Adipose depot-specific modulation of angiotensinogen gene expression in diet-induced obesity

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Adipose tissue is an important source of angiotensinogen (AGT). We investigated the effect of obesity induced by a high-fat diet on the expression of mouse (mAGT) and human AGT (hAGT) genes in liver, kidney, and heart and different adipose depots in normal mice (C57BL/6j), and in transgenic mice expressing the hAGT gene under the control of its own promoter. Mice were fed a high-fat diet (45% kcal) or normal chow (10% kcal) for 10 and 20 wk. The expression of mAGT and hAGT mRNA was quantified using an RNase protection assay. Mice on the high-fat diet exhibited increased weight, fat mass, and plasma leptin. Expression of mAGT or hAGT genes was not affected by high-fat diet in nonadipose tissues, brown adipose tissue, or subcutaneous white fat. In contrast, high-fat diet increased both mAGT and hAGT gene expression in visceral adipose depots (omentum, reproductive, and perirenal fat). Thus obesity-induced by a high-fat diet is associated with a tissue-specific increased expression of both mouse and human AGT genes in intra-abdominal adipose tissue. Our findings also suggest that 1.2 kb of regulatory sequences present in the hAGT transgene are sufficient to transcriptionally respond to a high-fat diet in an adipose-specific and depot-specific manner.

OBESEITY IS ASSOCIATED WITH a high risk of developing cardiovascular and metabolic disorders, such as coronary heart disease, hypertension, diabetes, and dyslipidemia (16). Weight loss induced by gastric bypass (4) or low-calorie diet (25) reduces arterial pressure and improves diabetes and other comorbidities associated with obesity, suggesting a role for the adipose tissue in these disorders. Adipocytes synthesize and secrete a variety of peptides and factors that may act locally to affect adipocyte growth and differentiation or may be released into the circulation to act elsewhere. Adipose tissue represents an important source of angiotensinogen (AGT), the precursor of angiotensin II (8). This AGT produced by adipose tissue appears to be involved in the regulation of blood pressure and renal function. Indeed, compared with AGT knockout mice, which have no detectable AGT plasma levels and lower blood pressure, transgenic mice expressing AGT only in the adipose tissue (on an otherwise AGT-deficient genetic background) have some circulating AGT (~10% that of the wild type) that are normotensive and exhibit restored renal function (18). Moreover, mice that overexpress adipose AGT have increased levels of circulating AGT compared with wild-type mice and are hypertensive (18).

Blockade of angiotensin-converting enzyme or angiotensin type 1 receptors prevents the development of high blood pressure in mice on a high-fat diet, suggesting a role for the renin-angiotensin system in obesity hypertension (22). Furthermore, the positive correlation between plasma AGT levels and body mass index in different human populations suggests that increased adipose AGT production could explain the high circulating level of this protein in obesity (2, 5). In obese subjects, adipose AGT gene transcription was found by some investigators to be upregulated (29), whereas others reported its downregulation (10). In animal models, the patterns of AGT gene expression appear also to vary substantially and in opposite direction between different models of obesity. Frederich et al. (9) demonstrated that AGT mRNA was threefold higher in the epididymal fat pad of genetically obese mice (ob/ob and db/db) than in lean controls. In obese Zucker rats, Jones et al. (14) found a significant decrease in adipose AGT gene expression. Hainault et al. (11) have recently shown that the production of AGT by inguinal and retroperitoneal adipose tissue increases more dramatically with age in obese Zucker rats compared with lean controls. Jones et al. (14) also found that AGT gene expression was significantly lower in adipose tissue of obese agouti yellow mice. Therefore, the effect of obesity on adipose AGT gene expression is currently unclear.

In the present study, we evaluated the expression of the mouse AGT (mAGT) gene in different tissues in the common model of diet-induced obesity. To study the effect of diet-induced obesity on human AGT (hAGT) gene expression, we used transgenic mice that express the hAGT gene under the control of its own endogenous promoter (1.2-kb regulatory sequence) (34). In these transgenic mice, the hAGT gene was expressed in appropriate tissue and cell types, consistent with the expression of the rodent AGT genes.

MATERIALS AND METHODS

Animals. All mice used in these studies (transgenic hAGT mice and their wild-type littermates) were obtained from the Transgenic Facility of the University of Iowa. All mice were maintained by consecutive backcross breeding to C57BL/6j. The hAGT-transgenic mice can be considered C57BL/6j congenics because they have been back-crossed for over seven generations. Animals were housed in a temperature-, humidity-, and light-controlled room (12:12-h light-dark cycle) with free access to chow and tap water. All experimental animals were weaned at 21 days and housed in the Animal Research Facility, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242 (E-mail: curt-sigmund@uiowa.edu).

