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## Deficiency of Angiotensin Type 2 Receptor Rescues Obesity But Not Hypertension Induced by Overexpression of Angiotensinogen in Adipose Tissue

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Increased angiotensinogen (AGT) production by white adipose tissue has been related to not only obesity but also hypertension. Several studies have highlighted the importance of the angiotensin II type 2 receptor (AT2) in the regulation of blood pressure and fat mass, but the relevance of this transporter in a physiopathological model of increased AGT production, as it occurs in obesity, has not yet been investigated. We used transgenic mice that display either a deletion of AT2 (AT2 KO), an overexpression of AGT (OVEX), or both compound mutants (KOVEX). Results demonstrated that adipocyte hypertrophy and increased lipogenic gene expression induced by adipose AGT overproduction was rescued by deletion of AT2. In line with AGT overexpression, KOVEX and OVEX mice have similar increased plasma AGT levels. However, KOVEX mice display a higher blood pressure than OVEX mice. In kidney, renin expression was clearly reduced in OVEX mice, and its expression was normalized in KOVEX mice. Taken together, we demonstrated that the loss of AT2 expression was sufficient to rescue obesity induced by adipose tissue AGT overexpression and confirmed the necessary role of AT2 for the onset of obesity in this model. Furthermore, despite a reduction of adipose mass in KOVEX, AT2 deficiency caused increased renin production, further worsening the hypertension caused by AGT overexpression. (*Endocrinology* 150: 1421–1428, 2009)

**O**besity, a prevalent epidemic problem worldwide, is a major risk factor for the development of a cluster of metabolic disorders leading to type 2 diabetes, hypertension, and atherosclerosis. Although the association between obesity and hypertension is well recognized (1, 2), the molecular mechanisms remain elusive.

During the last decade, adipose tissue received much attention due to its recently discovered endocrine function. Fat tissue produces a variety of adipocytokines that have been implicated in several complications of obesity, including hypertension. One molecule with a potential role in obesity-related hypertension is angiotensinogen (AGT), the precursor of the bioactive pressor peptide angiotensin II (Ang II). AGT is reportedly produced by

adipose tissue in humans and rodents, and its production is increased in the obese state (3–6). Recent studies from our group have demonstrated that transgenic mice with overexpression of AGT in adipose tissue exhibited an increased fat mass and developed high blood pressure, revealing for the first time a key endocrine role of AGT in obesity-related hypertension (7).

Most of the known physiological functions and vascular pathologic effects associated with Ang II are primarily mediated by the angiotensin type 1 receptor (AT1). In contrast, the functions of the angiotensin type 2 receptor (AT2) are less established and are still being elucidated. AT2 is expressed abundantly during fetal development and declines after birth; however, its expression has been reported in several adult tissues, including

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Abbreviations: AGT, Angiotensinogen; Ang II, angiotensin II; aP2, adipose fatty acid protein 2; AT1, angiotensin type 1 receptor; AT2, angiotensin II type 2 receptor; AT2 KO, transgenic mice displaying deletion of AT2; FAS, fatty acid synthase; KOVEX, transgenic mice displaying both compound mutants; OVEX, transgenic mice displaying overexpression of AGT; RAS, renin-angiotensin system; SREBP, sterol regulatory element-binding protein; VEGF, vascular endothelial growth factor; WT, wild type.

kidney, heart, and vessel wall (8). Previous *in vitro* and *in vivo* studies also highlighted an important local role for AT2 in mediating up-regulation of lipogenesis and fat mass enlargement (9–11). Together, these effects could potentially contribute *in vivo* to the enlargement of adipose tissue induced by local overexpression of AGT in adipose tissue (7). However, direct *in vivo* evidence that AT2 is directly involved in the regulation of fat mass expansion induced by local AGT overproduction is missing.

The enlargement of adipose tissue has been associated with an increased production of inflammatory cytokines (12). Ang II is also considered as a major mediator in the inflammatory response, and some evidence suggests that AT2 participates in the inflammatory process in renal and vascular tissues (13, 14). Also, we hypothesized that increased AGT production in adipose tissue stimulates local cytokines production and that AT2 could mediate this inflammatory effect in adipose tissue.

The renin-angiotensin system (RAS) acting through AT1 plays a critical role in the regulation of blood pressure. As in an endocrine feedback regulatory system, Ang II could potentially regulate the circulating levels of AGT by increasing tissues' AGT expression and decreasing kidney renin expression. Most studies pointed out a role for AT1 in mediating this feedback regulation, but an increasing number of reports now support a role for AT2 as a cardiovascular and renal regulator (15). Thus, in presence of an overproduction of AGT by adipose tissue such as in obesity, the contribution of AT2 in the control of blood pressure must be reconsidered.

