Adipose tissue-specific increase in angiotensinogen expression and secretion in the obese \(fa/fa\) Zucker rat

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Hainault, Isabelle, Guillaume Nebout, Sophie Turban, Bernadette Arduin, Pascal Ferré, and Annie Quignard-Boulangé. Adipose tissue-specific increase in angiotensinogen expression and secretion in the obese \((fa/fa)\) Zucker rat. *Am J Physiol Endocrinol Metab* 282: E59–E66, 2002.—We investigated angiotensinogen (AGT) expression in adipose tissue and liver of Zucker rats during the onset of obesity. The developmental pattern of AGT expression (protein and mRNA) in liver was similar in both genotypes. In inguinal adipose tissue, AGT cell content was similar in suckling and weaned pups in lean rats, whereas it continuously increased with age in obese rats. AGT amount in adipocytes was unaffected by the genotype until weaning. Thereafter, adipocytes from obese rats displayed a significant increase in AGT content that was strengthened with age. Compared with the cell content, the amount of secreted AGT over 24 h was higher, and a genotype effect was observed as early as 14 days of age. Using fat cell populations differing by size, we showed that this AGT oversecretion was not solely related to adipocyte hypertrophy. Our results demonstrate that the \(fa\) genotype exerts a control on the production of AGT in a tissue-specific manner, suggesting a local role of AGT in the overdevelopment of adipose tissue.

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The view that the adipocyte acts only as a passive storage site for energy in the form of triacylglycerols is now obsolete after the discovery that adipose cells secrete a variety of active factors. Among them, angiotensinogen (AGT), in addition to its role in the regulation of systemic blood pressure, appears to be involved in preadipocyte differentiation processes (34, 35). According to previous studies, the link between AGT production and the control of adipose mass involves angiotensin II (ANG II), which has been implicated in the differentiation of adipose precursors by a paracrine/autocrine mechanism (12). The role of a local production of ANG II has been linked to control of adipocyte number and size, suggesting an important role of AGT production by adipose cells in adipose tissue development.

In rodents, results from different models of obesity are divergent, showing an increased AGT production by adipose cells from genetically obese \((ob/ob)\) mice, whereas AGT gene expression is reduced in adipose tissue from \(fa/fa\) rats and the viable yellow (Avy) mouse (17, 23). However, all of these studies were performed in adult animals, which displayed an established hyperplasia of adipose tissue. The obesity in the Zucker rat is an autosomal and recessive inheritance and is caused by a leptin receptor missense mutation resulting in a failure of leptin signaling in \(fa/fa\) rats (9). This rat develops a syndrome with multiple metabolic and hormonal disorders that shares many features with human obesity. Hyperphagia, hyperinsulinemia, hypertriglyceridemia, hypertrophy, and hyperplasia of fat cells, as well as insulin resistance, renal complications, and hypertension are features common to both species (5). In contrast to the complex syndrome exhibited by the adult obese Zucker rat, we have previously demonstrated that, early in life, the obese \(fa/fa\) rat presents an overdevelopment of fat deposits, which is related to only a moderate adipocyte hypertrophy (3). After weaning, the enlargement of adipocytes is accelerated, and the later emergence of hyperplasia exacerbates fat accretion (31). The fact that all of the previous observations in obese models were performed when hyperplasia was already present did not allow a real evaluation of the potential influence of RAS in the onset of obesity. Moreover, although AGT appears to be secreted only constitutively by the liver, it is unclear whether the ability of adipose cells to secrete the protein is entirely related to its gene expression. A lack of relationship between gene expression and protein se-

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creatin has been previously demonstrated for the emergence of AGT during the adipocyte differentiation process of Ob1771 cells (33). All of these observations prompted us to evaluate AGT production and gene expression by adipocytes during the developmental changes that led to adipocyte hyperplasia. Interestingly, a linkage between intra-abdominal fat accumulation and hypertension has been found in obese Japanese women independently of age or body mass index, suggesting a possible site-specific variation in adipose tissue AGT secretion (24). Furthermore, we investigated whether levels of AGT production were comparable in subcutaneous and visceral fat deposits. Then we compared the developmental pattern of AGT expression in adipose tissue and in liver to estimate the relative importance that these tissues play in the control of circulating AGT at different phases of obesity.

