Angiotensinogen gene expression in adipose tissue: analysis of obese models and hormonal and nutritional control

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Jones, Brynn H., Melissa K. Standridge, James W. Taylor, and Naïma Moustaïd. Angiotensinogen gene expression in adipose tissue: analysis of obese models and hormonal and nutritional control. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R236–R242, 1997.—Synthesis of angiotensin II (ANG II) has recently been described in adipose cells and has been linked to regulation of adiposity. Angiotensinogen (AGT), the substrate from which ANG II is formed, was previously shown to be elevated in adipose tissue of obese (*ob/ob* and *db/db*) mice and regulated by nutritional manipulation. It is unknown, however, whether overexpression of adipose AGT can be extended to other models of obesity and whether hormonal and/or nutritional factors directly regulate AGT expression in adipocytes. We investigated these possibilities by analyzing AGT mRNA levels in adipose tissue of obese Zucker rats, viable yellow (A^{vy}) mice, and humans and by treating 3T3-L1 adipocytes with insulin, glucose, and a β-adrenergic agonist. We demonstrate that AGT mRNA is decreased by ~50 and 80%, respectively, in adipose tissue of obese vs. lean Zucker rats and A^{vy} mice. We also report that AGT is expressed at variable levels in human adipose tissue. Finally, we show that AGT mRNA is upregulated by insulin and downregulated by β-adrenergic stimulation in adipocytes.

human adipose tissue; 3T3-L1 adipocytes; Zucker rat; A^{vy} mouse

IN RECENT YEARS, the synthesis of angiotensin II (ANG II) has been described in various tissues. In addition to the classical pathway of ANG II synthesis involving the liver, kidney, and endothelium, tissue renin-angiotensin systems (RAS), which consist of all components necessary for ANG II synthesis, have been identified in numerous peripheral tissues. Accordingly, the classical function of ANG II as a modulator of vascular tone and hemodynamics has been expanded: in many tissues the function of locally produced ANG II has been linked to control of tissue growth and development (28).

Adipose tissue is one of the tissues shown to express its own RAS. Expression of the angiotensinogen gene (AGT), the precursor from which ANG II is formed, has been reported in murine adipocyte cell lines and in rat and mouse adipose tissue (1, 9, 31). Synthesis and secretion of ANG II has been confirmed in adipocyte cell lines (32). Consistent with other tissues expressing RAS, adipocyte ANG II has also been linked to control of adipose tissue growth and development. Several clinical and laboratory studies using angiotensin-converting enzyme (ACE) inhibitors to control blood pressure have reported weight loss as a side effect (8, 12, 22, 23, 29). Crandall et al. (6) specifically investigated this issue by treating rats with an oral ANG II receptor antagonist (losartan) and measuring the effects on adiposity. Losartan-treated rats displayed significantly reduced fat pad weights and adipocyte size, independent of food intake. Harp and DiGirolano (11) recently reported that AGT mRNA and protein levels were positively related to relative rates of adipocyte growth in rats. In humans, linkage between genetic variation at the AGT locus and waist-to-hip ratio was recently reported in a population of male Hutterites (13). According to a recent report by Darimont et al. (7), cellular mechanisms that link adipocyte ANG II to control of adipose mass may involve recruitment of preadipocytes to differentiate into mature fat cells. These authors demonstrated that ANG II treatment of mature adipocytes (Ob 1771) induced differentiation of cocultured preadipocytes. In agreement with these studies, we have recently demonstrated that ANGII increases lipogenesis in murine and human adipocytes (17a).

In light of a potential role for ANG II in control of adiposity, it is particularly interesting that AGT synthesis was recently shown to be threefold higher in adipose tissue of genetically obese mice (*ob/ob* and *db/db*) than in lean controls (9). However, it is unknown whether these results can be extrapolated to other models of obesity. The serine protease adipsin, for example, was reported to be much lower in *ob/ob* and *db/db* mice vs. lean controls and was originally linked to obesity; however, no significant differences were seen in adipsin levels between lean and obese Zucker rats (reviewed in Ref. 20) or in obese compared with lean humans (25). We therefore were interested to determine whether the obese-specific regulation of AGT in adipose tissue described by Fredrich et al. (9) could be extended to other animal models of genetic obesity, specifically the Zucker obese rat and the viable yellow (A^{vy}) mouse. We further investigated whether AGT mRNA was detected in human adipose tissue and, if so, whether these levels were elevated in obese compared with lean human subjects. Finally, we determined whether hormonal and nutritional factors known to regulate adipocyte metabolism also modulated AGT expression in adipose cells.