To investigate further whether AT2 mediates the *in vivo* effects of Ang II in fat mass expansion and blood pressure regulation, we generated a compound transgenic mouse model that overexpresses AGT in adipose tissue but lacks AT2. Analysis of these animals demonstrated that AT2 deficiency protected against obesity triggered by adipose AGT overexpression but exacerbated the high blood pressure.

## Materials and Methods

### Animals

Transgenic mice overexpressing adipose tissue angiotensinogen, *aP2-AGT<sup>+/-</sup>* (OVEX) were originally generated using a transgenic construct containing the adipocyte-specific adipose fatty acid protein 2 (*aP2*) promoter as described previously (7). By crossing OVEX mice in ICR-CD1 background with AT2-deficient mice in C57BL/6 background (16) for more than six generations, we generated double-transgenic mice into the C57BL/6 background that were deficient for AT2 with a targeted overexpression of AGT in adipose tissue (KOVEX). To obtain wild type (WT), OVEX, AT2 knockout (AT2 KO), and KOVEX littermates, *aP2-AGT<sup>+/-</sup>/AT2<sup>+/-</sup>* females were crossed with WT mice. The identification of transgenic mice in the offspring was achieved by PCR of genomic DNA using specific oligonucleotides. AT2 deficiency was screened as previously described (10) and *aP2-AGT* transgene was screened with transgene-specific (5'-CTTTGCCCTTCTCTCCACAG-3') and intron-specific (5'-TTATCTCGCAGGCTCTCTC-3') oligonucleotides, which produce a 300-bp DNA fragment. All mice were housed in a controlled environment with a 12-h light, 12-h dark cycle and constant temperature (22 C) and had free access to water and laboratory chow diet (UAR, Meylan, France). Animal studies were conducted according to the French Guidelines for Care and Use of Experimental Animals, France. Results of

16-wk-old male littermates issued from six breedings were shown in this study.

### Blood pressure measurement

Systolic blood pressure was measured noninvasively by the tail-cuff method, using piezoelectric transducers connected to a PowerLab/S system and chart version 3.4/s software (ADInstruments, Phymep, France). Nonanesthetized mice, warmed to 30 C, were accustomed to the restrainers and tail-cuff inflation daily for 2 d before blood pressure determination. Blood pressure was recorded daily (20 determinations in a row) over a 2-d period. Results were expressed as millimeters of mercury.

### Adipose tissue cellularity

After the animals were killed, inguinal sc and epididymal adipose tissue were removed and weighed. Cellularity of epididymal adipose tissue was determined as previously described (17). Briefly, images of isolated adipocytes were acquired from a light microscope fitted with a camera, and the measurement of approximately 400 cell diameters was performed using Perfect Image software (Numeris, Paris, France). Tissue triglyceride content was measured from a sample of adipose tissue using a commercial kit (Sigma Chemical, St. Louis, MO). Fat cell number was estimated by dividing the tissue lipid content by the fat cell weight.

### Adipose cell culture

To evaluate the level of AGT secretion, a portion of the epididymal adipose tissue from transgenic mice was digested with collagenase, and isolation and culture of mature adipocytes were performed as previously described (17). The culture medium was collected 24 h later for assay of AGT as described later. AGT secretion was expressed by  $10^6$  cells for 24 h of culture.

### Isolation and analysis of RNA

Total RNA was extracted from epididymal adipose tissue and cDNA was synthesized from 1  $\mu$ g of total RNA with superscript reverse transcriptase (Invitrogen, Cergy, France). Real-time PCR was performed using a Bio-Rad Thermocycler (Bio-Rad, Richmond, CA) and PCR was carried out as described elsewhere (18). Ribosomal 18S RNA amplifications were used to account for variability in the initial quantities of cDNA. The primer sequences were previously published (19).

### Immunohistochemistry

Sections (7  $\mu$ m thick) of paraformaldehyde-fixed, paraffin-embedded kidneys from transgenic male mice were deparaffinized in xylene and rehydrated in graded ethanol and water. Endogenous peroxidase was inactivated in 3% hydrogen peroxide in PBS buffer. Sections were incubated successively in the antirenin monoclonal antibody (20) [a gift from Pierre Corvol, Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 833, College de France Paris, France], as previously described (21) and subsequently exposed to peroxidase-conjugated secondary immunoglobulins. Peroxidase activity was visualized using 0.035% diaminobenzidine (Polysciences, Warrington, PA) as a chromogen, and slides were counterstained with toluidine blue. Images were analyzed by ImageJ software [National Institutes of Health (NIH), Bethesda, MD] for immunostaining intensity and expressed as arbitrary units.