MATERIALS AND METHODS

Animals. Lean (Fa/+) and obese (fa/fa) Zucker rat litters were obtained in our animal house by breeding heterozygous lean females and homozygous obese males. Pups were weaned at 28 days of age on a regular chow diet (UAR, Epinay-sur-Orge, France). Before weaning, obese and lean pups were identified on the basis of ob receptor mutation as previously described (30). All animals were housed in a temperature-controlled condition with a 12:12-h light-dark period (0700–1900) and free access to regular food and tap water. Experiments were undertaken according to the Guidelines for Care and Use of Experimental Animals. After animal decapitation, blood was collected for serum determinations. The inguinal, epididymal, and retroperitoneal adipose tissues and liver were removed and used for further determinations or directly frozen in liquid N2 for total RNA extraction.

Adipose cell culture. To evaluate the AGT secretion, a portion of adipose tissue from suckling and weaned rats was digested with collagenase, and isolation of mature adipocytes and preadipocytes was performed as previously described (6). Mature adipocytes were maintained for 24 h in DMEM supplemented with 1% FCS, 2% BSA, antibiotics, and 15 mM insulin. In a preliminary experiment, we verified that the amount of AGT secreted in the medium was linear during a 24-h incubation (data not shown). To study the effect of adipocyte size on its ability to secrete AGT, we isolated different cell populations according to their size. After isolation and dilution, mature adipocytes issued from one fat pad were separated by successive filtration through one or three nylon meshes (from 190- to 30-μm meshes). By this procedure, we obtained three or four cell populations of different mean size. Cells from each population (2–4 × 10⁶) were suspended in standard medium and incubated for 24 h to estimate their AGT protein production.

Adipose tissue cellularity. Size and number of adipocytes were determined as previously described (6). Fat cell size was determined by a procedure derived from a microphotometric method (26). Briefly, images of isolated cells were acquired from a light microscope fitted with a camera, and the measurement of cell diameters was performed using a computer equipped with an analyzing program (Perfect-Image, Numeris, Nanterre, France). The mean fat cell volume was calculated as previously described (26), and the mean fat cell weight was determined by calculation of triolein density (0.92). Fat cell number was estimated on an aliquot of adipocyte suspension or a portion of adipose tissue by dividing the lipid content by average fat cell weight.

Measurement of AGT protein. In whole adipose tissue or in primary cultures, the AGT protein content was measured in a cellular extract. Briefly, adipose tissue or cells were homogenized in a 1,500-μl buffer containing 250 mM Tris-acetate, pH 7, 5 mM EDTA, 3% BSA, 100 μM catalopril, and 50 μM protease inhibitor [4-(2-aminoethyl)benzenesulfonyl fluoride], centrifuged at 4°C for 15 min at 3,000 g, and used for AGT determination. AGT concentration in whole tissues, isolated cells, and the culture medium was determined by incubating the samples with an excess of porcine renin (500 U) for 90 min at 37°C and measuring the generated ANG I by RIA (REN-CT2, Cis-Bio International, Gif-sur-Yvette, France). For each sample, we verified that there was no detectable ANG I in the incubation medium without addition of exogenous renin. Results were expressed as nanograms of ANG I liberated by 10⁶ cells.

Western blotting. Aliquots of proteins from medium and adipocyte cell homogenate were electrophoresed on a 12% SDS-polyacrylamide gel and electroblotted overnight onto a nitrocellulose membrane at room temperature. The membrane was further incubated at room temperature for 1 h with a 1:5,000 dilution of anti-AGT antibody [rabbit antibody against rat AGT (2), a kind gift from Prof. P. Corvol]. The blot was washed and exposed for 1 h to horseradish peroxidase-conjugated anti-rabbit IgG. The immune complex was detected by luminescent visualization (ECL, Amersham Pharmacia Biotech, Orsay, France). Molecular weight standards were obtained from Bio-Rad (Richmond, CA).