MATERIALS AND METHODS

Animals. Lean Zucker rats (*Fa/?*) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and bred to produce lean and obese rats. Pups were weaned and sexed at 21 days; obese and lean pups were identified on the basis of body weight. C57BL/6J-A^{vy} mice were purchased from the Jackson Laboratory and maintained at the Oak Ridge National Laboratory (Oak Ridge, TN) by mating A^{vy}/a mice to nonagouti a/a black siblings. Obese (A^{vy}/a) mice and lean control nonagouti (a/a) mice were identified on the basis of coat color (yellow or

black, respectively). All animals were fed standard rat or mouse chow (Ralston Purina, St. Louis, MO) and housed in a climate-controlled environment (21°C) with a 12-h light-dark cycle. Four or five male animals of each phenotype (lean and obese), age 14 wk, were included in this study. This protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee. Animals were anesthetized with pentobarbitol sodium and killed by exsanguination while under anesthesia. Liver and abdominal adipose tissue were snap-frozen into liquid nitrogen for subsequent RNA isolation.

3T3-L1 cells. 3T3-L1 cells (American Type Culture Collection, Rockville, MD) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (standard medium) in 100-mm culture dishes, as previously described (16). Cells were induced to differentiate into an adipocyte phenotype by a modification of the method of Rubin et al. (30). At confluency (*day 0*), the medium was supplemented with 250 nM dexamethasone and 0.5 mM isobutyl-methylxanthine for 2 days, after which cells were returned to standard medium. Differentiation was considered to be complete at *day 5*, at which time cells were incubated in either serum-free medium (SFM) containing 1% bovine serum albumin (BSA; insulin and isoproterenol experiments) or glucose-free medium (GFM) supplemented with 10% FBS (glucose experiments) for 36 h. Insulin (0.1 nM, 10 nM, or 1 μM) and isoproterenol (10 or 100 nM) treatments were carried out in SFM for 48 h; controls consisted of cells in SFM + BSA (1%) only. In the glucose experiments, cells were either switched to standard medium containing 0.5, 1.0, or 2.5 mM glucose or were maintained in GFM for 48 h. After treatment, adipocytes were harvested and prepared for RNA isolation using the guanidine/CsCl method, as we previously described (16). Individual culture dishes (100 mm) of cells were used for each RNA sample within treatments, and 3 or 4 samples were included in each treatment group in an experiment. All experiments were repeated at least twice.

Humans. Subcutaneous abdominal adipose tissue was taken from 12 female, Caucasian patients, 19–58 years of age, undergoing elective cosmetic surgery. Samples were obtained in compliance with a protocol approved by the Institutional Review Board for Human Subjects and by the Committee for Research Protocols at the University of Tennessee, Knoxville. All patients were nondiabetic and nonhypertensive, with no known metabolic abnormalities. Patients with a body mass index (BMI) >28 were considered obese; seven nonobese and five obese patients were included in this study. Tissue samples to be used for RNA isolation were frozen immediately until use. Human adipocytes were isolated by collagenase digestion and filtration of adipose tissue, as we recently described (24). Mature, lipid-filled adipocytes were separated from stromal-vascular cells by centrifugation, the latter of which were discarded. Floating cells (mature adipocytes) were maintained in primary culture in DMEM supplemented with 1% FBS for 2 wk. Viability during culture was confirmed by Trypan blue exclusion, glucose consumption, and activity levels of glycerol-3-phosphate dehydrogenase and fatty acid synthase enzymes (24).

RNA analysis. RNA was prepared by centrifugation of tissue homogenates through CsCl density gradients. RNA from all samples within an experiment were electrophoresed on the same agarose gel, transferred to positively charged nylon membranes, and analyzed by Northern blotting. Membranes were hybridized with ^{32}P-labeled cDNA probes for rat AGT (kindly provided by Dr. K. Lynch, University of Virginia) or human AGT (kindly provided by Dr. E. Clauser, College de France, Paris, France), stearoyl-CoA desaturase (SCD; kindly provided by Dr. P. Smith, Veterans Affairs Medical Center, East Orange, NJ), and β-actin (American Type Culture Collection). Unbound probe was removed by washing membranes in 2× saline-sodium phosphate-EDTA (SSPE) for 30 min at 25°C and then in 0.1× SSPE-0.1% sodium dodecyl sulfate for 60 min at 65°C. After washing, membranes were exposed to X-ray film (New England Nuclear, Boston, MA). Autoradiograms were analyzed by densitometric scanning, and data were expressed as the ratio of AGT to β-actin. Results are reported as means ± SE.