### In situ hybridization

*In situ* hybridization for renin mRNA was performed essentially as previously described (21). Briefly, paraffin sections (7  $\mu$ m) were deparaffinized, rehydrated, boiled in a sodium citrate solution (microwave oven), and digested with proteinase K (Roche Diagnostics, Meylan, France) before hybridization with the  $^{35}$ S-labeled riboprobes. Hybridization was performed overnight at 50 C and followed by several washes and an ribonuclease treatment to remove single-strand nonhybridized cRNA strands. Sense probes were used as controls. Sections were ex-

posed in the dark, processed for autoradiography, and counterstained with toluidine blue. Observation was performed under dark-field or bright-field illumination.

### Blood parameters

After the animals were killed, blood was collected by cardiac puncture. Plasma leptin and Ang II were determined using commercial kits (Clinisciences, Montrouge, France, and SPI-bio, Montigny Le Bretonneux, France, respectively). The concentration of AGT in plasma was determined by measuring the production of Ang I concentrations at 37°C in the presence of added renin, as previously described (18).

### Statistical analysis

Data are given as means  $\pm$  SEM. Comparison of mean values between groups was evaluated by Student's two-tailed unpaired *t* test or one-way ANOVA with a Tukey multiple comparison posttest (GraphPad software, San Diego, CA). Differences were considered significant at  $P < 0.05$ .

## Results

### Body weight and adiposity of mice

To determine the role of AT2 in mediating the effect of Ang II on adiposity, we determined total body weight and fat pad weights on each animal group, *i.e.* WT, obese mice overexpressing adipose AGT (OVEX), AT2-deficient mice (AT2 KO), and the double transgenic mice overexpressing adipose AGT and lacking AT2 (KOVEX). Table 1 shows that body weight was slightly but significantly increased in OVEX male mice at 16 wk of age compared with the other genotypes. Although AT2 KO mice exhibited similar epididymal fat pad weight as WT controls, the 20% increase in epididymal fat pad weight observed in OVEX mice was prevented by the deletion of this receptor (Table 1). Similar results were obtained when sc fat pad weight and adiposity were analyzed, although a significant decrease in sc fat pad weight also occurred in AT2 KO mice (Table 1). Consistent with the changes in adipose tissue mass, AT2 deficiency completely reversed the hyperleptinemia induced by adipose AGT overexpression (Table 1). This occurred despite a constant increase in AGT secretion level as shown in Fig. 1D. Taken together, these results reveal that AT2 is a main player in the development of fat mass induced by Ang II *in vivo*.

### Genotype effect on adipose tissue cellularity and lipogenesis

Figure 1 shows the adipocyte size and number from mice of different genotypes. Consistent with our previous findings, we found reduced adipose cell size in AT2 knockout mice and increased adipose cell size in OVEX mice. Accordingly, we next asked whether deletion of AT2 might control the adiposity of the OVEX mice by altering fat cell size (*i.e.* adipocyte metabolism) or the number of adipocytes (*i.e.* preadipocyte proliferation and differentiation). In AT2 knockout mice, the 2-fold increase in adipose cell number per fat pad fully compensated for the reduced adipose cell size. In contrast, the number of adipose cells per fat pad in mice overexpressing adipose AGT (OVEX) was reduced by approximately 30%, but this was not sufficient to compensate for the adipose cell hypertrophy (2-fold increase in adipose cell weight), leading to higher adiposity in these mice compared with the other genotypes (Table 1). Interestingly, deficiency of AT2 abolished both the hypertrophy and hypoplasia induced by the overexpression of adipose AGT. Changes in the mRNA levels of the fatty acid synthase (FAS), a key enzyme of the lipogenic pathway, and its major regulatory transcriptional factor, sterol regulatory element-binding protein (SREBP)-1c, closely paralleled changes in the adipose cell size of the different genotypes (Fig. 2, A and B). These results show the first *in vivo* evidence that AT2 mediates the lipogenic effect of Ang II, confirming our previous *in vitro* findings (9, 10).

