

# Deletion of the Angiotensin Type 2 Receptor (AT2R) Reduces Adipose Cell Size and Protects From Diet-Induced Obesity and Insulin Resistance

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The renin-angiotensin system with its active metabolite angiotensin (Ang) II has been related not only to hypertension but also to obesity and insulin resistance. Recent evidence obtained *in vitro* suggests that the type 2 Ang II receptor (AT2R) mediates the trophic action of Ang II on adipocyte differentiation and lipogenesis. We used AT2R<sup>0/0</sup> mice to delineate a potential role of AT2R in adipose tissue development and metabolism. AT2R<sup>0/0</sup> mice had a normal adiposity but displayed a striking adipose tissue phenotype characterized by small adipocytes and an increase in cell number. In muscle, the expression of several genes involved in lipid metabolism, including fatty acid translocase, uncoupling protein-3, peroxisome proliferator-activated receptors ( $\alpha$ ,  $\delta$ ), and carnitine palmitoyl transferase-1, was increased in AT2R-deficient mice. In response to high-fat feeding, these mice were protected against obesity and obesity-related glucose intolerance, as assessed by glucose tolerance tests. Moreover, lipid oxidation assessed by indirect calorimetry was higher in AT2R-deficient mice than in wild-type mice, irrespective of the diet. This suggests that AT2R-dependent signaling exerts a direct or indirect negative control on lipid utilization in muscles. These data support the idea that AT2R-dependent Ang II signaling increases adipose cell mass and glucose intolerance and thus could participate to the deleterious effects of a high-fat diet. *Diabetes* 54: 991–999, 2005

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AGT, angiotensinogen; Ang, angiotensin; aP2, adipocyte fatty acid protein; CPT, carnitine palmitoyl transferase; FAS, fatty acid synthase; HFD, high-fat diet; LFD, low-fat diet; LPL, lipoprotein lipase; PPAR, peroxisome proliferator-activated receptor; RQ, respiratory quotient; SREBP-1C, sterol regulatory element-binding protein-1C; UCP, uncoupling protein; WAT, white adipose tissue; WT, wild-type.

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Obesity represents a major public health problem because of its epidemic proportions worldwide. Populations that have a sedentary lifestyle and consume a Western diet are predisposed to obesity, a condition associated with increased insulin resistance and hypertension.

During the last decade, the role of white adipose tissue (WAT) as an endocrine organ has been well established. WAT produces a variety of bioactive peptides that have been implicated in several complications of obesity, including insulin resistance and hypertension. WAT is an important extra-hepatic production site of angiotensinogen (AGT) in rodents (1–3) and humans (4–6). Moreover, adipose AGT production is increased in the obese state (2,3). AGT is the precursor of the bioactive peptide angiotensin (Ang) II, produced via the action of renin and ACE. Ang II is reportedly involved in the development of both hypertension and insulin resistance. Agents that inhibit either the biosynthesis or the action of Ang II in hypertensive and insulin-resistant individuals not only normalize blood pressure but also restore insulin sensitivity (7,8). In addition, *in vitro* studies have shown that Ang II negatively modulates muscle insulin signaling, leading to a decrease in insulin-stimulated glucose uptake (9,10). These studies highlight the interactions between the insulin and Ang II signaling systems in the control of glucose homeostasis (11,12).

Several reports suggest the existence of a functional renin angiotensin system in WAT (5,13,14). Recent studies have demonstrated that transgenic mice with overexpression of AGT in the adipose tissue exhibited an increased fat mass due to adipocyte hypertrophy (15). Moreover, *in vitro* studies indicate a stimulatory effect of Ang II on lipogenesis in 3T3-L1 and human adipocytes (16,17). Thus, one could speculate that Ang II acts locally to control adipose tissue mass.

The biological effects of Ang II are mediated by cell surface receptors that belong to the large family of G-protein-associated receptors (18). Ang II receptors exist in the form of two major subtypes: type 1 (AT1R) and type 2 (AT2R). Several studies using specific antagonists suggest that the major vascular functions of Ang II are primarily mediated by the AT1R. By contrast, the physiological functions of AT2R have not been clearly defined. Nevertheless, in adipose cells, recent *in vitro* studies have

implicated AT2R in the lipogenic effect of Ang II (16). AT2R also mediates the effect of Ang II to induce the production and release of prostacyclin from adipocytes, which in turn stimulates differentiation of adipose precursor cells (19,20). All together, this *in vitro* evidence suggests that Ang II could have a trophic role in WAT, favoring both hypertrophy and hyperplasia of adipocytes.