Statistics. Student's *t*-test (Figs. 1 and 2) and analysis of variance (ANOVA; Fig. 4) were used to compare overall group means. The Bonferroni multiple-comparisons procedure (26) was used to compare pairs of means following a significant *F* test (Fig. 4). All tests were conducted using a 95% confidence interval.

RESULTS

Expression of the AGT gene in liver and adipose tissue of obese Zucker rats. Northern blot analysis of RNA isolated from Zucker rat liver and adipose tissue revealed a single band of the expected size (~1.8 kb) for AGT. Hepatic levels of AGT mRNA, obtained by densitometric scanning and normalized to β-actin, are shown in Fig. 1*A*. No significant differences in hepatic AGT mRNA levels between lean and obese rats were detected (2.35 ± 0.46 vs. 2.34 ± 0.43, respectively). These data are consistent with expression of AGT in liver of genetically obese (*ob/ob* and *db/db*) and lean mice, in which no effect of obesity was observed (8).

Figure 1*B* represents Northern blot analysis of adipose tissue AGT mRNA in lean and obese Zucker rats. AGT mRNA content in adipose tissue from obese rats was ~50% lower than in lean rats (3.61 ± 0.68 vs. 1.79 ± 0.15; $P < 0.05$). The signal for SCD mRNA, which we have previously reported to be significantly elevated in adipose tissue of Zucker rats (17), is shown as a control.

Expression of the AGT gene in liver and adipose tissue of lean (a/a) and obese (A^{vy}) mice. Figure 2*A* depicts hepatic AGT mRNA levels in lean and obese (A^{vy}) mice. As in *ob/ob* and *db/db* mice (9) and in Zucker rats (Fig. 1*A*), there was no effect of obesity on AGT mRNA levels in liver. Adipose tissue levels of AGT mRNA are shown in Fig. 2*B*. Like the obese Zucker rat, A^{vy} mice displayed significantly reduced expression of AGT (0.686 ± 0.17 vs. 0.102 ± .00004; $P < 0.01$) in adipose tissue relative to lean controls. These data are in direct contrast to previous reports regarding AGT expression in adipose tissue of obese mice (*ob/ob* and *db/db*), in which AGT mRNA and protein levels were increased two- to four-fold over lean controls (9). The signal for SCD mRNA, which we recently reported to be increased in obese A^{vy} mice (16), is shown as a control.

Expression of the AGT gene in human adipose tissue and primary cultures of human adipocytes. The results of Northern blot analysis for AGT mRNA levels in human adipose tissue are shown in Fig. 3*A*. Hybridization of human adipose tissue RNA with the human AGT cDNA revealed a band of the expected size [~2.0 kb (4)]. AGT mRNA was detectable by Northern blot in adipose tissue of 7 (*lanes 1, 4, 5, 7-10*) of the 12 patients examined. Among these seven patients, expression varied from low (*lanes 4* and *10*) to high (*lanes 1* and *9*)