### Expression of angiogenic and inflammatory markers in adipose tissue

To gain further insight into the role of AT2 in adipose tissue development, we next evaluated the expression of various growth factors and cytokines such as vascular endothelial growth factor (VEGF), TNF $\alpha$ , IL-6, or IL-1 $\beta$  in adipose tissue that might modulate the vessel wall integrity. A coordinated up-regulation of VEGF, TNF $\alpha$ , IL-6, or IL-1 $\beta$  mRNAs (2- to 4-fold) was observed in OVEX mice (Fig. 3). By contrast, whereas the lack of AT2 in wild-type genotype (AT2 KO) did not modify the expression of these adipokines, deletion of AT2 in the transgenic mice (KOVEX) normalized their mRNA expression (Fig. 3). The serum levels of these inflammatory cytokines were barely detect-

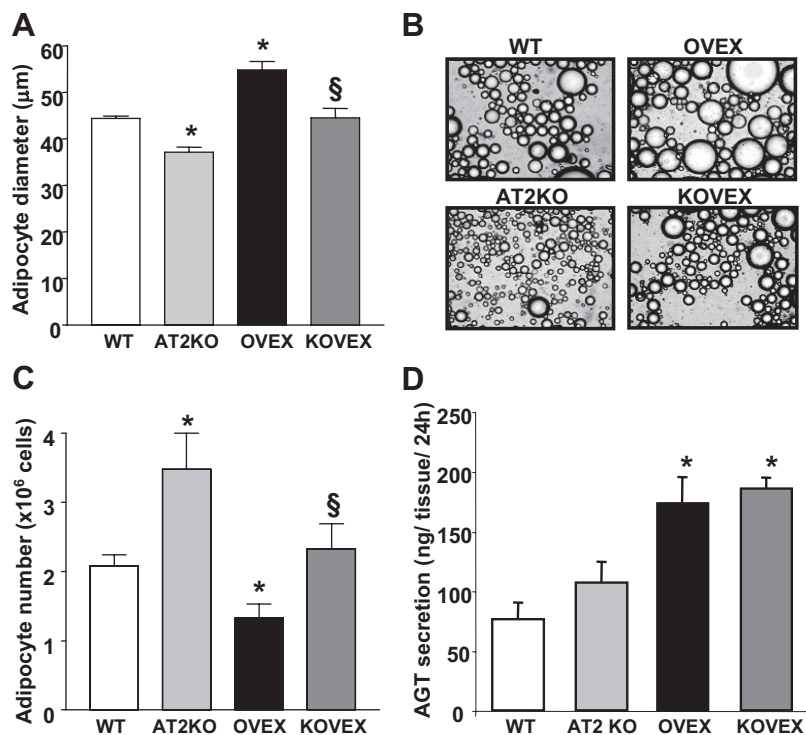
**TABLE 1.** Adiposity and metabolic parameters in different transgenic mice

	WT (n = 9)	AT2 KO (n = 9)	OVEX (n = 10)	KOVEX (n = 8)
Body weight (g)	25.2 $\pm$ 1.1	25.9 $\pm$ 0.7	27.4 $\pm$ 1.1 <sup>a</sup>	25.7 $\pm$ 0.6 <sup>b</sup>
EAT				
mg per two pads	242 $\pm$ 12	235 $\pm$ 14	296 $\pm$ 16 <sup>a</sup>	198 $\pm$ 12 <sup>b</sup>
mg per g body weight	8.8 $\pm$ 0.3	8.5 $\pm$ 0.4	10.7 $\pm$ 0.6 <sup>a</sup>	7.9 $\pm$ 0.6 <sup>b</sup>
Adipocyte weight (ng)	42 $\pm$ 1.5	25 $\pm$ 2.3 <sup>a</sup>	79 $\pm$ 7.6 <sup>a</sup>	44 $\pm$ 5.7 <sup>b</sup>
Inguinal SCAT				
mg/two pads	131 $\pm$ 3	112 $\pm$ 9 <sup>a</sup>	151 $\pm$ 11 <sup>a</sup>	129 $\pm$ 5 <sup>b</sup>
mg per g body weight	5.2 $\pm$ 0.3	4.3 $\pm$ 0.1 <sup>a</sup>	5.7 $\pm$ 0.2 <sup>a</sup>	5.0 $\pm$ 0.2 <sup>b</sup>
Plasma leptin (ng/ml)	0.99 $\pm$ 0.07	0.81 $\pm$ 0.05 <sup>a</sup>	1.43 $\pm$ 0.10 <sup>a</sup>	0.90 $\pm$ 0.04 <sup>b</sup>

Data are means  $\pm$  SEM. The number of mice used in this study (n) was obtained from six independent litters. Weight of epididymal (EAT) and inguinal sc adipose tissue (SCAT) were expressed by whole tissue and gram of body weight.