The purpose of the present study was to test the hypothesis that AT2R mediates the effects of Ang II on adipose tissue development *in vivo*. Whole-body glucose disposal and components of energy expenditure were performed using AT2R<sup>+/−</sup> mice (21) exposed to a nutritional challenge known to induce obesity and insulin resistance in rodents. We show that, in the absence of AT2R, mice develop normal adipose mass in spite of adipocyte hypertrophy because of an increased number of adipose cells. In addition, AT2R<sup>+/−</sup> mice exhibit an increased lipid oxidation capacity and are protected from the development of high-fat diet-induced obesity and associated abnormalities such as insulin resistance and hypertension.

## RESEARCH DESIGN AND METHODS

**AT2R-deficient mouse strains.** The generation of the AT2R gene-targeted mouse has been previously described (21). Male C57BL/6 AT2R<sup>+/−</sup> and wild-type (WT) littermates were obtained by crossing male WT and female AT2R<sup>+/−</sup> mice (a gift from Dr. Ichiki). Transgenic mice were screened by PCR of genomic DNA using exon 2-specific oligonucleotides (sense: 5'-AAA AGG TGT AAG AAT TTG GAG TTG CTG CAG-3'; antisense: 5'-AAG GGG AAC TAC ATA AGA TGC TTG CCA GGG-3') on both sides of the neomycin cassette, which produce a 700-bp DNA fragment for WT or a 1-kb fragment for AT2R<sup>+/−</sup> mice. Animals had free access to food and water and were housed in a controlled environment with a 12-h light-dark cycle and constant temperature (22°C). At weaning, the mice were fed either a low-fat diet (LFD) (4% fat wt/wt) or a high-fat diet (HFD) (25% fat wt/wt) as previously described (22). Body weight was recorded throughout the experimental period. Food consumption was measured daily for 2 weeks, between the age of 10 and 12 weeks. All animal protocols were undertaken according to the Guidelines for Care and Use of Experimental Animals, France.

**Energy expenditure.** *In vivo* indirect open circuit calorimetry was performed in metabolic chambers. The 12-week-old animals were randomly and alternatively placed into experimental chambers at 25°C ± 1 with free access to food and water. Constant airflow (0.5 l/min) was drawn through the chamber and monitored by a mass-sensitive flowmeter. To calculate oxygen consumption (V<sub>O<sub>2</sub></sub>), carbon dioxide production (V<sub>CO<sub>2</sub></sub>), and respiratory quotient (RQ: ratio of V<sub>CO<sub>2</sub></sub> to V<sub>O<sub>2</sub></sub>), gas concentrations were monitored at the inlet and outlet of the scaled chambers. Total metabolic rate (energy expenditure) was calculated from oxygen consumption and carbon dioxide production using Lusk's equation and expressed as watts per kilogram to the 0.75 power of body weight. Glucose and lipid oxidation were calculated as previously described (23).

**Glucose tolerance tests.** After 12 h of fasting, mice were injected intraperitoneally with D-glucose (2 g/kg body wt), and blood samples were collected at different times by tail bleeding. Blood glucose was assayed with a blood glucose meter (Roche Diagnostics, Meylan, France), and serum insulin was determined by enzyme-linked immunosorbent assay (Eurobio, Courtaboeuf, France). An index of insulin resistance was calculated from the product of the area under the glucose and insulin curves × 10<sup>−3</sup> as previously described (24).

**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 v3.4/s software (ADInstruments, Phymep, France). Nonanesthetized mice were accustomed to the restrainers and tail-cuff inflation for 2 days before blood pressure determination. Blood pressure was recorded daily (20 determinations in a row) over a 2-day period. Results are expressed as millimeters of mercury.

**Blood parameters.** Plasma leptin was determined using a commercial kit (Clinisciences, Montrouge, France). Plasma insulin was determined by radioimmunoassay using a commercial kit with a rat insulin standard (CIS Biointernational, Gif/Yvette, France).

**Adipose tissue cellularity.** Cellularity of epididymal adipose tissue was determined as previously described (22). Briefly, images of isolated adipocytes were acquired from a light microscope fitted with a camera, and the

measurement of ~400 cell diameters was performed using Perfect Image software (Numeris, Paris, France), allowing calculation of a mean fat cell weight. 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.