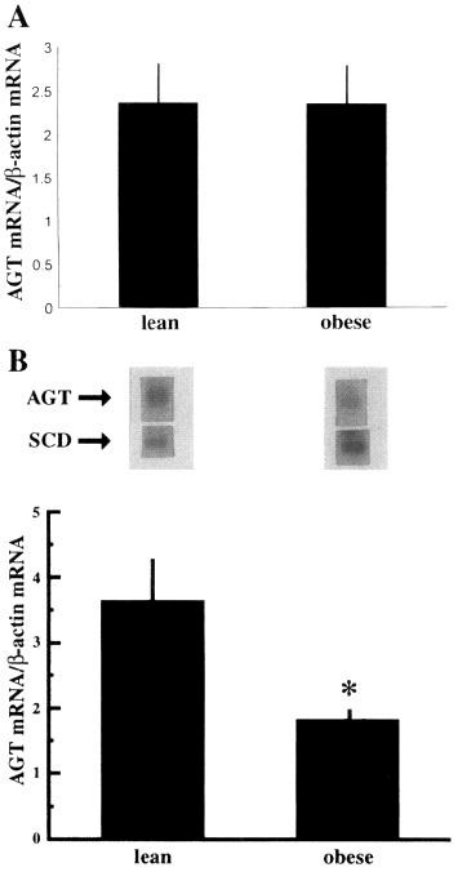

Fig. 1. Northern blot analysis of angiotensinogen (AGT) mRNA levels in liver and adipose tissue of lean and obese Zucker rats. RNA was extracted and analyzed as described in MATERIALS AND METHODS. Twenty-five micrograms of total RNA was loaded in each lane. Values for AGT and β-actin mRNA levels depicted in bar graphs were obtained by densitometric scanning of autoradiograms. Data are presented as the mean ± SE of AGT/β-actin for 4 (obese) or 5 (lean) animals from each phenotype. *A*: AGT mRNA levels in liver. *B*: AGT mRNA levels in adipose tissue. Bands shown are from representative animals of each phenotype. Corresponding signal for stearoyl-CoA desaturase (SCD) from the same blot is shown as a comparison to AGT. * Significantly different from lean, $P < 0.05$ (Student's *t*-test).

levels. Although the amount of RNA loaded as indicated by β-actin levels did vary considerably, the wide range of AGT mRNA content between patients was not due to these differences: *lanes 2*, *11*, and *12*, for example, had high levels of β-actin mRNA but expressed almost no AGT. We did not find a consistent obesity-related pattern of AGT expression in adipose tissue of lean and obese subjects. *Lanes 1-5* represent samples taken from obese patients. Only three of those patients exhibited detectable levels of AGT, with one patient (*lane 1*) displaying high levels and one relatively low levels (*lane 4*).

To determine whether the variation in AGT mRNA levels among patients was due to circulating factors, we analyzed expression of AGT in human adipocytes maintained in primary culture (24). On the basis of Fig. 3*A* we included adipocytes from one patient (*lane 1*) in which AGT was clearly expressed in adipose tissue and one patient (*lane 2*) in which AGT mRNA was absent. These results are reported in Fig. 3*B*. The lane numbers correspond to the patient's adipose tissue sample represented on the Northern blot shown in Fig. 3*A*. Interestingly, AGT was expressed at similar levels in isolated adipocytes maintained in primary culture from both patients. This pattern of expression is in sharp contrast to that in freshly isolated adipose tissue (Fig. 3*A*), in which a wide degree of patient-to-patient variability was exhibited and in which AGT expression was limited to 7 of 12 patients. Consistent with the results from two patients in Fig. 3*B*, we have analyzed AGT expression in primary adipocytes from 12 additional patients, both nonobese and obese, not included in Fig. 3*A*: after isolation and maintenence in similar in vitro conditions, AGT was expressed in adipose cells of all patients studied to date (data not shown). We propose that the variability in adipose tissue AGT mRNA levels reflects inhibition of AGT expression by in vivo factors, such as diet and hormonal status, for which we could not control in this study; accordingly, these differences disappeared when adipocytes were isolated and main-