<sup>a</sup>  $P < 0.05$  vs. WT.

<sup>b</sup>  $P < 0.05$  KOVEX vs. OVEX mice.



**FIG. 1.** Effects of adipose *Agt* overexpression and *Agtr2* deficiency on epididymal adipose tissue. **A**, Adipocyte size was expressed as cell diameter. **B**, Micrographs of isolated epididymal adipose cells (magnification,  $\times 20$ ). **C**, The number of adipocytes was calculated for two gonadal fat pads. **D**, AGT secretion from cultured adipocytes isolated from epididymal adipose tissue. Results are mean  $\pm$  SEM. The number of mice in each genotype is given in Table 1, except for AGT secretion (where  $n = 3$ ). \*,  $P < 0.05$  vs. WT; §,  $P < 0.05$  KOVEX vs. OVEX mice.

able in these mice, suggesting a local but not a systemic inflammatory state. Thus, deficiency of AT2 in OVEX mice not only reduced lipid stores but also decreased the local inflammation in adipose tissue.

#### Blood pressure and circulating angiotensinogen

Given the potential role of obesity in hypertension and our finding that AT2 gene deficiency was able to rescue obesity and local inflammation in OVEX mice, we hypothesized that AT2 deficiency might also rescue the hypertension induced by adipose AGT overexpression. As previously established, these OVEX mice were hypertensive and exhibited a 25% increase in plasma AGT levels due to the presence of the *aP2*-AGT transgene (Fig. 4A). However, deficiency of AT2 did not protect OVEX mice from the hypertension induced by adipose AGT overexpression; rather it enhanced the blood pressure by approximately 20 mm Hg in KOVEX mice (Fig. 4A). This occurred along with an increase in plasma Ang II levels (Fig. 4C) despite comparable plasma AGT levels in KOVEX and OVEX mice (Fig. 4B). Thus, KOVEX mice constitute a novel model of increased blood pressure that was fully dissociated from obesity and local adipose tissue inflammation associated with obesity.

#### Renal renin expression

The fact that plasma Ang II levels were increased in mice overexpressing AGT but lacking AT2 prompted us to evaluate the effect of AT2 deficiency on expression of renin, the rate-limiting step in Ang II production, in OVEX mice. Renin mRNA

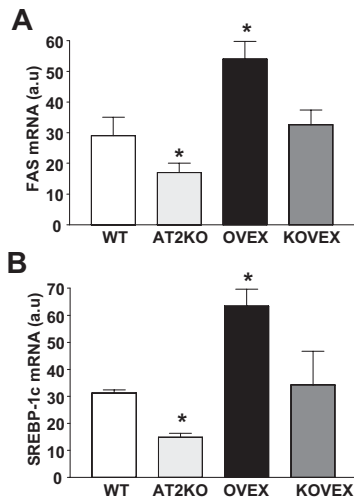
and protein, detected by *in situ* hybridization and immunostaining, respectively, were mainly localized in the juxtaglomerular apparatus of WT mice (Fig. 5, D–G). Overexpression of adipose AGT appeared to cause a decrease in renin mRNA expression (Fig. 5, B and E, vs. 5, A and D) and in the number of juxtaglomerular complexes labeled by immunostaining of renin (Fig. 5H vs. 5G). Surprisingly, the loss of AT2 in OVEX mice normalized the renin mRNA and protein levels in the juxtaglomerular complexes in kidneys (Fig. 5, C, F, and I). Quantification of the immunostaining intensity indicated that loss of AT2 prevented the approximate 2-fold decrease in renin protein content observed in the kidney of OVEX mice [immunostaining intensity (arbitrary units) in WT:  $29386 \pm 2316$ ; OVEX:  $16372 \pm 1557$ ; and KOVEX:  $25615 \pm 2198$ ]. These changes occurred without significant variation in the mRNA levels of AT1 (data not shown). Together these data suggest that the enhanced blood pressure observed in KOVEX mice reflects an exaggerated vasopressor response due to high plasma Ang II, which was secondary to restoring renin expression to the WT levels.