Biochemical assays. Plasma renin activity was measured by RIA (28). Briefly, 4 μl of plasma were incubated for 90 min at 37°C and used for ANG I assay. For measurement of plasma AGT concentration, 100 μl of plasma were incubated for 90 min at 37°C in the presence of renin as described, and the generated ANG I was assayed by RIA. To estimate the level of leptin secretion by the adipocytes, leptin concentration was measured in the culture medium by RIA (Linco Research, St. Charles, MO).

RNA extraction and Northern blot analysis. Total RNA was extracted according to Chomczynski and Sacchi (8). Equal amounts of total RNA were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde and transferred by capillarity onto nylon membranes (Membrenes N+-Eurobio, Les Ulis, France). Hybridization was performed at 65°C overnight with the 32P-labeled complementary RNA probe from plasmid containing rat AGT cDNA (pGEM4Z/AGT from American Type Culture Collection). Blots were autoradiographed at −70°C for 6–8 h. Each Northern blot was hybridized with a ribosomal 18S probe to verify that equivalent amounts of total RNA were loaded in each lane. The relative amount of AGT mRNA was quantified by densitometry and normalized to the 18S rRNA level.

Data analysis. Data are expressed as means ± SE. All data from developmental studies were compared using a one-way analysis of variance, followed by Tukey’s and Fisher’s tests. Comparison of adipose sites data was performed using Student’s t-test.

RESULTS

There was no difference between lean and obese rats in body weight and liver weight throughout this developmental period (Table 1). In agreement with the well-known genotype effect on adipose tissue, we observed that inguinal adipose tissue weight increased in fa/fa
Developmental changes in AGT protein and mRNA in liver and adipose tissue. Figure 1A depicts AGT protein levels in the liver of lean and obese growing Zucker rats. The developmental pattern was quite similar in both genotypes, increasing until weaning and decreasing thereafter. Northern blot analysis of liver AGT mRNA revealed that AGT gene expression tended to be decreased with age and that no significant differences in AGT mRNA levels between lean and obese Zucker rats were detected at all ages studied (Fig. 1B).

In adipose tissue, the developmental changes of AGT amount were different and followed in parallel the enlargement of adipocytes (Fig. 2A; Table 1). In lean rats, the amount of AGT in cells increased from 21 days of age. During the suckling period, adipose cell size continuously increased in fa/fa rats, whereas it remained unchanged in Fa/fa rats, and there was no difference in cell number between genotypes. During the weaning period, the enlargement of adipocytes was manifested in both genotypes but with a greater extent in obese than in lean rats (+80 vs. +200% in Fa/fa and fa/fa rats, respectively), and cell number was 28% lower in fa/fa than in lean rats at 28 days of age. By contrast, adipocyte hyperplasia was evident in 56-day-old fa/fa rats. This is in agreement with the primary obesity effect of fa genotype (3) and gives evidence of a late onset of adipocyte hyperplasia. We also evaluated the RAS in the serum and, as shown in Table 1, plasma renin activity and plasma AGT in obese rats were comparable to values in age-matched lean rats.

Developmental changes in AGT protein and mRNA in liver and adipose tissue. Figure 1A depicts AGT protein levels in the liver of lean and obese growing Zucker rats. The developmental pattern was quite similar in both genotypes, increasing until weaning and decreasing thereafter. Northern blot analysis of liver AGT mRNA revealed that AGT gene expression tended to be decreased with age and that no significant differences in AGT mRNA levels between lean and obese Zucker rats were detected at all ages studied (Fig. 1B).