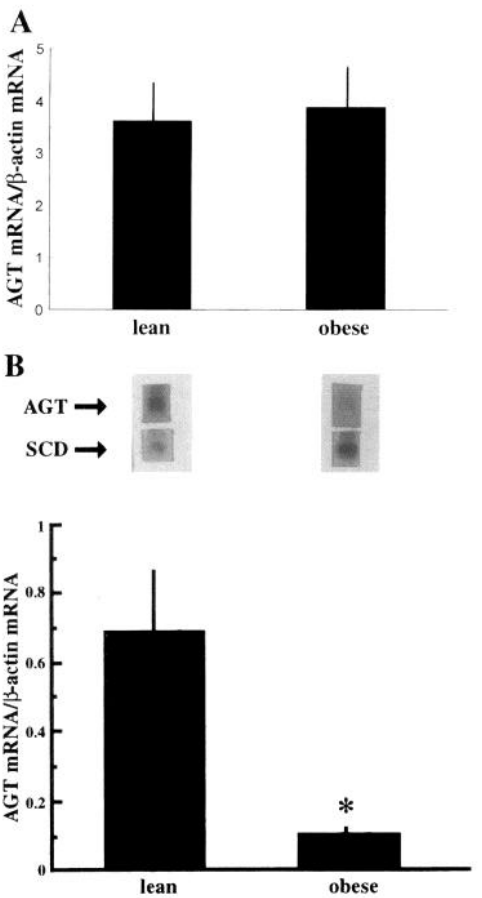

Fig. 2. Northern blot analysis of AGT mRNA levels in liver and adipose tissue of lean and obese A^{vy} mice. RNA was extracted and analyzed as described in MATERIALS AND METHODS. Twenty-five micrograms of total RNA was loaded in each lane. Values for AGT and β-actin mRNA levels depicted in bar graphs were obtained by densitometric scanning of autoradiograms. Data are presented as the mean ± SE of AGT/β-actin for 4 (obese) or 5 (lean) animals from each phenotype. *A*: AGT mRNA levels in liver; *B*: AGT mRNA levels in adipose tissue. Bands shown are from representative animals of each phenotype. Corresponding signal for SCD from the same blot is shown as a comparison to AGT. * Significantly different from lean, $P < 0.05$ (Student's *t*-test).

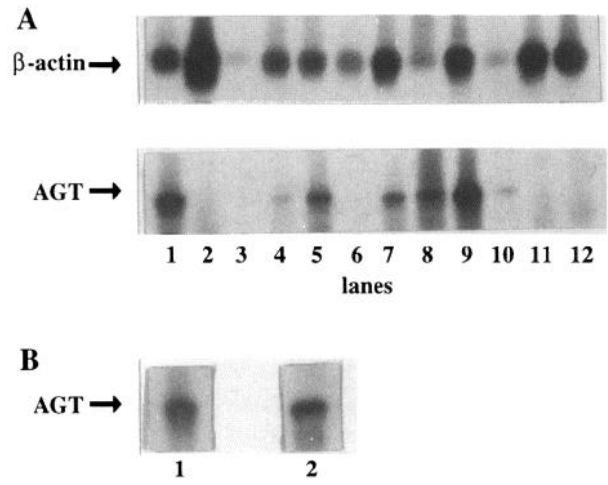

Fig. 3. *A*: expression of AGT mRNA in human adipose tissue. Total RNA from adipose tissue of 12 patients was analyzed by Northern blotting (as described in MATERIALS AND METHODS). Variable amounts of RNA were loaded onto the gel, as indicated by the variability in the hybridization signal for β-actin. Autoradiograms were exposed to film for 3 days (AGT) or 4 h (β-actin). *B*: expression of AGT in human adipocytes maintained in primary culture for 4–7 days under similar conditions. About twenty micrograms of total RNA was analyzed from adipocytes from 2 of the 12 patients represented in *A*, including one patient with (*lane 1*) and one without (*lane 2*) AGT expression in adipose tissue; the number corresponds to the lane of the adipose tissue RNA sample for that patient in *A*; the autoradiogram was exposed for 24 h.

tained as viable, metabolically active cells in culture for several days.

Nutritional and hormonal regulation of AGT expression in 3T3-L1 adipocytes. In light of *1*) downregulation of AGT expression in adipose tissue of obese Zucker rats and A^{vy} mice and *2*) variable regulation of AGT in human adipose tissue, we then wanted to determine whether factors known to regulate adipocyte metabolism influenced expression of AGT in adipose cells. For these studies we used the 3T3-L1 murine adipocyte cell line, which exhibits biochemical and physiological characteristics similar to those of mature adipocytes. Initially, we evaluated the effects of glucose on AGT expression. We were interested in glucose because *1*) AGT mRNA in adipose tissue was shown to be regulated by fasting and refeeding (9), *2*) hyperglycemia is a common feature of obese models (15), and *3*) several of the glycolytic and lipogenic genes with which AGT is regulated in parallel during adipocyte differentiation are also regulated by glucose (reviewed in Ref. 14). For these experiments, 3T3-L1 adipocytes were incubated for 36 h in GFM, after which they were either treated with media containing 0.5–2.5 mM glucose or maintained in glucose-free conditions for 48 h. As shown in Fig. 4*A*, we saw no effect of the paradigm described above on AGT mRNA levels in cultured adipocytes. By contrast, expression of an important lipid-metabolizing gene (SCD) was dramatically decreased in the absence of glucose and was restored by glucose treatment in a dose-dependent manner (33). Similar results with AGT were obtained with longer duration of both the glucose-free pretreatment phase (48 h) and the glucose treatment period (72 h) and with higher concentrations (5 and 25 mM) of glucose (data not shown).