#### Discussion

In the present study, we investigate the contribution of AT2 to obesity and hypertension induced by adipose-driven overexpression of AGT. This work was driven by our previous findings showing that adipose overexpression of AGT caused obesity and hypertension (7), whereas AT2 or AGT deficiency prevented diet-induced obesity (10, 11). In this work, we created a novel compound transgenic mouse model overexpressing AGT but lacking AT2. We demonstrated that lack of AT2 rescued the obesity induced by an adipose overproduction of AGT, revealing the first *in vivo* evidence that AT2 has a major role in mediating the local Ang II action on fat mass enlargement. Surprisingly, high blood pressure displayed by OVEX mice was aggravated by the deletion of AT2, and this was associated with higher plasma Ang II secondary to a normalization of the renin expression in kidney.

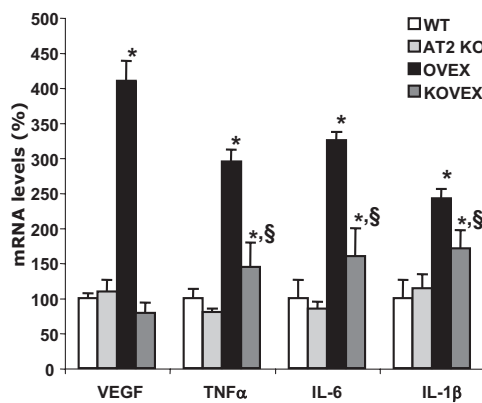
#### Implication of AT2 in obesity induced by adipose angiotensinogen overexpression

Initial studies in rodent and human reported a positive correlation between adipose AGT expression and adipose mass (3, 5, 6, 22). The generation of transgenic mice overexpressing AGT in adipose tissue, leading to the development of obesity, has been a major contribution in favor of Ang II playing the role of an endocrine effector in obesity *in vivo* (7). Later studies revealed that mice lacking either AT1 or AT2 were protected from high-

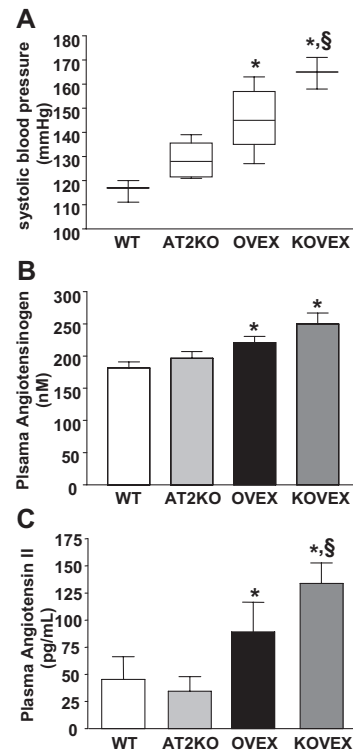


**FIG. 2.** Epididymal adipose tissue lipogenic gene expression in 16-wk-old WT, AT2 KO, OVEX, and KOVEX mice. A, FAS mRNA levels. B, SREBP-1c mRNA levels. Values were normalized to ribosomal 18S amount. Results are mean  $\pm$  SEM [arbitrary units (a.u.)]. The number of mice in each genotype is given in Table 1. \*,  $P < 0.05$  vs. WT.

fat diet-induced obesity, suggesting that both receptors may contribute to the fat mass enlargement induced by Ang II *in vivo* (10, 23). Interestingly, Kim *et al.* (24) recently reported that elevating adipose AGT expression (OVEX mice) was associated with reduced AT2 expression, reflecting most likely a negative feedback mechanism to protect adipose tissue from excessive lipid storage and emphasized the importance of this receptor *in vivo*. However, conflicting *in vitro* data exist as to the contribution of AT1 and AT2 in the control of adiposity through their ability to modulate both preadipocytes differentiation and mature adipocyte metabolism, and it remains unclear whether *in vivo* these receptors could mediate the Ang II-mediated fat mass enlargement. For instance, conflicting data exist as to the role of Ang II in preadipocyte differentiation *in vitro* (25–29). The present study showed that overexpression of AGT in mice led to a slight decrease in adipose cell number and this effect was partially mediated by AT2, suggesting an inhibitory effect of Ang II on preadipocyte differentiation *in vivo*. However, this effect seems to be



