

Angiotensinogen-Deficient Mice Exhibit Impairment of Diet-Induced Weight Gain with Alteration in Adipose Tissue Development and Increased Locomotor Activity

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White adipose tissue is known to contain the components of the renin-angiotensin system, which gives rise to angiotensin II from angiotensinogen (AGT). Recent evidence obtained *in vitro* and *ex vivo* is in favor of angiotensin II acting as a trophic factor of adipose tissue development. To determine whether AGT plays a role *in vivo* in this process, comparative studies were performed in AGT-deficient ($agt^{-/-}$) mice and control wild-type mice. The results showed that $agt^{-/-}$ mice gain less weight than wild-type mice in response to a chow or high fat diet. Adipose tissue mass from weaning to adulthood appeared altered rather specifically, as both the size and the weight of other organs were almost unchanged. Food intake

was similar for both genotypes, suggesting a decreased metabolic efficiency in $agt^{-/-}$ mice. Consistent with this hypothesis, cellularity measurement indicated hypotrophy of adipocytes in $agt^{-/-}$ mice with a parallel decrease in the fatty acid synthase activity. Moreover, AGT-deficient mice exhibited a significantly increased locomotor activity, whereas metabolic rate and mRNA levels of uncoupling proteins remained similar in both genotypes. Thus, AGT appears to be involved in the regulation of fat mass through a combination of decreased lipogenesis and increased locomotor activity that may be centrally mediated. (*Endocrinology* 142: 5220–5225, 2001)

ANGIOTENSINOGEN (AGT), the unique substrate of renin, is the precursor of angiotensin I (AngI) that gives rise to active angiotensin II (AngII) through the action of AngI-converting enzyme. The renin-angiotensin system is known to have a major role in the regulation of blood pressure and fluid and sodium homeostasis (1). White adipose tissue (WAT) is an important extrahepatic production site of AGT (2), and several reports have suggested the existence of a functional renin-angiotensin system in this tissue. In isolated adipocytes and cultured adipose cells from rodents and human, recent data have shown the presence of 1) renin, by RT-PCR, and renin-like activity; 2) AngI-converting enzyme, by RT-PCR and Western blot; and 3) AngII production (3–5). In addition to the systemic effect of AngII in the regulation of blood pressure, various roles of AGT via locally produced AngII have been proposed: 1) at the time of adipose tissue development, AngII appears to be a trophic factor that is involved in organogenesis of rodent, primate, and human fetuses (6); 2) AngII has been implicated in cell cycle progression of human preadipocytes, the cell type that precedes the formation of nondividing adipocytes (7); 3) both *in vitro* and *in vivo*, AngII stimulates the production and release of prostacyclin from adipocytes, which, in turn, stimulates adipogenesis of adipose precursor cells (8, 9); and 4) AngII increases lipogenesis and triglyceride accumulation in

3T3-L1 preadipose cells and human adipocytes (10), consistent with the observation that rats treated with an oral AngII receptor antagonist (losartan) exhibit a decrease in adipocyte size (11). In contrast to liver cells, in which numerous hormones have been shown to enhance AGT mRNA levels and AGT secretion, adipose cells respond only to fatty acids and glucocorticoids, which are known to be implicated in the hyperplastic and hypertrophic growth of WAT (12, 13). Collectively, these studies indicate that AngII plays a local role in the development of adipose tissue and its cellularity, *i.e.* fat cell number and size. To gain a better understanding of the effects of AngII on adipose tissue growth, we examined the comparative development of WAT and brown adipose tissue (BAT) in wild-type (WT) and AGT-deficient ($agt^{-/-}$) mice in response to a standard chow or a high fat diet.

Materials and Methods

Mice

Generation of $agt^{-/-}$ mice has been previously described. Briefly, chimeric mice were backcrossed with ICR mice for at least 10 generations (14), then 5 $agt^{-/-}$ males and 15 $agt^{-/-}$ females were bred to generate further generations. AGT-deficient mice reproduced normally. Although some perinatal lethality occurred, as previously reported (15), their life expectancy after weaning was not different from that of WT mice.