**Glucose metabolism studies.** Isolated adipocytes were incubated with 5.5 mmol/l [<sup>14</sup>C]glucose (specific radioactivity 9.7 GBq/mmol) in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3% serum albumin without or with 10 nmol/l Ang II in the presence or not of 1 μmol/l losartan (AT1R antagonist) or 1 μmol/l PD 123319 (AT2R antagonist). After 2 h, the generated <sup>14</sup>C<sub>2</sub> and the <sup>14</sup>C incorporation into total lipids were measured.

**Enzymes activities.** Fatty acid synthase (FAS) activity was measured by spectrophotometric assay of the cytosolic fraction of a crude homogenate of epididymal adipose tissue as previously described (22). Data are expressed as nanomoles NADPH oxidized per minute (mU) per 10<sup>6</sup> cells. Carnitine palmitoyl transferase (CPT-1 and -2) activities were determined in the mitochondrial fraction of hindlimb skeletal muscle homogenate by spectrophotometric assay according to Bieber et al. (25). Data are expressed as millimoles per minute (mU) per milligram mitochondrial proteins.

**Isolation and analysis of RNA.** Total RNA was extracted from tissues as previously described (26), and cDNA was synthesized from 1 μg total RNA with superscript reverse transcriptase (Invitrogen, Cergy, France). Real-time PCR was performed using a Light Cycler apparatus (Roche Diagnostics), and the PCR reaction was carried out as previously described (27). A list of primer sequences is available upon request (quignard@bhd.c.jussieu.fr). Ribosomal 18S RNA expression was used to account for variability in the initial quantities of mRNA.

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

## RESULTS

**Effect of the AT2R gene disruption on growth and adipose tissue development.** On an LFD, AT2R<sup>+/−</sup> mice showed no significant difference in weight gain or fat pad mass compared with WT mice (Fig. 1). Despite similar tissue weight, adipose cell size was twofold lower in the epididymal adipose tissue from AT2R<sup>+/−</sup> mice compared with WT mice (Fig. 1C, Table 1). This adipose cell hypotrophy was fully compensated by a twofold increase in the number of adipose cells per fat pad compared with WT mice. FAS activity, a key lipogenic enzyme, was threefold lower in adipocytes from AT2R<sup>+/−</sup> mice compared with WT mice (Table 1). This was associated with a significant decrease in sterol regulatory element-binding protein-1C (SREBP-1C) and FAS mRNA levels in epididymal adipose tissue (Fig. 2A). The mRNA levels of other adipocyte genes, namely lipoprotein lipase (LPL), fatty acid transporters CD36 and adipocyte fatty acid protein (aP2), and peroxisome proliferator-activated receptor (PPAR)-γ, were also reduced by approximately twofold in epididymal adipose tissue of AT2R<sup>+/−</sup> mice (Fig. 2B and C). By contrast, the mRNA of adiponectin and interleukin-6 were unchanged by the deletion of AT2R (Fig. 2D).

**Lack of effect of an AT1R antagonist on adipocyte glucose metabolism.** In adipose tissue, AT1R expression was clearly higher than AT2R, and the level of its mRNA was not altered in epididymal adipose tissue of AT2R<sup>+/−</sup> mice, suggesting that AT2R deficiency did not promote a compensatory increase in AT1R expression (Fig. 3A). Further, the rates of [<sup>14</sup>C]glucose oxidation and incorporation into lipids by the adipocytes in basal conditions were similar in both genotypes but showed different Ang II responsiveness between genotypes: in adipocytes from WT mice, we observed a stimulating effect of Ang II on both glucose oxidation and glucose conversion into lipids, which is unmodified by the presence of losartan and