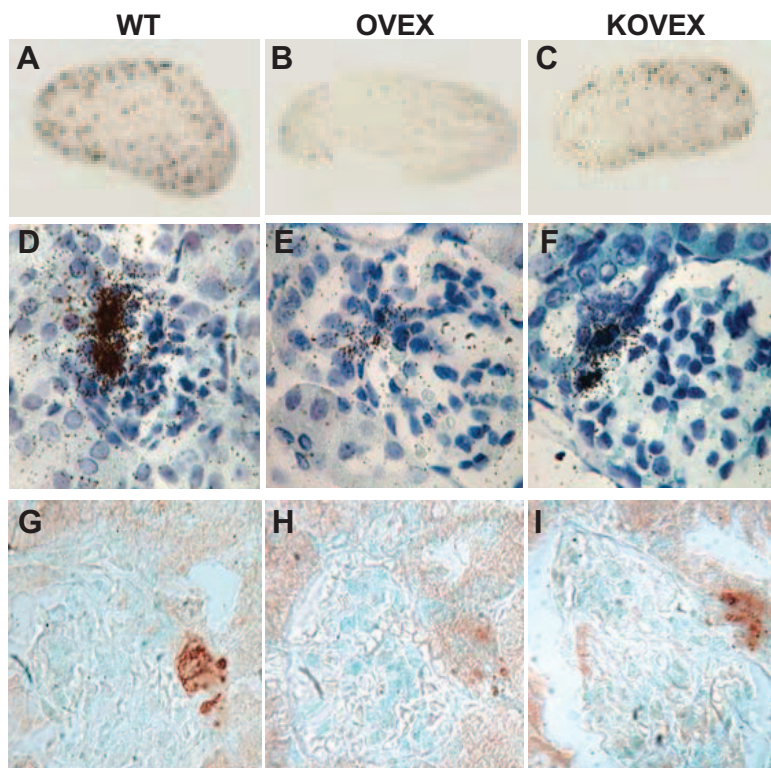
**FIG. 3.** Epididymal adipose tissue angiogenic and inflammatory markers expression in 16-wk-old WT, AT2 KO, OVEX, and KOVEX mice. VEGF, TNF $\alpha$ , IL-6, and IL-1 $\beta$  mRNA levels were normalized to ribosomal 18S amount and expressed as percentage over WT. The number of mice in each genotype is given in Table 1. Values are mean  $\pm$  SEM. \*,  $P < 0.05$  vs. WT; §,  $P < 0.05$  KOVEX vs. OVEX mice.



**FIG. 4.** Effects of adipose *Agt* overexpression and *Agtr2* deficiency on blood pressure and AGT levels. A, Systolic blood pressure. B, Plasma AGT levels. C, Plasma Ang II. The number of mice in each genotype is given in Table 1. Data are mean  $\pm$  SEM. \*,  $P < 0.05$  vs. WT; §,  $P < 0.05$  KOVEX vs. OVEX mice.

marginal because overexpression of AGT promoted fat mass enlargement and rather a role of Ang II in favoring adipocyte metabolism appeared to be the main factor contributing to the development of the adipose tissue *in vivo*.

Ang II acts as an antilipolytic hormone via AT1 (18, 30) or a lipogenic hormone via AT2 in both human and murine mature adipocytes (9, 10). The antilipolytic role of Ang II via AT1 may contribute to some extent to the phenotype of KOVEX mice because deletion of AT2 in OVEX mice did not totally recapitulate the phenotype of the AT2-deficient mice but rather normalized the fat mass to the level of WT mice. This observation was consistent with recent findings, suggesting a possible contribution of both AT1 and AT2 in the development of adipose tissue (31), but this possibility warrants further investigation because other mechanisms such as the ACE2/Ang (1–7)/mas receptor pathway may also be involved in the regulation of fat metabolism (32, 33). The generation of transgenic mice overexpressing AGT and lacking AT1 should help to clarify this issue. However, our data clearly demonstrate that the obesity induced by adipose AGT overproduction can be rescued when Ang II signaling through AT2 is eliminated. Clearly the profound decrease in fat mass, fat cell size, FAS, and SREBP-1c gene expression in KOVEX compared with OVEX mice cannot be attributed to the antilipolytic role of Ang II via AT1. Rather, our results point to a central role of the lipogenic effects of AT2 in the development of obesity induced by excessive local production of adipose AGT *in vivo*.



**FIG. 5.** Renin mRNA expression by *in situ* hybridization (A–F) and protein by immunoperoxidase staining (G–I) in juxtaglomerular apparatus. Macroscopic image of the hybridization signal on x-ray film (A–C) shows the intensity and density of the labeling. Microscopic image of the same section shows a sharp decrease in renin mRNA (D–F) and protein (G–I) of OVEX (B, E, and H) compared with KOVEX (C, F, and I) and WT (A, D, and G); magnification:  $\times 200$  (quantification is given in *Results*).