Only male $agt^{-/-}$ mice were used in the experiments herein, and ICR-CD1 control WT mice were purchased from Harlan (Gammat, France). Animals were housed five per cage and had free access to food and water in a controlled environment with a 12-h light, 12-h dark cycle and constant temperature (22 C). At weaning, the mice were fed either

Abbreviations: AGT, Angiotensinogen; AngII, angiotensin II; BAT, brown adipose tissue; FAS, fatty acid synthase; UCP-1, uncoupling protein-1; WAT, white adipose tissue; WT, wild-type.

a standard laboratory chow diet or a high fat diet containing 1% cholesterol, 30% corn oil (representing 65% of calories as fat), 27% carbohydrates, 11.5% proteins, and 1.9% minerals (UAR, Villemoisson, France). Body weight was assessed weekly for up to 46 wk. At the indicated times mice were killed by cervical dislocation according to French Centre National de la Recherche Scientifique ethical guidelines. Epididymal WAT, BAT, and hind limb skeletal muscle were rapidly removed and immediately used for RNA preparation.

Food consumption and feces analysis

Mice were housed individually in metabolic cages (Marty Technology, Marcilly-sur-Eure, France) for 1 wk, fed *ad libitum* with a standard or high fat diet, and given free access to water in a controlled environment at 22 C with a 12-h light, 12-h dark cycle. Food consumption was measured during the last 4 d as the difference between the amount of food given and that removed from the cage after the amount of any food spilled was taken into account. Similarly, feces were collected during the last 4 d of feeding, and the weight of pooled feces was determined after drying at 70 C to a constant weight. The fat content of the feces was determined by the Soxhlet extraction method using petroleum benzene.

Body weight and body composition

Body weight was measured at the same time each day. For body composition, mice were killed by cervical dislocation, and the whole carcasses were incised, dried to a constant weight at 70 C, then subsequently homogenized. Total body fat content was determined by the Soxhlet extraction method as described above. The results are presented as absolute weight (grams) and as percentage of total body weight. The fat-free mass, which includes mineral content (which accounts for ~2% of fat-free mass in mice) was obtained by subtraction of body fat content from dry weight.

Adipose tissue cellularity

The size and number of adipocytes were determined as previously described (16). Briefly, fat cell size was determined by a procedure derived from a microphotometric method; micrographs of isolated cells were taken with a light microscope, and measurement of cell diameters was performed using a computer equipped with an image analyzer. Fat cell number was estimated from a portion of adipose tissue by dividing the lipid content by the average fat cell weight.

Fatty acid synthase (FAS) activities

FAS activities were assayed spectrophotometrically in crude cytosolic extracts of epididymal fat pads by measuring the oxidation of NADPH in the presence of acetyl coenzyme A and malonyl coenzyme A (17). Data are expressed as nanomoles of NADPH oxidized per min/mg, *i.e.* millimoles per mg cytosolic proteins, which were assayed by the method of Bradford (17).

Isolation and analysis of RNA

RNA was extracted using the RNeasy Midi kit according to the manufacturer's protocol (QIAGEN, Courtaboeuf, France). Northern blot analysis was performed as described previously (18). Autoradiographs were quantified using a Fujix PhosphorImager (Tokyo, Japan). All results were normalized to β -actin signals.

Measurements of metabolic rate and locomotor activity

For locomotor activity and metabolic rate measurements, mice were randomly and alternatively placed into the respective experimental chambers; at least 1 wk separated successive testing. Metabolic rate was measured by indirect calorimetry during 24 h. An open circuit calorimeter, as described in detail previously (19), equipped with a sensitive mass flow meter (model 5875, Brooks Instrument, Veenendaal, The Netherlands) was used. Food and water were available during testing. The ambient temperature was set at 22 C. The data were recorded every 5 sec by an on-line computerized data acquisition system (SICMU, CMU, Geneva, Switzerland). The metabolic rate was calculated using Weir's

equation and expressed in terms of watts per kg BW to the 0.75 power. For each mouse, the mean metabolic rate was calculated for the last 23 h.