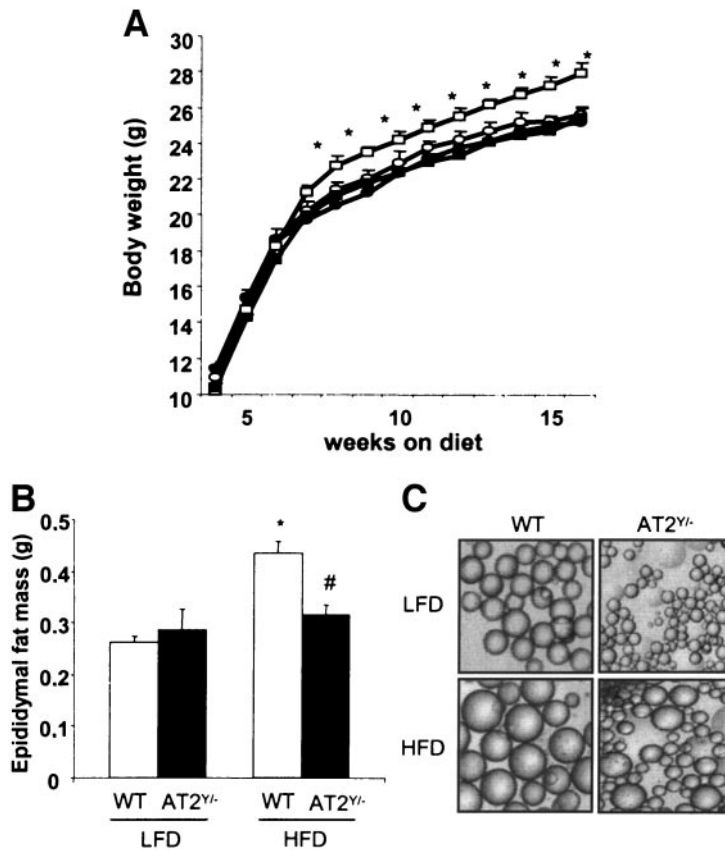


FIG. 1. Body growth and fat accumulation in WT and AT2R<sup>-/-</sup> mice fed an LFD or HFD. **A**: Body weight gain from weaning to 16-week-old WT mice fed an LFD (○) or HFD (□) and AT2R<sup>-/-</sup> mice fed an LFD (●) or HFD (■). \**P* < 0.05, WT vs. AT2R<sup>-/-</sup> in HFD-fed mice. **B**: Epididymal fat mass of WT (□) and AT2R<sup>-/-</sup> (■) mice fed an LFD or HFD. Data are means ± SE (*n* = 6 per group). \**P* < 0.01, HFD vs. LFD; #*P* < 0.01, AT2R<sup>-/-</sup> vs. WT. **C**: Micrographs of isolated epididymal adipose cells of WT and AT2R<sup>-/-</sup> mice fed either an LFD or HFD (representative of *n* = 6 in each group).

decreased in the presence of PD 123319. By contrast, the rates of these two metabolic pathways in AT2R<sup>-/-</sup> mice were unchanged in the presence of Ang II alone or combined with the AT1R antagonist (Fig. 3B and C).

**AT2R deficiency protects against obesity induced by high-fat feeding.** In response to 12 weeks of high-fat feeding, WT mice exhibited a significant increase in the rate of body weight gain when compared with age-matched animals fed an LFD (Fig. 1A). By contrast, AT2R<sup>-/-</sup> mice fed an HFD demonstrated a reduced rate of weight gain compared with WT mice. The difference was significant by the 3rd week of diet and was maintained throughout the feeding period. Epididymal adipose tissue weight was increased by >60% in WT mice fed an HFD versus an LFD, while no diet-induced changes in adipose tissue weight were observed in mice lacking AT2R (Fig. 1B). Consistent with the maintenance of adipose tissue mass, AT2R<sup>-/-</sup> mice did not exhibit the HFD-induced hyperleptinemia that was evident in WT mice (Table 1). Cellularity analysis indicated that the hypotrophic-hyperplastic cellular phenotype of adipose tissue exhibited in mice lacking AT2R was maintained on an HFD (Table 1). High-fat feeding induced a large decrease in FAS activity

regardless of the genotype. Nevertheless, AT2R<sup>-/-</sup> mice maintained the lowest FAS activity compared with WT mice (Table 1). Similarly, lower levels of FAS, LPL, CD36, aP2, SREBP-1C, and PPAR-γ mRNA in the adipose tissue of AT2R<sup>-/-</sup> were induced by high-fat feeding (Fig. 2). The levels of adiponectin and interleukin-6 mRNA in WAT were down- and upregulated, respectively, by high-fat feeding in the WT mice but were unchanged in the AT2R<sup>-/-</sup> mice (Fig. 2D).