### AT2 gene deletion prevents local inflammation-induced by adipose angiotensinogen overexpression

Growing evidence indicates that obesity involves a low-grade inflammatory process and that inflammation plays a significant role in the onset of its complications such as diabetes or hypertension. Besides being a potent vasocative peptide, Ang II exerts proinflammatory effects in various tissues, including adipose tissue, by inducing integrins, adhesion molecules, cytokines, and growth and profibrotic mediators (34–37). We documented an increased expression of a variety of angiogenic and inflammatory cytokines in adipose tissue of OVEX mice that was rescued by deletion of AT2. VEGF, a major angiogenic factor in adipose tissue, is regulated by proinflammatory cytokines in adipocytes (38, 39). Increased TNF $\alpha$ , IL-6, and IL-1 $\beta$  expression from OVEX adipose tissue is likely to trigger local insulin resistance and alterations in adipocyte function (40, 41). Together, our findings suggest a new role of AT2 in the local inflammation induced by Ang II in adipose tissue. Further experiments should help to clarify whether this effect is directly linked to a signaling pathway through AT2 or the consequence of the reduced fat mass.

### AT2 disruption increases the pressor action induced by adipose AGT overexpression

Our second goal was to determine whether AT2 gene deficiency contributes to the regulation blood pressure in the hypertensive state. In agreement with the reported positive relation-

ship between plasma AGT levels and blood pressure (7), our data show that elevation of AGT production in adipose tissue of OVEX leads to hypertension. Surprisingly, despite equivalent high levels of plasma AGT produced by adipose tissue of OVEX and KOVEX mice, deficiency of the AT2 gene exacerbated the hypertension observed in OVEX mice. This also occurred despite reversal of obesity, suggesting that this response most likely mimicked increased vasopressor response in AT2 KO mice because it was previously observed after Ang II infusion (16, 42). Together our study excludes the contribution of adipose tissue in the enhanced hypertension observed in absence of AT2 and rather suggests an increased vasopressor response in KOVEX that might be the consequence of change in some component(s) of the RAS system.

At equivalent circulating AGT levels, the rate-limiting step for Ang II generation resides in the kidney through the renin action, which cleaves AGT into Ang I. A previous study implicated AT2 in modulating RAS activity through a reduction of renin synthesis (43). This observation prompted us to investigate the expression of renin in transgenic mice. Our data show that increased AGT formation in fat tissue of OVEX mice

provoked a negative feedback loop on the renin expression in the kidney as previously reported (7). Interestingly, this feedback was disrupted when AT2 was absent in OVEX mice, consistent with the previous unforeseen role of AT2 in the regulation of renin expression (43). So far, AT1 has been considered the main receptor in the control of blood pressure, in part through its positive effect on renin production (44, 45). However, comparable levels of AT1 mRNA expression occurred in the kidney from both OVEX and KOVEX mice, suggesting that AT1 was not involved in this regulation in our model (data not shown). It is still possible that loss of antagonist effects of AT2 may favor the antinatriuretic actions of AT1 as well its regulatory functions in glomerular filtration. The antinatriuretic actions of AT1 in our model is uncertain because we previously reported similar sodium excretion in mice overexpressing AGT in adipose tissue when fed a low-salt chow diet, but the role for AT1 in regulating glomerular filtration remains to be investigated (7). However, when compared with OVEX mice, increased plasma Ang II levels in KOVEX mice were related to the increase in renin expression. As a consequence, elevated Ang II will most likely target Ang II-sensitive tissues expressing primarily AT1 such as the adrenal glands (46), the central nervous system (47), and/or the arterial wall (48, 49), leading to an exaggerated acute pressor response. Similar observation has been made in mice lacking the AT2 gene after pharmacological injection of Ang II (16, 42, 50). Together our findings strongly suggest that AT2 mediates the vasodilator effect of Ang II in presence of an overproduction of AGT, in part

by modulating both kidney renin expression and the vasodilator response in tissues.

In conclusion, our compound transgenic mouse model overexpressing AGT and lacking AT2 provides a unique model in which obesity and hypertension are dissociated. Our studies point out a clear involvement of AT2 in the regulation of both adipose tissue development and blood pressure in mice, such that inactivation of AT2 increased blood pressure and decreased adiposity. Whereas the relevance of our findings in humans remains to be defined, our results provide new insight into the understanding of pathophysiology and therapy of obesity-related hypertension such as metabolic syndrome and provide evidence for a potential important role for AT2 in these diseases.

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