In adipose tissue, the developmental changes of AGT amount were different and followed in parallel the enlargement of adipocytes (Fig. 2A; Table 1). In lean rats, the amount of AGT in cells increased from 21 days of age, leading to a twofold increase in AGT content between 21 and 56 days of age. In obese rats, AGT level increased continuously during the development of adipose tissue, and a genotype-related increase occurred as soon as 21 days of age. This genotype-mediated difference was dramatically amplified at 56 days of age. A similar genotype effect was found when AGT amount was expressed per the whole inguinal adipose depot (data not shown). To document further the amount of AGT, an analysis of cytosolic fraction from

Table 1. Characteristics of lean and obese Zucker rats

<table>
<thead>
<tr>
<th>Age, days</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fa/fa</td>
<td>Fa/fa</td>
<td>Fa/fa</td>
<td>Fa/fa</td>
<td>Fa/fa</td>
<td>Fa/fa</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>12.2 ± 0.4</td>
<td>22.2 ± 0.5</td>
<td>30.1 ± 1.4</td>
<td>45.0 ± 0.4</td>
<td>205.5 ± 4.2</td>
</tr>
<tr>
<td>Liver, g</td>
<td>0.3 ± 0.03</td>
<td>0.6 ± 0.01</td>
<td>1.1 ± 0.1</td>
<td>2.0 ± 0.6</td>
<td>10.9 ± 0.4</td>
</tr>
<tr>
<td>Inguinal adipose tissue, mg/2 pads</td>
<td>53 ± 9</td>
<td>132 ± 10</td>
<td>180 ± 10</td>
<td>363 ± 30</td>
<td>1,630 ± 110</td>
</tr>
<tr>
<td>Adipocyte weight, mg/cell</td>
<td>12 ± 2</td>
<td>12 ± 1</td>
<td>10 ± 1</td>
<td>18 ± 2</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Adipocyte no., (¹²⁵I)² pads</td>
<td>2.1 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>10.8 ± 0.6</td>
<td>13.1 ± 1</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>PRA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>pAGT</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are means ± SE of 4–12 independent experiments. Plasma levels of renin (PRA) and angiotensinogen (pAGT) were determined as described in MATERIALS AND METHODS and expressed as nanograms of ANG I per milliliter of plasma. Comparisons between lean (Fa/fa) and obese (fa/fa) littermates were performed using ANOVA; NS, not significant; ND, not determined. *P < 0.05; †P < 0.01; ‡P < 0.001.
lean and obese rat adipose cells by Western blot was performed at 28 days of age. As it was described for rat liver (7), we observed the existence of two AGT precursors (approximate molecular weights, 52,000 and 56,000) in adipocyte homogenates (Fig. 3A). There was no genotype effect on the pattern of migration of AGT protein, but the signal intensities from obese adipose cells were threefold stronger than those from lean rats, showing an increase in the intracellular pool of AGT in adipocytes from obese rats. In the medium, the 56-kDa signal was prominent, in agreement with the secretion of a glycosylated protein, and a similar genotype effect on AGT secretion level by adipose cells was found (Fig. 3B).

During the suckling period, adipose tissue AGT mRNA increased similarly in both genotypes, and no significant difference between the genotypes could be detected (Fig. 2, B and C). After weaning, the level of AGT mRNA was highly depressed in both genotypes, and a significant increase in the amount of AGT mRNA was observed in adipose tissue from obese rats compared with that from lean rats. The discrepancy between the AGT protein content (increased by the fa genotype) and its corresponding mRNA (no significant change) can be explained by a greater amount in total RNA displayed by adipose tissue or cells from obese rats, as previously shown (14). To confirm that the mature adipocyte was the main source of AGT, we analyzed the amount of AGT mRNA in isolated adipocytes and stromal cells. Our results indicate that AGT expression in adipose tissue must be related only to the mature adipose cells, because no AGT mRNA could be detected in cells from the stroma-vascular fraction that contains preadipocytes (Fig. 2D). These findings were in agreement with a late emergence of this protein during the differentiation process (34, 36).