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procedures were approved by the Animal Care and Use Committee at the University of Iowa.

Experimental protocol. Transgenic hAGT mice and their wild-type littermates at the age of 6–10 wk were randomly assigned to either normal chow (10% kcal) or high-fat diet (45% kcal) for 5–20 wk. All mice received water ad libitum. Body weight was measured at the beginning of the study and each week after the start of the diet. As soon as mice were killed, blood was collected in tubes containing 2.5% sodium citrate and centrifuged at 1,400 g for 5 min, and plasma was frozen at −80°C. Different adipose depots, including brown adipose tissue, subcutaneous white adipose tissue, reproductive fat (epididymal in males and parametrial in females), omental fat, and perirenal fat, as well as the kidneys and heart, were excised, and tissue samples from the liver were collected. The subcutaneous white adipose tissue was collected from the same location in all mice: abdominal left and right sides. Tissues were immediately frozen in dry ice, weighed, and stored at −80°C.

Resulting from these procedures, the liver, kidney, heart, and subcutaneous white adipose tissue were selected for subsequent analysis. Omental and perirenal adipose tissues were excised, and the kidneys and heart were dissected. These tissues were immediately frozen in dry ice and stored at −80°C. Leptin and insulin assays. Plasma concentrations of leptin and insulin were measured by radioimmunoassay using commercially available kits (from Linco).

Statistical analysis. Results are expressed as means ± SE. Data were analyzed by Student’s t-test and one- or two-way ANOVA with a Bonferroni test for post hoc comparison. P < 0.05 was considered to be statistically significant.

Table 1. Effects of 10 and 20 wk of HF diet on weight gain, fat mass, and plasma leptin in WT and transgenic hAGT mice

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Diet</th>
<th>n (M,F)</th>
<th>Weight Gain, g</th>
<th>BAT</th>
<th>Omental</th>
<th>Reproductive</th>
<th>Perirenal</th>
<th>Leptin, ng/ml</th>
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<tbody>
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<tr>
<td>WT mice</td>
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</tr>
<tr>
<td>10</td>
<td>NC</td>
<td>7 (3,4)</td>
<td>4.06±0.59</td>
<td>0.34±0.05</td>
<td>1.12±0.17</td>
<td>1.39±0.25</td>
<td>0.90±0.14</td>
<td>6.6±1.26</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>13 (6,7)</td>
<td>6.02±0.85</td>
<td>0.48±0.03*</td>
<td>1.83±0.16*</td>
<td>2.76±0.49</td>
<td>1.43±0.13*</td>
<td>12.5±1.14*</td>
</tr>
<tr>
<td>20</td>
<td>NC</td>
<td>9 (6,3)</td>
<td>6.84±0.77</td>
<td>0.41±0.02</td>
<td>1.07±0.07</td>
<td>2.10±0.32</td>
<td>1.03±0.07</td>
<td>7.53±0.67</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>11 (8,3)</td>
<td>11.34±1.63*</td>
<td>0.65±0.09*</td>
<td>2.03±0.2*</td>
<td>4.44±0.66*</td>
<td>2.08±0.20*</td>
<td>16.18±2.74*</td>
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<tr>
<td>hAGT mice</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>NC</td>
<td>8 (3,5)</td>
<td>3.18±0.50</td>
<td>0.34±0.02</td>
<td>1.29±0.08</td>
<td>1.57±0.15</td>
<td>0.92±0.14</td>
<td>6.47±0.9</td>
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<tr>
<td></td>
<td>HF</td>
<td>10 (5,5)</td>
<td>5.34±0.75*</td>
<td>0.52±0.04*</td>
<td>1.72±0.09*</td>
<td>2.32±0.36</td>
<td>1.25±0.12*</td>
<td>11.06±1.37*</td>
</tr>
<tr>
<td>20</td>
<td>NC</td>
<td>8 (3,5)</td>
<td>6.07±0.93</td>
<td>0.42±0.03</td>
<td>1.10±0.06</td>
<td>2.30±0.26</td>
<td>1.08±0.06</td>
<td>7.27±0.54</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>9 (6,3)</td>
<td>9.02±1.42*</td>
<td>0.75±0.06*</td>
<td>2.39±0.18*</td>
<td>4.64±0.72*</td>
<td>2.25±0.18*</td>
<td>15.67±1.69*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. hAGT, human angiotensinogen; WT, wild type; BAT, brown adipose tissue; NC, normal chow; HF, high-fat diet; n, no. of mice per group; (M, F), no. of male and female mice per group; *P < 0.05 vs. NC.