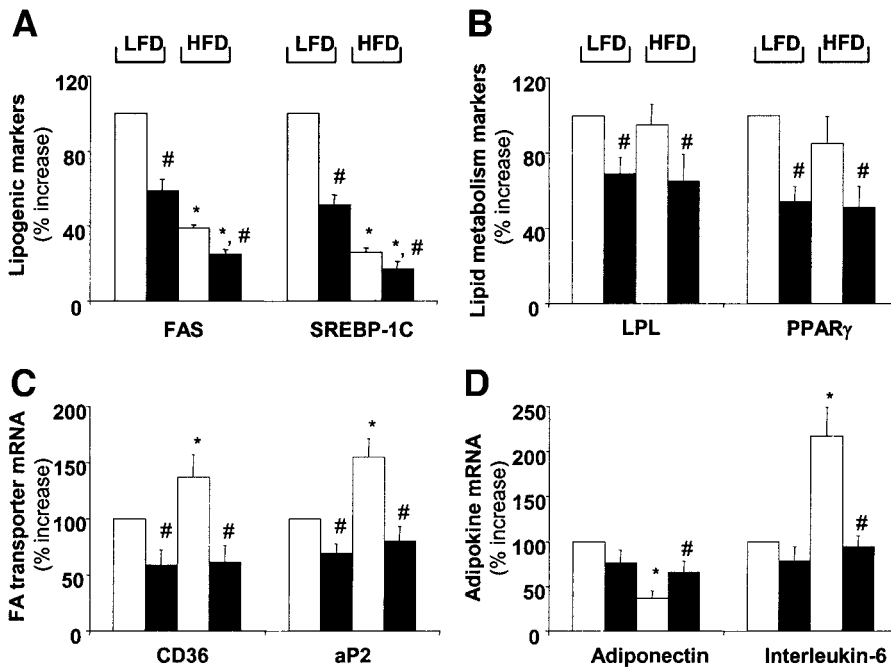
**AT2R deficiency protects against hypertension induced by high-fat feeding.** Blood pressure was similar in mice of both genotypes fed an LFD (137.6 ± 5.5 and 134.4 ± 6.5 mmHg in WT and AT2R<sup>-/-</sup> mice, respectively; NS). However, AT2R deficiency prevented the increase in systolic blood pressure induced by high-fat feeding in WT mice (156.5 ± 3.5 and 133.6 ± 2.3 mmHg in WT and AT2R<sup>-/-</sup>, respectively; *P* < 0.05).

**AT2R deletion influences energy balance.** In low fat-fed mice, neither food intake nor total energy expenditure significantly differed between genotypes (Table 2, Fig. 4A), as reflected by their similar body weights. However, AT2R<sup>-/-</sup> mice exhibited a lower RQ than WT mice during the light phase, as the result of a 30% increase in lipid

TABLE 1  
Effects of AT2R deficiency on epididymal adipose tissue cellularity, FAS activity, and plasma leptin

	WT LFD	AT2R <sup>-/-</sup> LFD	WT HFD	AT2R <sup>-/-</sup> HFD
Fat cell number (×10 <sup>6</sup> )	1.3 ± 0.1*	2.8 ± 0.6†	1.6 ± 0.3*	2.4 ± 0.3†
Fat cell weight (ng)	81.2 ± 7.3*	48.1 ± 3†	110.5 ± 13.3‡	61.9 ± 6.7*
FAS activity (mU/10 <sup>6</sup> cell)	54 ± 7.6*	17.4 ± 3.3‡	26.9 ± 4.2‡	10.6 ± 3.4§
Plasma leptin (ng/ml)	1.7 ± 0.3*	1.6 ± 0.4*	2.7 ± 0.6†	1.5 ± 0.2*

Data are means ± SE (*n* = 6 per group). Data with different symbols (\*, †, ‡, §) are significantly different (*P* < 0.05).