For locomotor activity, the system used has been previously described (19). The home-cage traveled distance was measured during either the lights on or lights off cycle. When placed in this experimental set-up, mice did not have access to food or water. Quantitative analyses of the distance traveled during the entire period were made off-line. The fraction of time spent in activity was calculated by measuring the time during which the animal showed a displacement of its center of mass of at least 1 cm. All calculations were made using 386-Matlab (Mathworks, Sherborn, MA).

Blood parameters

Mice were anesthetized 2 h after lights on with 60 μ l xylene/ketamine (1:4, vol/vol). Blood was collected by eye puncture into tubes containing citrate at a final concentration of 0.01 M. After 10 min in ice, plasma was separated by centrifugation at 10,000 \times g for 10 min and stored at -20 C. A volume of 100 μ l of a 1:2 dilution was used for mouse leptin assays using a commercial kit (R&D Systems, Inc., London, UK). Glucose, triglycerides, total cholesterol, and free T₃ were determined using standard laboratory procedures. Insulin was determined by RIA using a commercial kit with a rat insulin standard (CIS Biointernational, Gif-sur-Yvette, France).

Statistical analysis

All data are expressed as the mean \pm SEM. The values were examined by the one-way ANOVA or *t* test with the computer software STATISTIX, version 4.0 (Analytical Software, Tallahassee, FL).

Results

AGT-deficient mice gain less weight than WT mice in response to diets

Male *agt*^{-/-} and WT mice were fed a chow diet or a high fat diet from weaning up to 46 wk of age. Figure 1 shows that *agt*^{-/-} mice exhibited, at weaning, a lower body weight than WT mice. This difference remained throughout development and was still observed at adulthood. At 6 wk of age and thereafter, *agt*^{-/-} mice fed a chow diet weighed 21% less than WT mice. At that age, the epididymal fat pad mass of chow-fed *agt*^{-/-} mice was 2-fold lower than that of WT mice (see Table 2), and at 20 wk of age (Table 1, Exp 1), the total fat mass of *agt*^{-/-} mice, determined on whole carcasses, was

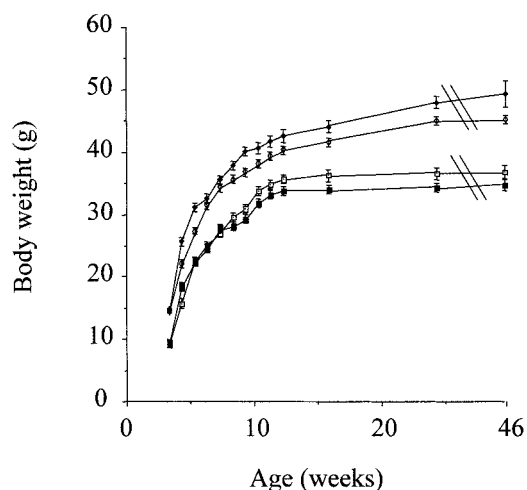


FIG. 1. Body weights of WT (◇ and ◆) and AGT-deficient (□ and ■) mice fed a chow diet (◇ and □) or a high fat diet (◆ and ■) from weaning onward (n = 30).

1.9-fold lower. Due to the trophic role of AngII in organogenesis, it is of interest to note that the effect of AGT deficiency was mainly confined to adipose tissue. At 6 wk of age, no effect on body length was observed (8.8 ± 0.4 cm for WT *vs.* 9.0 ± 0.5 for $agt^{-/-}$ mice; $n = 20$), and only a modest effect (<1.2 -fold) was detected on the weights of heart, kidney, and liver. Although fat-free mass was lower in $agt^{-/-}$ mice, leanness (expressed as protein mass per g BW) was similar in $agt^{-/-}$ and WT mice in adulthood (Table 1). In WT mice, a weight gain of 10–15% was induced by high fat feeding; in contrast, no weight gain but, instead, a slight decrease (6%) was observed in $agt^{-/-}$ mice compared with mice fed a chow diet (Fig. 1). The lack of weight gain in $agt^{-/-}$ mice fed a high fat diet could be due to lower food intake and/or defective