RESULTS

Although the body weights were comparable at baseline, both wild-type and hAGT-transgenic mice on the high-fat diet for 10 and 20 wk exhibited a greater weight gain than the controls on normal chow (Table 1). Fat mass and plasma leptin at 10 and 20 wk were also higher in mice fed the high-fat diet than in those fed normal chow (Table 1). Plasma insulin was increased 1.5- to 2.8-fold, reflecting an insulin resistance associated with obesity. The weight of nonadipose organs remained comparable. For example, in the wild-type mice, the weight of the kidneys was 0.37±0.02 and 0.34±0.03 g after 20 wk of normal chow and high-fat diet, respectively. After only 5 wk, there was no difference in body weight, fat mass, or plasma leptin between mice on the high-fat diet and those on normal chow (data not shown).

The expression of mAGT in normal mice and hAGT in transgenic mice was not affected by high-fat diet at either 10 or 20 wk in nonadipose tissues: liver, kidney, and heart (Fig. 1). In contrast, the high-fat diet caused a substantial and significant increase in mAGT transcript in the intra-abdominal fat of
neous white fat (Fig. 4). The increase in mAGT mRNA at 10 and 20 wk, respectively, was ~2.8- and 6.6-fold in the omental fat, 3.8- and 5.7-fold in the reproductive fat, and 3.8- and 4.5-fold in the perirenal fat. The induction of mAGT gene expression in intra-abdominal fat by dietary obesity does not depend on sex, because the mAGT mRNA increased similarly in males and females. For instance, at 10 wk, the increase in mAGT mRNA in the omental fat was ~2.7-fold in males and 2.8-fold in females, and at 20 wk, mAGT mRNA increased in this tissue ~6.4-fold in males and 6.7-fold in females. Just as in nontransgenic control mice, 20 wk of high-fat diet increased mAGT gene expression in hAGT-transgenic mice ~5.3-fold in the perirenal fat (P = 0.007), 6.5-fold in the omental fat (P = 0.013), and 7.5-fold in the reproductive fat (P = 0.002). These data suggest that the presence of the hAGT does not alter the mAGT response to high-fat diet.

In hAGT-transgenic mice, a marked and significant increase in hAGT gene expression was also observed in the intra-abdominal fat (Fig. 3). The expression of the hAGT gene at 10 and 20 wk was increased, respectively, ~48.6- and 6-fold in the omental fat, 5.6- and 6.3-fold in the reproductive fat, and 3.9-and 7.4-fold in the perirenal fat. As for mAGT, no sex difference in the induction of hAGT in the intra-abdominal fat was observed (data not shown). The expression of mAGT and hAGT genes was comparable in mice fed normal chow and those fed the high-fat diet for only 5 wk. The change in mAGT and hAGT mRNA in mice on 5 wk of high-fat diet compared with controls on normal chow was, respectively, +14 and +38% in the omental fat, ~24 and +2% in the reproductive fat, and +49 and ~13% in the perirenal fat.

Levels of mAGT and hAGT mRNA were not significantly affected by high-fat diet in brown adipose tissue and subcutaneous white fat (Fig. 4).

**DISCUSSION**

There are two major findings from the present investigation of the effect of obesity on the expression of the AGT gene. First, obesity induced by high-fat diet in mice is associated with an intra-abdominal adipose tissue-specific increase in the expression of both mAGT and hAGT genes. Second, 1.2 kb of regulatory sequences present in the hAGT transgene are sufficient to transcriptionally respond to the obesity induced by high-fat diet in an adipose-specific and a depot-specific manner.

Our finding of a differential effect of high-fat diet and obesity on AGT gene expression strongly suggests that regional tissue AGT synthesis is regulated through different mechanisms. Nutritional regulation of regional AGT gene transcription is also tissue specific. In a previous study, fasting resulted in a significant reduction, and refeeding led to a marked increase, in adipose tissue AGT gene expression in Sprague-Dawley rats (9). These local changes in adipose AGT gene expression were not accompanied by variation in hepatic AGT gene expression (9). Our study is the first to demonstrate that diet-induced obesity selectively increases AGT gene expression in intra-abdominal fat. The absence of changes in the mAGT gene expression after 5 wk of high-fat diet (before the development of obesity) indicates that the increase in AGT transcript observed at 10 and 20 wk is likely to be due to obesity rather than to the high-fat diet per se. The reported lipopolysaccharide-induced increase in mAGT in all tissues except the brain and the distinct temporal profile of angiotensin II-stimulated AGT synthesis in the liver compared with cardiac myocytes (3) also support the existence of regional variations in the regulation of AGT gene transcription in other tissues.