Because hyperinsulinemia is a common feature of obese models, we then chose to evaluate the effects of

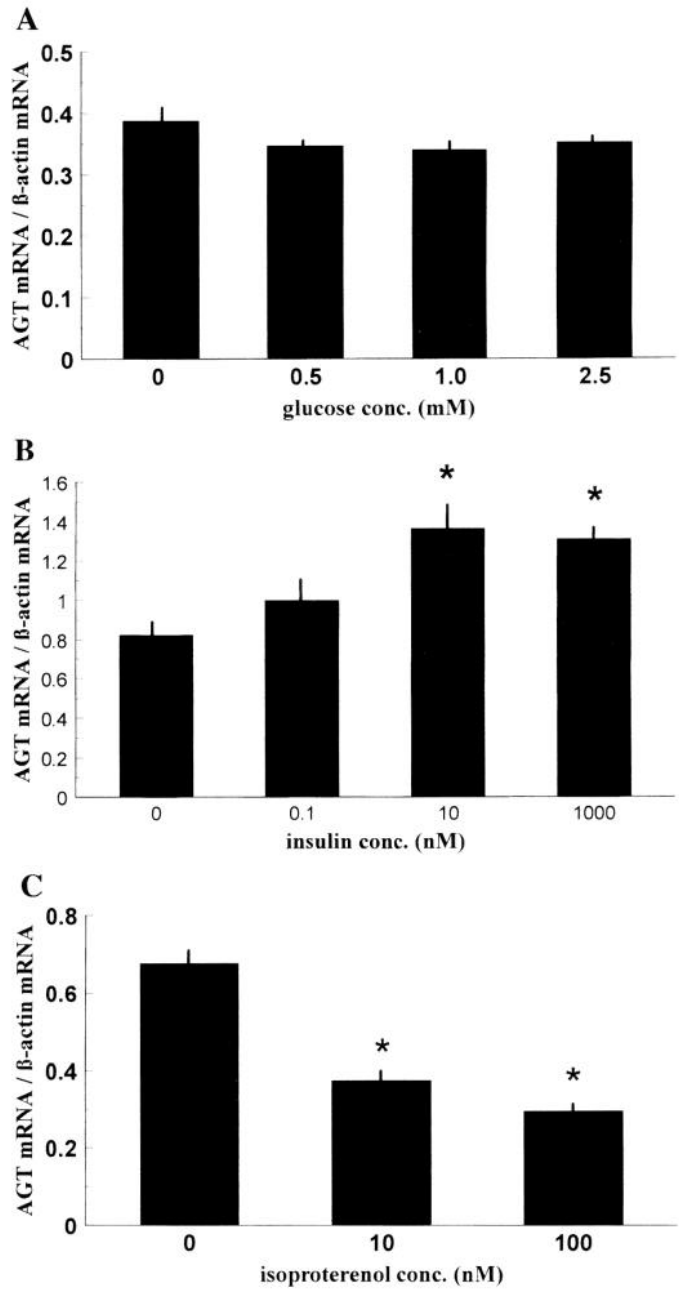

Fig. 4. AGT expression in 3T3-L1 adipocytes treated with glucose, insulin, or isoproterenol. As described in MATERIALS AND METHODS, cells for glucose experiment were incubated for 24 h in glucose-free medium before treatment. Cells used for insulin and isoproterenol experiments were incubated for 24 h in serum-free medium before treatment. All treatments were carried out for 48 h. Data were derived from densitometric scanning of the AGT and β-actin autoradiogram signals and are presented as means ± SE for 3 or 4 100-mm culture dishes within each treatment. *A*: effects of media glucose concentration (0, 0.5, 1.0, or 2.5 mM). *B*: effects of insulin (0.1 nM, 10 nM, or 1 μM). $^*P < 0.05$ vs. 0 insulin (Bonferroni multiple-comparison procedure). *C*: effects of β-adrenergic receptor stimulation with isoproterenol (10 or 100 nM). $^*P < 0.01$ vs. 0 isoproterenol (Bonferroni multiple-comparison procedure). conc, Concentration.

insulin on AGT expression. Although physiological concentrations of insulin (0.1 nM) have been shown to increase AGT expression in a hepatocyte cell line (H4IIE) (2), no studies have reported the effects of insulin on AGT expression in adipocytes. As shown in Fig. 4*B*, treatment of 3T3-L1 adipocytes for 48 h with insulin caused an increase in AGT mRNA levels. ANOVA indicated a significant effect of insulin dosage ($P < 0.05$); post hoc comparisons revealed that both 10 nM and 1 μM insulin significantly increased AGT expression (control: 0.82 ± 0.1; 10 nM: 1.37 ± 0.14; 1 μM: 1.31 ± 0.06; $P < 0.01$) over control levels. There was a slight but not significant increase in AGT expression in adipocytes treated with 0.1 nM insulin.