intestinal absorption. As shown in Table 1, this was excluded, as food intake was higher in chow-fed $agt^{-/-}$ than in WT mice and even higher in $agt^{-/-}$ mice when corrected for body mass to the 0.75 power, consistent with similar levels of circulating leptin (see Table 3). In high fat-fed animals, food intake was slightly lower in $agt^{-/-}$ mice, but, when expressed per body mass to the 0.75 power, both values were similar. Feces analysis did not show any difference in daily quantity or fat content expressed as a percentage of dry weight between WT and $agt^{-/-}$ mice. Because the body composition data in Table 1 indicate that the lower body weight was partly due to a lower fat mass, a detailed analysis of WAT cellularity was performed.

AGT deficiency leads to alterations in WAT cellularity

When fed a standard chow diet, a 2-fold difference was observed in the weight of epididymal fat pads between WT mice and $agt^{-/-}$ mice. This was due to hypotrophy of adipocytes in $agt^{-/-}$ mice, as the number of adipocytes was similar in both genotypes. Upon high fat feeding, *i.e.* under conditions of increased supply of exogenous fatty acids to adipose tissue, the size of adipocytes increased in both genotypes, but to a greater extent in WT mice. A significantly lower weight of epididymal fat pads and fat cell size persisted in $agt^{-/-}$ mice (Table 2). Importantly, in $agt^{-/-}$ mice only, adipose tissue hypoplasia was observed in high fat-fed mice compared with chow-fed mice.

To gain some insights on the metabolic pathways leading to triglyceride accumulation in adipocytes, measurement of endogenous fatty acid synthesis was performed by determining FAS activities of cytosolic extracts of epididymal fat pads of WT and $agt^{-/-}$ mice. Data from Table 2 show that FAS activity was 2.2-fold higher in extracts from WT mice than in those from $agt^{-/-}$ mice, consistent with the 2.6-fold increase observed in adipocyte weight. This observation is also in accordance with a report showing that AngII regulates lipogenesis by increasing FAS activity (10). Upon high fat feeding, it is known that the exogenous supply of fatty acids from chylomicrons increases dramatically, leading to a down-regulation of FAS activity. As shown in Table 2, this down-regulation was taking place in WT mice, but not in $agt^{-/-}$ mice, suggesting that this modulation was AngII related.

TABLE 1. Body weight, food intake, body composition, and feces analysis in wild-type and AGT-deficient mice (16–20 wk old)

	Wild-type (n = 6)	AGT-deficient (n = 9)	P
Exp 1: chow diet			
BW (g)	48.5 ± 1.7	34.2 ± 1.2	<0.005
Food intake (g/day)	5.3 ± 0.3	5.4 ± 0.2	NS
Food intake (g/day·g BW ^{0.75})	0.3 ± 0.01	0.4 ± 0.02	<0.05
Fat mass (g)	4.5 ± 0.7	2.4 ± 0.3	<0.01
Adiposity (% of BW)	9.1 ± 1.2	6.9 ± 0.6	<0.05
Fat-free mass (g)	11.2 ± 0.3	8.1 ± 0.2	<0.001
Leanness (% of BW)	23.1 ± 0.3	23.7 ± 0.2	NS
Feces (g/day)	1.3 ± 0.1	1.1 ± 0.1	<0.01
Feces fat content (% of dry wt)	2.4 ± 0.2	2.5 ± 0.2	NS
	Wild-type (n = 8)	AGT-deficient (n = 8)	P
Exp 2: high fat diet			
BW (g)	48.5 ± 1.2	30.7 ± 1.3	<0.001
Food intake (g/day)	5.0 ± 0.2	4.0 ± 0.45	<0.01
Food intake (g/day·g BW ^{0.75})	0.3 ± 0.01	0.3 ± 0.02	NS
Feces (g/day)	1.1 ± 0.1	1.1 ± 0.1	NS
Feces fat content (% of dry wt)	4.1 ± 0.2	3.9 ± 0.2	NS

Mice were fed either a chow diet (Exp 1) or a high fat diet (Exp 2) from weaning onward. For body composition, mice were weighed and killed at 20 weeks of age, and total carcass fat content and fat-free dry mass were determined as described in *Materials and Methods*. All results are the means \pm SE. n, Number of animals.