A difference in the mAGT and hAGT mRNA responses to diet-induced obesity was observed between subcutaneous and intra-abdominal fat depots. Other investigators have reported a higher AGT gene expression in visceral fat compared with subcutaneous fat (7, 30). Regional differences in adipose tissue mRNA have also been demonstrated for several other genes, including those encoding for leptin and tumor necrosis factor-α (1). Although there is no obvious explanation for the regional difference in AGT gene expression between the subcutaneous and intra-abdominal fat depots, it could be due to differences between the anatomic and biochemical characteristics of visceral and subcutaneous adipocytes. For example, the catecholamine-induced increase in intracellular cAMP (23) and lipolysis (31) is higher in omental adipocytes than in subcuta-
Obesity is associated with an increase in adipose and circulating levels of several factors that can stimulate AGT gene expression, such as hyperglycemia, glucocorticoids, free fatty acids, and tumor necrosis factor-α (32). The similar responses of both mAGT and hAGT genes to 10 and 20 wk of high-fat diet in the present study suggest that the 1.2-kb promoter of the hAGT transgene used to generate our mice contains all the regulatory elements necessary to respond to diet-induced obesity and in an adipose tissue-specific manner. A number of elements that match consensus sequences for regulatory motifs for transcription factors have been identified in the upstream region of the AGT gene (21). The 5′-flanking region of the rat AGT gene between nucleotides −615 and −470 appears to contain a multihormone-responsive enhancer that integrates hormonal signals from both glucocorticoids and cytokines. Within this enhancer, two glucocorticoid response elements were located within the sequence spanning nucleotides −584 to −570, whereas the sequence located between nucleotides −557 and −531 is required for cytokine induction of AGT promoter activity (3). The induction of the AGT gene transcription during adipocyte differentiation is also transcriptionally mediated through the activation of the promoter. Tamura and colleagues (27, 28) have shown that the proximal promoter region of the hAGT gene, from nucleotides −96 to +22 of the transcriptional start site, was sufficient to confer adipogenic activation. These authors also identified three cis-acting elements that contribute to the transcriptional activity of AGT promoter in adipocytes, namely AGE1 (−399 to −139), AGE2 (−96 to −52) and AGE3 (−6 to +22). Although the AGE2 site seems to be important for the basal transcription of the AGT promoter, as well as for its differentiation-induced activation in adipocytes, mice carrying a mutation in the AGE2-binding site had unaltered AGT gene expression in tissues including adipose tissue (35). It is important to recognize that the effect of the AGE2 mutation on the induction of AGT mRNA caused by dietary obesity in the intra-abdominal fat has not been tested.

The substantial increase in AGT gene expression in intra-abdominal fat is consistent with the strong association between visceral fat accumulation, hypertension, sympathoactivation, and other cardiovascular diseases (16, 17, 32). Selective reduction of intra-abdominal visceral fat is accompanied by improvement in cardiovascular diseases associated with obesity, including hypertension (15). Increased intra-abdominal AGT could, therefore, be a factor in the obesity-related disorders. Furthermore, increased production of AGT in visceral fat could exacerbate further obesity because adipose AGT could be converted locally to angiotensin II, which promotes adipocyte growth and differentiation (6, 8, 18). However, this notion of angiotensin II acting to promote growth of adipose tissue is based mainly on rodent studies, because angiotensin II does not promote the growth or differentiation of human preadipocytes or adipocytes (13).

We recognize that the study leaves some unanswered questions that need to be addressed in the future. First, it is unclear whether adipocytes or other cell types account for the increase in AGT gene expression observed in the intra-abdominal fat tissues. Given the rich vascularization of visceral adipose tissue, a potential increase in vascular AGT gene expression may contribute to the observed increase in the AGT gene expression in adipose tissue. This is unlikely because it would increase the AGT gene expression in other tissues with higher vascularization, such as the heart or the kidney, where the AGT gene expression was not affected by the high-fat diet. Second, our study does not address whether, in our model, the obesity induced by high-fat diet is associated with an increase in blood pressure. However, the association between obesity induced by high-fat diet and hypertension has been demonstrated in several species, including mice (12, 20, 26, 33). Overexpression of the adipose AGT gene of the same magnitude as in our study has also been reported to cause an increase in blood pressure in mice (18, 19).

In conclusion, the results of the present study indicate that high-fat diet and obesity produce a selective increase in the transcription of the AGT gene in intra-abdominal fat. This might represent a potential link between visceral obesity and cardiovascular diseases.

GRANTS
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REFERENCES