We also evaluated the effect of β-adrenergic stimulation on AGT expression in the 3T3-L1 adipocyte model. Activation of sympathetic β-receptors in adipose cells is known to increase lipolysis, and β-adrenergic stimulation has been shown to modulate AGT expression in other cell types (19, 33). As shown in Fig. 4*C*, isoproterenol treatment significantly reduced AGT mRNA levels by ~50% (ANOVA; $P < 0.01$). Both concentrations (10 and 100 nM) of the β-agonist elicited a similar and significant reduction (control: 0.67 ± 0.04; 10 nM: 0.37 ± 0.03 100 nM: 0.29 ± 0.02; $P < 0.01$) in AGT mRNA levels compared with controls.

DISCUSSION

We conducted this study in part to determine whether overexpression of the AGT gene in adipose tissue, previously reported in obese mice (*ob/ob* and *db/db*), could be extended to another murine model of obesity (A^{vy}) or to obese models of other species (Zucker rats and humans). We were interested in this issue because of recent data suggesting a role for ANG II in control of adiposity. ANG II has been shown to increase adipocyte differentiation (7), and antagonism of ANG II receptors reduced adipose mass and adipocyte size by ~40% in rats (6) and prevented ANGII-stimulated lipogenesis in 3T3-L1 adipocytes (17a). This apparent ability of ANG II to modulate fat mass, coupled with increased expression of the adipose tissue RAS in obesity, could thus provide insight into control of adiposity. Although the report by Frederich et al. (9) described obese-specific expression of AGT in two different models of obesity, both models have etiologies related to the recently identified protein leptin (10, 27). Overexpression of AGT in adipose tissue found in this report could thus be unique to models with a defect in the leptin signaling pathway. Alternatively, the differences in AGT expression described by Frederich et al. (9) could be unique to mouse models of obesity. To determine whether this pattern of AGT expression was common to numerous obese models, or if this pattern was due to leptin- or species-related factors, we analyzed adipose tissue expression of AGT in *1*) a rodent model (Zucker rat) with a genetic mutation homologous to that of the *db/db* mouse (3) and *2*) a mouse model (A^{vy}) in which obesity is due to ectopic overexpression of the agouti gene (35) and is unrelated to the leptin pathway. In direct contrast to Frederich's results in obese mice, AGT expression in the obese Zucker rat was significantly decreased (~50%) in adipose tissue relative to lean controls (Fig. 1*B*). AGT mRNA levels were also significantly lower (~80%) in adipose tissue of obese A^{vy} mice compared with lean controls (Fig. 2*B*). Hepatic levels of AGT were not different between lean and obese animals of either model (Figs. 1*A* and 2*A*). We also found no differences in renal AGT mRNA levels between lean and obese animals (unpublished data). These results demonstrate that decreased AGT expression in these two models of obesity is tissue specific.

We also wanted to determine whether the AGT gene was expressed in human adipose tissue because no previous studies had addressed this issue. In addition, we wanted to determine whether there were differences in AGT mRNA levels between lean and obese humans. Linkage between genetic variation near the AGT locus on human chromosome 1 and waist-to-hip ratio in male Hutterites was recently reported (13), which implies that the AGT gene might be involved in body fat distribution in humans. We have reported here that AGT was expressed in human adipose tissue taken from 7 of 12 patients studied. These observations complement recent studies that demonstrated the presence of ANG II receptors (5) and ACE (18) in human adipocyte membranes and adipose tissue, respectively. Five of the 12 patients that we studied were classified as obese according to BMI. We found no consistent obesity-related pattern of AGT expression in these obese subjects. Two of the five obese subjects displayed detectable levels of AGT mRNA; levels were relatively high in adipose tissue from one of these two patients but low in the other. Although it is not possible to make any conclusions regarding expression of AGT in adipose tissue of all obese humans based on samples from five subjects, our data do not suggest an association between obesity and levels of AGT mRNA in human adipose tissue.