AGT secretion by adipose cells. To determine whether increased levels of AGT content exhibited by the adipocytes from fa/fa rats were concomitant with an increased rate of AGT secretion, we measured AGT protein content in culture medium of mature adipocytes. Figure 4 shows that the amount of secreted protein per day was 30–60 times higher than the cellular content, regardless of the genotype. In lean rats, we found similar secretion rates of AGT by adipocytes from suckling (14 days) or weaned (28 days) rats. In contrast, we observed an age-related increase (40%) in AGT secretion in adipocytes from obese rats. Moreover, adipocytes from 14-day-old obese rats, despite the lack of genotype effect on the AGT expression (mRNA and protein content; see Fig. 2), displayed a clear twofold increase in the amount of secreted protein compared with that of lean rats. These data suggested that the secretion level is a better index to evaluate the
AGT production by adipocytes is higher than mRNA or protein concentrations.

**Effect of adipose tissue site on secretion capacity.** Because some site-specific variations in the storage and release of lipids from adipose tissue have been described, we investigated the AGT secretion by adipocytes of 56-day-old rats from three different sites: subcutaneous (inguinal) and visceral (epididymal and retroperitoneal) adipose tissues. Table 2 shows that different sites of fat depots from lean rats exhibited a similar capacity to produce and secrete AGT. By contrast, obese rats exhibited significant intersite differences in AGT content, since retroperitoneal adipose tissue displayed a twofold increase in intracellular AGT compared with the other sites. This site effect was also obvious when the secretion rates were compared, showing a specific effect of the retroperitoneal localization. The greater potency to secrete AGT exhibited by adipocytes from the retroperitoneal site was also observed for leptin secretion (Table 2). Thus adipocytes from obese rats exhibit a higher capacity to secrete different factors, and this capacity is largely related to the localization. The influence of the size of the amplification of the genotype effect was also observed at the mRNA levels (Table 2).

**Effect of the adipose cell size on angiotensinogen secretion.** To assess whether the genotype-related effect on AGT secretion by adipose cells could be related to the cell hypertrophy per se, we examined the production of AGT by adipocytes according to their size. In obese rats, we observed that adipocytes from the retroperitoneal site exhibited higher levels of AGT production than from other sites, whereas no change in adipocyte size could be detected (Table 2). We further estimated the AGT secretion by three to four populations of adipocytes, which differed in their cell diameter distribution. Using a successive filtration procedure, we were able to isolate within the same tissue (inguinal) three or four populations on the basis of the fat cell size distribution, as presented in Fig. 5A. In each population, AGT secretion was measured, and the results show that there was a negative relationship between AGT secretion and cell size in both Faa/ffafa and fa/fa genotypes (Fig. 5B). Moreover, when we compared the secretion rates by adipocytes of the same size (14–16 ng) but issued from different genotypes, there was a fivefold increase in the AGT secretion rate (6 ± 2.5 and 30 ± 12 for lean and obese genotypes, respectively; n = 3). These findings led us to exclude an important role of cell hypertrophy in the overproduction of AGT by adipocytes from fa/fa rats.

**DISCUSSION**

Because the secretion of AGT by adipose tissue has been involved in the differentiation of adipose tissue through a paracrine effect, we have explored in a genetic model of obesity whether adipose tissue AGT expression and secretion differ during the early onset of obesity. Previous studies have yielded conflicting results regarding the effect of obesity on AGT expression in adipose tissue. Both increased and decreased AGT expression in adipose tissue has been reported in ob/ob mice or fa/fa rats, respectively (17, 23). We document here an early and clear-cut increase in the content and secretion of AGT by obese fa/fa rat adipocytes. Conversely, AGT content in the liver is not affected, showing the tissue-specific character of this