TABLE 2. Cellularity of epididymal fat in 6-wk-old mice fed a chow or a high fat diet after weaning

	Chow diet			High fat diet		
	Wild-type	AGT-deficient	n	Wild-type	AGT-deficient	n
BW (g)	30.4 ± 0.2	24.3 ± 0.3^a	50	33.3 ± 0.6^b	23.8 ± 0.6^c	33
Epididymal fat (mg)	248.8 ± 13.0	124.5 ± 7.0^a	36	365.0 ± 32.0^b	$251.5 \pm 17.0^{d,g}$	8
Fat cell no. ($\times 10^6$) ^e	4.6 ± 0.5	5.9 ± 0.7	10	3.6 ± 1.4	3.9 ± 0.5^d	6
Cell diameter (μ m)	48 ± 1	31.2 ± 1^a	10	53 ± 2.6^b	48 ± 2.6^d	6
Cell weight (ng)	55.8 ± 4.4	21.2 ± 1.5^a	10	81.3 ± 6.9^a	$56.8 \pm 8.4^{f,g}$	6
FAS activity (mU/mg)	28.3 ± 3.5	12.8 ± 1.5^a	5	11.7 ± 3^a	10.5 ± 4.3^d	6

FAS, Fatty acid synthase.

^a $P < 0.001$ *vs.* chow-fed wild-type.

^b $P < 0.01$ *vs.* chow-fed wild-type.

^c $P < 0.001$ *vs.* high fat-fed wild-type.

^d $P < 0.005$ *vs.* chow-fed AGT-deficient.

^e Fat cell number is expressed per two epididymal fat pads.

^f $P < 0.001$ *vs.* chow-fed AGT-deficient.

^g $P < 0.01$ *vs.* high fat-fed wild-type.

AGT deficiency and thermogenesis-related parameters

Comparative analysis of the main metabolic blood parameters is shown in Table 3. Compared with WT mice, *agt*^{-/-} mice exhibited a moderate decrease in circulating levels of cholesterol and triglycerides. Although statistically significant, the increase observed in glucose levels in *agt*^{-/-} mice was slight and could not be considered physiologically important. In addition, insulin levels were similar. Upon high fat feeding, some interesting features emerged. First, glucose levels of *agt*^{-/-} mice remained unchanged, thus abolishing the slight difference observed between the two genotypes. Second, triglyceride levels were significantly decreased, whereas cholesterol levels were increased, in agreement with the responsiveness to dietary fat and cholesterol reported in various strains of inbred mice (20). Free T₃ was also determined, as this hormone has been long known to be involved in thermogenesis (21). Table 3 shows similar levels of free T₃ in chow-fed WT and *agt*^{-/-} mice. However, upon high fat feeding, the levels were significantly increased in both genotypes. Interestingly, a significant increase (1.3-fold) was observed in the level of free T₃ in *agt*^{-/-} mice compared with that in WT mice. The levels of insulin and leptin were similar in both genotypes when fed a standard chow or a high fat diet. Moreover, these data are in agreement with the fact that at 6 wk of age, no statistically significant difference between the two genotypes was observed with respect to interscapular BAT weight (59.4 ± 2.9 mg for WT mice vs. 61.9 ± 2.2 mg for *agt*^{-/-} mice; n = 12) and uncoupling protein-1 (UCP-1) mRNA levels (Fig. 2). Northern blot analysis was also performed for UCP-2 from epididymal fat pads and for UCP-3 from skeletal muscle. Using β-actin mRNA levels as an internal standard and taking an arbitrary unit of 1 for WT mice, the values for *agt*^{-/-} mice were, respectively, 1.09 for UCP-1 (n = 9; P = NS), 1.13 for UCP-2 (n = 6; P = NS), and 1.24 for UCP-3 (n = 3; P = NS).

