</td>
<td>0.000001–0.00355</td>
</tr>
<tr>
<td>PAI-1-β-actin mRNA ratio</td>
<td>0.0037±0.0007</td>
<td>0.000005–0.0153</td>
</tr>
<tr>
<td>Adiponectin-β-actin mRNA ratio</td>
<td>2.89±0.29</td>
<td>0.34–4.85</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE or ranges as shown; n = 20 for all variables. PAI-1, plasminogen activator inhibitor type 1.

Cytokine gene expression between women with and without metabolic syndrome. The number of metabolic syndrome (MS) components was counted in all 20 women, following the NCEP criteria (9): 1) waist circumference >88 cm, 2) TG ≥150 mg/dl, 3) HDL-C <50 mg/dl for women, 4) blood pressure ≥130/85 mmHg or on antihypertensive medicine(s), and 5) fasting glucose ≥110 mg/dl or on antidiabetic medicine(s). The distribution of the MS component number in the entire cohort was as follows (from 0 to 5): 0, 4, 8, 5, 1, 2. Therefore, there were 12 women without MS (<3 components) and 8 women with MS (≥3 components).

Descriptive characteristics, body composition, abdominal fat distribution, aerobic fitness, lipids, glucose, and other OGTT variables and adipose tissue cytokine gene expression levels were compared between women with and without MS. Plasma TG (138.0±10.2 vs. 94.2±6.3 mg/ml, P < 0.01) and fasting glucose (113.0±8.0 vs. 89.1±3.7 mg/ml, P < 0.01) were significantly higher, and plasma HDL-C (47.9±2.1 vs. 57.6±2.8 mg/dl) was significantly lower, in the MS group. In addition, adiponectin gene expression was significantly lower (adiponectin-β-actin ratio: 2.26±0.46 vs. 3.31±0.33, P < 0.05) in the MS group (Fig. 5). There were no group differences in all other variables.

Fig. 1. Relationship of adipose tissue leptin (A; r = −0.46, P < 0.05) and adiponectin (B; r = −0.38, P = 0.09) gene expression to visceral fat volume (n = 20).
DISCUSSION

Our data showed significant relationships between metabolic and obesity cardiovascular disease risk factors and abdominal subcutaneous adipose tissue gene expression in obese women. Specifically, the quantity of visceral fat was negatively related to leptin and adiponectin abdominal adipose tissue gene expression. In addition, hyperinsulinemia, as indicated by fasting insulin and 2-h insulin during the OGTT, was positively associated with adipose TNF-α and IL-6 gene expression. Both hyperinsulinemia and glucose intolerance during the OGTT were negatively related to adipose adiponectin expression. Our data also showed that adipose adiponectin gene expression was higher in women with vs. without the metabolic syndrome.

Both leptin and adiponectin are proteins that are predominantly produced by adipose tissue (17). Leptin is important in the regulation of appetite and energy balance, as shown by animal studies (11). In humans, circulating levels of leptin are elevated with total obesity (8, 37). Adiponectin is one of the most abundantly expressed adipokines and plays a key role in inflammatory reactions and metabolic disturbances (27, 45). Several studies show that there is regional variation in leptin and adiponectin expression in adipose tissue, with expression of both proteins being higher in subcutaneous fat compared...
with visceral fat (23, 30). Therefore, it is likely that subcutaneous fat production of the two proteins contributes more to their circulating levels and systemic physiological functions. Our findings indicate that subcutaneous adipose tissue expression of both genes is lower in women with increased visceral fat volume. This finding offers important information, since viscero-obese women have lower adipose leptin production, which may lead to an impaired feedback response on energy intake (11). Both visceral fat accumulation and hypoadiponectinemia are associated with an elevated metabolic risk (27). Our findings indicate that these two factors may be linked by adiponectin gene expression in subcutaneous abdominal adipose tissue. Because these are cross-sectional data, our findings do not imply that visceral fat is causally linked to leptin and adiponectin subcutaneous adipose gene expression. Animal studies have shown that leptin (3) or adiponectin (26) administration significantly reduces visceral adiposity in rodents. Moreover, circulating leptin and adiponectin levels are strongly influenced by abdominal fat distribution (33).

Current evidence supports that metabolic risk factors, including dyslipidemia, glucose intolerance, and hyperinsulinemia, are linked with circulating levels of inflammatory and thrombogenic cytokines (4, 6, 25, 45). Relationships between cytokine gene expression in adipose tissue and metabolic risk are strongly influenced by abdominal fat distribution (33).

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