**Table 2. Effect of adipose tissue site on AGT and leptin production in 56-day-old lean and obese Zucker rats**

<table>
<thead>
<tr>
<th>Adipocyte size diameter, µm</th>
<th>Inguinal</th>
<th>Obese</th>
<th>Gonadal</th>
<th>Inguinal</th>
<th>Obese</th>
<th>Retroperitoneal</th>
<th>Inguinal</th>
<th>Obese</th>
<th>Gonadal</th>
<th>Inguinal</th>
<th>Obese</th>
<th>Retroperitoneal</th>
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<tbody>
<tr>
<td></td>
<td>Lean</td>
<td>Obese</td>
<td>Lean</td>
<td>Obese</td>
<td>Lean</td>
<td>Obese</td>
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<td>Obese</td>
<td>Lean</td>
<td>Obese</td>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td>36 ± 0.8</td>
<td>70 ± 1.3*</td>
<td>33 ± 2.1</td>
<td>60 ± 1.9*</td>
<td>31 ± 2.8</td>
<td>66 ± 1.2*</td>
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<tr>
<td>0.9 ± 0.13</td>
<td>2.2 ± 0.01**</td>
<td>1.2 ± 0.08</td>
<td>2.0 ± 0.03†</td>
<td>0.6 ± 0.07</td>
<td>4.1 ± 0.3†</td>
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<tr>
<td>34 ± 1.4</td>
<td>55 ± 1.8**</td>
<td>47 ± 5.8</td>
<td>62 ± 1.8**</td>
<td>27 ± 2.8</td>
<td>113 ± 9.0†</td>
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<tr>
<td>18 ± 6.6</td>
<td>59 ± 26**</td>
<td>8.5 ± 3.1</td>
<td>57 ± 22**</td>
<td>15 ± 1.0</td>
<td>352 ± 70†</td>
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</tbody>
</table>
| 0.7 ± 0.14*                | 1.0 ± 0.21* | 1.5 ± 0.21b | 2.0 ± 0.51a,b | 1.5 ± 0.11b | 2.7 ± 0.37b*

Data are means ± SE of 4–6 independent experiments. Isolated adipocytes were cultured for 1 day and used thereafter for angiotensinogen (AGT) content determinations. AGT and leptin concentrations were determined in culture medium to estimate the release capacity of adipose cells. Total RNA was extracted from adipose tissue, and Northern blots were quantified by densitometry scanning. AGT mRNA amounts relative to 18S rRNA are given as arbitrary units. Differences between lean and obese rat cells were assessed as described in MATERIALS AND METHODS: *P < 0.05; †P < 0.01. Comparisons between adipose sites within the same genotype were performed using a paired t-test, and values not sharing the same superscript are significantly different (P < 0.05).
mean diameters (μm) of these populations were: 128, 30, 18, and 15 μm, respectively. After separation, each cell population was separately maintained in a culture medium, and AGT concentration was determined in the medium after 24 h of culture. Each regression line corresponds to the values obtained in cell populations from an individual rat. This figure is representative of 3–4 experiments for each genotype.

Fig. 5. Effect of fat cell size on AGT secretion. A: frequency distribution of cell diameters from inguinal adipose tissue from 4-wk-old obese rats separated by successive filtrations. Each curve corresponds to different cell populations after separation through one or three nylon meshes as described in MATERIALS AND METHODS. The mean diameters (μm) of these populations were: 128 ± 27, 93 ± 13, 81 ± 18, and 15 ± 6 for filtration through 1 × 60, 3 × 60, 1 × 46 and 1 × 30 μm, respectively. B: relationship between adipocyte weight and AGT secretion rate in inguinal adipose tissue from lean (♦) and obese (●) rats. After separation, each cell population was separately measured in a culture medium, and AGT concentration was determined in the medium with 24 h culture. Each regression line corresponds to the values obtained in cell populations from an individual rat. This figure is representative of 3–4 experiments for each genotype.