AGT-deficient mice exhibit increased locomotor activity

To investigate whether changes in energy expenditure could account for the lower metabolic efficiency in AGT-deficient mice compared with WT mice, metabolic rate and locomotor activity were measured in chow-fed mice. When oxygen consumption was corrected for body weight, similar mean metabolic rates were obtained for both genotypes. Locomotor activity was significantly enhanced in *agt*^{-/-} mice; the distance covered during lights on and lights off was

increased by 69% and 32% compared with that in WT mice, respectively. The time spent in activity during the lights on periods was also increased in *agt*^{-/-} mice compared with WT mice (Table 4).

Discussion

To determine the role of AGT in body weight and composition, we performed a detailed comparison of *agt*^{-/-} vs. control WT mice. The phenotype of the AGT-null mouse line offers clues to the function of AGT as a secretory product from adipocytes. It is known that AngII plays a trophic role in fetal and/or postnatal development in rodents and humans (6). Our results provide evidence that in the absence of AGT, WAT development from weaning to adulthood is impaired, whereas both the length of the mice and the weight of other organs are only weakly altered, if at all. Contributing in part to the lower body weight are the smaller fat stores in *agt*^{-/-} mice compared with WT mice. Cellularity measurements of epididymal fat pads indicate that this was mainly

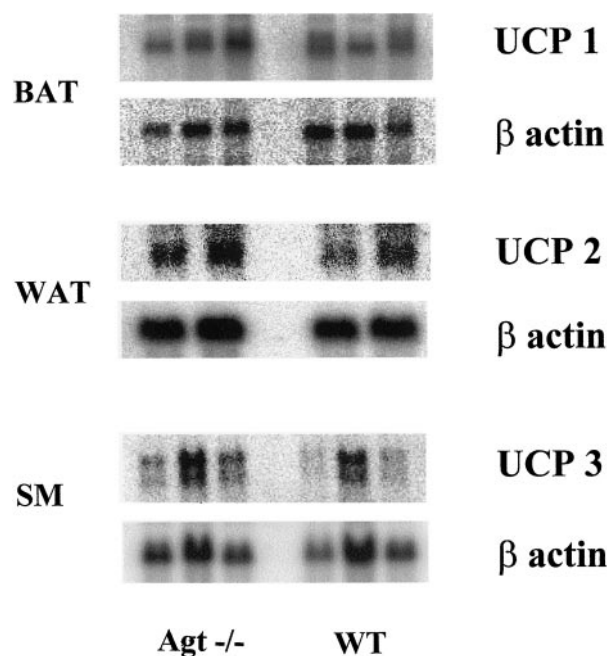


FIG. 2. UCP RNA content of interscapular BAT, epididymal WAT, and skeletal muscle (SM) of 6-wk-old WT and AGT-deficient (*agt*^{-/-}) mice.

TABLE 3. Blood parameters in 6-wk-old mice fed a chow or a high fat diet after weaning

	Chow diet			High fat diet		
	Wild-type	AGT-deficient	n	Wild-type	AGT-deficient	n
Triglycerides (g/liter)	1.04 ± 0.01	0.67 ± 0.07 ^a	18	0.28 ± 0.03 ^a	0.27 ± 0.05 ^b	18
Cholesterol (g/liter)	0.96 ± 0.03	0.74 ± 0.03 ^a	18	1.76 ± 0.08 ^a	1.57 ± 0.01 ^b	18
Glucose (g/liter)	2.3 ± 0.09	2.65 ± 0.14 ^c	18	3.09 ± 0.16 ^a	2.94 ± 0.14	18
Insulin (μU/ml)	34.3 ± 5.2	28.3 ± 5.7	12	30.4 ± 7.2	28.9 ± 5.5	12
Leptin (ng/ml)	2.09 ± 0.24	2.01 ± 0.22	18	1.9 ± 0.29	2.53 ± 0.49	12
T ₃ (pmol/liter)	4.43 ± 0.18	4.88 ± 0.40	10	5.18 ± 0.27 ^c	6.74 ± 0.54 ^{a,d}	6

^a P < 0.001 vs. chow-fed wild-type.

^b P < 0.001 vs. chow-fed AGT-deficient.

^c P < 0.01 vs. chow-fed wild-type.

^d P < 0.005 vs. chow-fed AGT-deficient.