When adipocytes were isolated and maintained in primary culture for 4–7 days, a very different pattern of AGT expression was observed: AGT mRNA was detectable at comparable levels in adipocytes from the two patients shown in Fig. 3*B*, despite the fact that one of the patients (*lane 2*) showed no expression of AGT in adipose tissue. The discrepancy between these data and those obtained in RNA from freshly isolated adipose tissue raises some interesting questions concerning in vivo regulation of adipose AGT. Apparently, elements found in vivo but not in vitro suppress AGT gene expression in some but not all patients. Preoperative factors such as diet, fasting, and medications were not controlled in the patients studied; many potential differences thus exist between patient status at the time of adipose tissue removal. These differences were effectively eliminated by isolating adipocytes and maintaining them in similar culture conditions. These findings may also impact the irregularity of AGT mRNA levels in obese humans as reported in Fig. 3*A*. It may thus be very difficult to demonstrate an association between obesity and AGT mRNA levels in human adipose tissue given that some factor(s) present in vivo apparently suppress AGT expression in certain patients.

Our data from obese Zucker rats, A^{vy} mice, and humans demonstrate that all obese models do not share increased expression of AGT in adipose tissue, as previously reported in *ob/ob* and *db/db* mice. AGT is thus regulated by a different complement of factors than the *ob* gene, the expression of which is increased in adipose tissue of all obese models examined to date. Downregulation in obese Zucker rats and A^{vy} mice (Figs. 1 and 2) and variable levels of expression in adipose tissue from human subjects (Fig. 3) demonstrate that AGT is subject to regulation in vivo by unknown factors. Little is known about regulation of the adipocyte RAS; glucocorticoid is the only hormonal factor that has been shown to modulate AGT expression in adipocytes (21). It is important to understand regulation of adipocyte AGT, the largest potential source of ANG II in the body (9), because of the potential role of ANG II in control of adipose mass obesity-related hypertension (19). The second purpose of our study was thus to investigate regulation of AGT mRNA levels in vitro using an adipocyte cell line. We found that 48 h of insulin treatment (10 nM and 1 μM) increased AGT expression in 3T3-L1 adipocytes (Fig. 4*B*). These results contrast with our findings in vivo showing that hyperinsulinemic, obese Zucker rats and A^{vy} mice have significantly lower levels of AGT mRNA in adipose tissue than do lean controls (Figs. 1*B* and 2*B*). Taken together, these results suggest that insulin is not a dominant factor in regulation of adipose tissue AGT expression in vivo, at least not in hyperinsulinemic, insulin-resistant animals. However, in animals with normal sensitivity to insulin, fluctuations in circulating insulin levels may directly regulate AGT expression in adipose tissue. Consistent with this possibility, Cassis (1) demonstrated that adipose tissue AGT mRNA levels were significantly reduced in streptozotocin-diabetic rats and were restored to control values by insulin replacement therapy.

We were unable to demonstrate regulation of adipocyte AGT mRNA levels by glucose (Fig. 4*A*) using an experimental paradigm comparable to fasting/refeeding. In contrast to the null effects of these manipulations on AGT, mRNA levels for SCD were markedly increased in a dose-dependent manner by glucose availability in the same set of experiments (33). Therefore, although these two genes are regulated in parallel during adipocyte differentiation, they appear to be under different control by carbohydrate and/or energy availability in mature adipocytes.

We have also reported that treatment of adipocytes with a β-adrenergic receptor agonist (isoproterenol) resulted in a marked decrease (~50%) in AGT expression. Sympathetic stimulation of adipocytes is known to increase lipolysis; these data thus suggest that AGT is inversely regulated with lipid mobilization. AGT mRNA levels have been shown to be increased by catecholamines in oppossum kidney cells (34) but decreased by catecholamines in rat hepatocytes (19). Further studies will be necessary to adequately characterize the relationship between β-adrenergic stimulation and the adipose tissue RAS.

In conclusion, we have demonstrated that adipose levels of AGT are not regulated in a consistent manner across all models of genetic obesity. Our data do suggest, however, that AGT is subject to control by physiological factors in vivo in both rat and human adipose tissue. We have also shown that insulin increases, while β-adrenergic receptor stimulation decreases AGT expression in 3T3-L1 adipocytes. Given the potential paracrine and endocrine actions of ANG II produced by adipocytes, it is important to identify the factors that regulate adipose tissue AGT production in vivo, particularly in human adipose tissue. Identification of these influences will be important in understanding the function of adipose tissue RAS and its regulation in both physiological and pathophysiological conditions.

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