These findings are at variance with those of Jones et al. (23), reporting a decreased expression of AGT in adipose tissue from obese adult Zucker rats. Such different findings could be explained by the age of the animals studied. In adults, in which hormonal and metabolic abnormalities are more pronounced, confounding factors such as hyperinsulinemia could be present, since insulin downregulated the AGT expression in cultured adipose cells (1). The fa genotype effect was visible as early as 14 days of age, in contrast to changes seen with mRNA level or protein content. Such a discrepancy has already been observed in the ob cell line, where AGT secretion was dramatically increased during the adipocyte differentiation process, whereas the cellular content remained stable during the same period (33). It could be postulated that this difference was due to the use of an indirect method of AGT assay. The presence of a potent proteolytic system in the tissue extract may provoke a degradation of ANG I during the renin incubation, as previously demonstrated in the liver (10). However, the difference between the intracellular pool and the secretion level of AGT persisted when the protein was detected by immunoblotting, suggesting that the quantification by RIA of secreted AGT by intact adipose cells is reliable. Thus these results also suggest that the measurement of AGT mRNA amount is not sufficient to estimate accurately the AGT production by the adipose tissue.

In adipocytes from obese rodents (fa/fa, ob/ob), the capacity of adipocytes to secrete different proteins, leptin, tumor necrosis factor-α, lipoprotein lipase, and AGT (present study) is enhanced, suggesting a potential relationship between cell hypertrophy and secretion capacity (4, 21, 27). In fact, our results do not support such a hypothesis, because in both lean and obese rat adipocytes, a negative relationship between cell size and secretion was found, and at comparable sizes, adipocytes of the fa genotype clearly secrete more AGT than those of the control one. The reasons why the production of AGT is specifically increased in adipose tissue of obese Zucker rats remain unclear. To our knowledge, AGT is secreted constitutively and is not stored in secretory vesicles (13). This implies that AGT production is controlled mostly at the transcriptional level (36). We have previously demonstrated that adipocytes from obese Zucker rats display a markedly increase in the transcription of a subset of genes related to the lipid storage pathway (15, 19). The present data support the hypothesis that, in obese rat adipocytes, the AGT gene could also be a target of the fa mutation. Whether defective lepin signaling is implicated in this feature remains to be determined. The lack of effect of exogenous leptin (100 ng/ml) on AGT release in lean rat adipose cells (data not shown) argues against a direct effect of this cytokine.

Adipose tissue is now well established as being heterogeneous in its metabolic activity, and regional variation in storage and mobilization capacities has been previously described in humans and rodents (18, 25, 29, 32). We found that adipocytes from retroperitoneal tissue of fa/fa rats displayed an increase in AGT expression and secretion rate. Such a site-related difference is consistent with a higher level of AGT mRNA in visceral than in abdominal fat found in obese subjects (16). The overproduction of both AGT and leptin found in retroperitoneal adipocytes from obese Zucker rats appears to be a part of a generalized increase in the adipocyte function of this localization as previously described (4, 18). Further studies are necessary to learn the underlying mechanism of this regional difference in AGT production in adipose tissue of obese fa/fa rats.

The present study raises questions about the potential impact of increased adipocyte AGT production. On the one hand, an excess of local production of ANG II...
by triggering adipocyte differentiation and lipid storage capacity may contribute to the onset of both hyper-trophy and hyperplasia of adipose tissue in obese rats (11, 12, 22). On the other hand, we can speculate that, in obese rats, AGT overproduction by adipose tissue is increasing with its development and could contribute to promoting a later onset of hypertension when the mass of adipose tissue represents an important part of the body weight (37).

In conclusion, the data presented here demonstrate that during the onset of obesity, there is an adipose tissue-specific increase in the production of AGT, which is concomitant with the overexpression of genes of lipid storage-related enzymes. These results raise the possibility that AGT, besides its role in vascular differentiation of preadipose cells: role of prostacyclin upon stimulation of adipose cells by angiotensin-II. Endocrinology 114: 776–785, 1984.


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