TABLE 4. Metabolic rate and locomotor activity in 16-wk-old wild-type and AGT-deficient mice fed a chow diet

	Wild-type (n = 6)	AGT-deficient (n = 9)	P
Metabolic rate (watts/kg ^{0.75})	5.1 ± 0.2	5.1 ± 0.2	NS
Distance covered (lights on; m)	29.5 ± 5.5	49.8 ± 3.8	<0.01
Distance covered (lights off; m)	83.6 ± 5.7	110.5 ± 6.8	<0.02
Activity (lights on; % of time)	31.6 ± 4.5	45.2 ± 3.0	<0.02
Activity (lights off; % of time)	55.0 ± 4.5	68.6 ± 4.3	NS

due to adipocyte hypotrophy in *agt*^{-/-} mice compared with WT mice. The lower triglyceride content of adipocytes in *agt*^{-/-} mice was tightly correlated with decreased endogenous lipogenesis through decreased FAS activity. This is in accordance with a report showing that AngII enhances triglyceride accumulation by stimulating FAS and glycerol-3-phosphate dehydrogenase activities (10).

In contrast to WT mice, which gained weight between 12 and 46 wk of age on high fat feeding, the body weight of *agt*^{-/-} mice remained stable. Under the conditions of augmented exogenous fatty acid supply, the weight of epididymal fat pads remained 1.8-fold higher in WT mice than in *agt*^{-/-} mice at 6 wk of age, and this increase became more evident at 16 wk of age. As anticipated, during high fat feeding, hypertrophy of adipocytes occurred in *agt*^{-/-} mice, but a large difference persisted, as the weights of adipocytes of WT mice remained much higher than those of *agt*^{-/-} mice. The stable body weight of *agt*^{-/-} mice appears at odds with the increase in adipocyte size observed in the epididymal depot. However, it cannot be ruled out that the growth of this depot may not be identical to that of the other adipose depots, in a way similar to the differential growth of different adipose depots in response to nutritional or environmental stimuli (22).

In search of additional factors that could explain the lower body weight of *agt*^{-/-} mice compared with WT mice in response to a chow or a high fat diet, deficient mice show a decrease in food efficiency, as estimated by the ratio of the mean 23-h metabolic rate divided by the energy content of the food eaten by the same animal. This ratio is significantly (*P* < 0.001) lower in *agt*^{-/-} mice than in WT mice. This finding suggests the occurrence of activation of futile cycles in the metabolic pathways of *agt*^{-/-} mice. In looking for possible mechanisms to explain the increased energy dissipation of these mice, our data probably exclude a difference in intestinal absorption, as both the amount and the fat content of feces were similar. In addition, leptin, which is known to increase sympathetic activity (23) and energy expenditure in *ob/ob* mice (24), is also excluded, as circulating leptin levels were similar in the two genotypes fed either a standard or a high fat diet. In agreement with this assumption, the sympathetic pathway did not seem to be altered, as shown indirectly by UCP-1 expression levels in BAT. Free T₃ levels were similar in *agt*^{-/-} mice and WT mice fed a chow diet. Upon high fat feeding, although both genotypes increased their levels of free T₃ compared with those in chow-fed animals, a significant hyperthyroidism was seen in *agt*^{-/-} mice compared with WT mice. This additional component may contribute to lower the metabolic efficiency of AGT-deficient mice.

Locomotor activity, as expressed by the distance covered, was clearly and significantly increased in *agt*^{-/-} mice compared with WT mice and may participate to some extent in the higher energy dissipation (19). Therefore, it is assumed that the more frequent and longer activity periods of AGT-deficient mice, in addition to decreased lipogenesis, are responsible for the decreased fat deposition. It has been reported in rats that brain AGT participates in a central regulation of blood pressure (25), and it can be hypothesized that AngII affects similarly the central pathway(s) leading to increased locomotor activity. In summary, our results show that, compared with WT mice, *agt*^{-/-} mice do not gain weight in response to a high fat diet and exhibit alterations in WAT development and locomotor activity, supporting the involvement of AngII in the regulation of body fat mass.

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