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

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Submitted 3 September 2004; accepted in final form 17 November 2004

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 tended to be negatively 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 comorbidities.

adipose cytokine; visceral fat; glucose; insulin

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...
(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), hyperglycemia (triglycerides >400 mg/dl), insulin-dependent diabetes, active cancer, liver, renal or hematological disease, or other medical disorders were excluded. Among the eight medicated women, five women were under antihypertensive therapy [Atllage (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 Glucotrol (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 (V̇OVO2 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 V̇OVO2 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 threshold cycle (CT) values. Relative gene expression was calculated using the formula (1/2)n cytokine + Ċ 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.
Relationship of adiposity and metabolic variables to cytokine gene expression levels. Visceral fat volume was negatively related to leptin (r = −0.46, P < 0.05) and tended to be related to adiponectin (r = −0.38, P = 0.09) gene expression (Fig. 1). No other measure of adiposity was related to expression of the genes measured.

Plasma levels of TG, TC, HDL-C, and LDL-C were not related to any adipose cytokine gene expression level in these women. However, in the 15 women with OGTT data, 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) were positively related to adipose tissue TNF-α gene expression (Fig. 2). In addition, 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, adipose tissue IL-6 gene expression in the 15 women (Fig. 3). On the other hand, 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, adipose tissue adiponectin gene expression (Fig. 4).

Table 2. Plasma metabolic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SE</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>TG, mg/dl</td>
<td>112.6 ± 7.5</td>
<td>54.0–176.0</td>
</tr>
<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·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>462.2 ± 78.4</td>
<td>138.9–1,132</td>
</tr>
<tr>
<td>Insulin area pmol·h⁻¹·h⁻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 all 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>Variable</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<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>0.00003–0.00269</td>
</tr>
<tr>
<td>IL-6-β-actin mRNA ratio</td>
<td>0.0015 ± 0.0003</td>
<td>0.00001–0.00355</td>
</tr>
<tr>
<td>PAI-1-β-actin mRNA ratio</td>
<td>0.0037 ± 0.0007</td>
<td>0.00009–0.0153</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 medication(s), and 5) fasting glucose ≥110 mg/dl or on anti-diabetic medication(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 ± 10.2 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.
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. Adiponectin expression was higher in women with vs. without the metabolic syndrome.

![Fig. 2](image1.png)  
**Fig. 2.** Relationship of fasting insulin (A; $r = 0.69, P < 0.01$), 2-h insulin (B; $r = 0.56, P < 0.05$), and homeostasis model assessment (HOMA) index (C; $r = 0.59, P < 0.05$) to adipose tissue TNF-α gene expression ($n = 15$).

![Fig. 3](image2.png)  
**Fig. 3.** Relationship of fasting insulin (A; $r = 0.54, P < 0.05$) and 2-h insulin (B; $r = 0.49, P = 0.06$) to adipose tissue IL-6 gene expression ($n = 15$).

![Fig. 4](image3.png)  
**Fig. 4.** Relationship of glucose area (A; $r = -0.56, P < 0.05$) and insulin area (B; $r = -0.49, P = 0.06$) to adipose tissue adiponectin gene expression ($n = 15$).

![Fig. 5](image4.png)  
**Fig. 5.** Adipose tissue adiponectin gene expression in women with metabolic syndrome (MS; $n = 8$) and without metabolic syndrome (NMS; $n = 12$). * $P < 0.05$ between groups.
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 viscerally 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 thrombotic cytokines (4, 6, 25, 45). Relationships between cytokine gene expression in adipose tissue and metabolic risk and insulin resistance have been reported as well (12, 13, 18, 19, 35). Abdominal adipose gene expression levels of TNF-\(\alpha\) (13, 18), IL-6 (35), and PAI-1 (19) are positively linked with insulin resistance and other cardiovascular risk factors, whereas adiponectin gene expression is negatively associated with metabolic variables (12). Our results were consistent with these previous findings and demonstrated that hyperinsulinemia was positively linked to adipose TNF-\(\alpha\) and IL-6 gene expression, and hyperinsulinemia and glucose intolerance were negatively linked to adipose adiponectin expression. Although these adipose-derived cytokines are traditionally viewed as the causes of the insulin resistance and metabolic risk (14, 35), recent evidence suggests that an elevated TNF-\(\alpha\) and IL-6 expression (22) and a decreased adiponectin expression (10) may also be a consequence of hyperinsulinemia. However, insulin infusion did not affect adiponectin gene expression in either healthy or type 2 diabetic individuals (20). Therefore, more studies are needed to clarify the underlying mechanisms of these associations.

Although one or more of the individual risk components of the metabolic syndrome are found in most obese people, the clustering of these components is not present in all obese individuals (34), suggesting that they are likely to occur together via a common underlying mechanism. Because adiponectin is a unique adipose-derived cytokine, adipose tissue production of adiponectin is a major factor responsible for its circulating levels. Current studies show that abdominal obesity (7), dyslipidemia (36), insulin resistance (38), and hypertension (15), as well as the cluster of the metabolic syndrome (45), are all associated with low circulating levels of adiponectin. Therefore, adiponectin is likely to be important in the pathophysiology of the metabolic syndrome (27). However, only a few studies investigated the relationship of metabolic variables to adipose tissue adiponectin gene expression (12, 40). Adiponectin gene expression in adipose tissue is negatively related with circulating levels of glucose and lipoprotein A, an important cardiovascular risk factor (12). In addition, adiponectin expression is reduced in type 2 diabetes patients compared with lean and obese normoglycemic subjects (40). Neither of these studies focused on the role of adipose adiponectin expression in the mechanisms of overall metabolic syndrome. Our findings indicate that adipose adiponectin gene expression is a crucial factor to distinguish obese women with and without the metabolic syndrome. Interestingly, this effect was independent of body composition, abdominal fat distribution, and plasma lipids. As mentioned above, hyperinsulinemia is a possible factor that results in a declined adiponectin expression (14). However, because of the small number of women with metabolic syndrome who had an OGTT, there was no group difference in plasma insulin. More studies are needed to investigate whether the lower adiponectin expression with metabolic syndrome is due to hyperinsulinemia.

The current study provides evidence that the quantity of visceral fat and glucose/insulin complications of obesity is related to abdominal subcutaneous adipose tissue cytokine gene expression. In addition, adipose adiponectin gene expression distinguishes obese individuals with and without the metabolic syndrome (defined by NCEP-ATPIII). However, it is notable that these findings are limited to postmenopausal women and need to be confirmed in other populations. Moreover, additional research is needed to discern whether abdominal subcutaneous gene expression of leptin, adiponectin, TNF-\(\alpha\), and IL-6 is causative for these risk factors or whether there is compensatory regulation of adipose tissue gene expression as a result of elevated visceral fat and/or insulin resistance. To answer this question, studies that focus on the effects of agents that increase adiponectin and decrease TNF-\(\alpha\) and IL-6 adipose tissue gene expression on treatment of metabolic syndrome are needed. Likewise, studies are needed to clarify whether a mechanism by which weight loss and exercise improve metabolic syndrome is through alteration of adipose tissue cytokine gene expression.

ACKNOWLEDGMENTS

We are grateful to the study coordinators, nurses, exercise physiologists, and laboratory technicians of the Section of Gerontology and Geriatric Medicine and the General Clinical Research Center at Wake Forest University School of Medicine for assistance in the conduct of this project. We also thank all women who volunteered to participate in this study.

GRANTS

This study was supported by National Institutes of Health (NIH) Grant RO1-AG/DK-20583, the Wake Forest University Claude D. Pepper Older Americans Independence Center (NIH Grant P30-AG-21332), and the Wake Forest University General Clinical Research Center (NIH Grant M01-RR-07122).

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