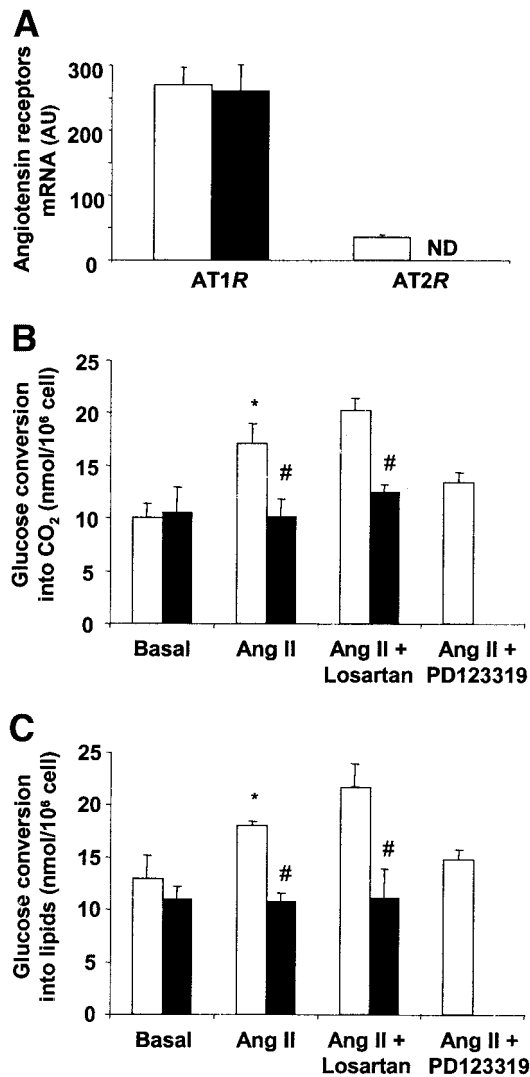
**FIG. 2.** Epididymal adipose tissue gene expression. Expression of mRNA in WT (□) and AT2R<sup>-/-</sup> (■) mice fed an LFD or HFD. **A:** FAS and SREBP-1C; **B:** LPL and PPAR-γ; **C:** CD36 and aP2; **D:** adiponectin and interleukin-6. Values were normalized to ribosomal 18S amount and expressed as percent over LFD-fed WT mice. Results are means ± SE (*n* = 6 per group). #*P* < 0.05, AT2R<sup>-/-</sup> vs. WT; \**P* < 0.05, HFD vs. LFD.

oxidation (Fig. 4B, Table 2). On an HFD, AT2R<sup>-/-</sup> mice displayed a significantly lower food intake (−15 and −25% as expressed per gram of body weight and per whole body, respectively) compared with WT mice (Table 2). High-fat feeding induced a similar threefold increase in lipid oxidation in both genotypes. As a result, the higher rate of lipid oxidation was maintained in AT2R<sup>-/-</sup> mice under high-fat feeding (Table 2). On an HFD, AT2R<sup>-/-</sup> mice also displayed a decreased RQ that specifically occurred during the dark phase at variance with mice fed an LFD (Fig. 4D). In addition, total energy expenditure was significantly increased in AT2R<sup>-/-</sup> mice (Table 2, Fig. 4C). These data indicate that decreased food intake and increased total energy expenditure accounted for resistance to an HFD-induced weight gain in AT2R<sup>-/-</sup> mice.

**AT2R disruption increases muscle β-oxidation and prevents HFD-induced muscle lipid storage.** Because skeletal muscle is the principal tissue using lipid as a fuel, we determined the expression of a subset of genes involved in fatty acid β-oxidation in hindlimb muscles from WT and AT2R<sup>-/-</sup> mice. A coordinated upregulation of CPT-1, uncoupling protein (UCP)-2, UCP-3, and CD36 mRNAs (two- to fourfold) was observed in LFD-fed AT2R<sup>-/-</sup> mice (Fig. 5). By contrast, FABP3 and CPT-2 mRNA levels were unchanged by the genotype. In addition, the expression of PPAR-α and PPAR-δ was increased twofold in muscle from AT2R<sup>-/-</sup> compared with WT mice (Fig. 5D). Increasing the supply of exogenous lipids by high-fat feeding induced a large increase in CPT, UCP, and fatty acid transporter gene expression in the muscle of WT mice. A similar effect was observed in AT2R<sup>-/-</sup> mice, with the exception of UCP-2 and CD36 mRNA, which were unchanged (Fig. 5). The increase in lipid oxidation gene expression due to AT2R deletion was maintained under high-fat feeding except for CD36. Muscle PPAR-α mRNA was increased fourfold in WT and twofold in AT2R<sup>-/-</sup> mice by an HFD, leading to a similar level of gene expression in both genotypes. By contrast, PPAR-δ mRNA was unaffected by an HFD regardless of the genotype and remained

higher in AT2R<sup>-/-</sup> mice than in WT high-fat-fed mice (Fig. 5D). In agreement with the higher CPT-1 mRNA levels, CPT activity was significantly increased in muscles from AT2R<sup>-/-</sup> mice compared with WT mice regardless of the diet (Table 3). To determine whether these modifications in the lipid oxidation capacity could interfere with lipid accumulation in muscle, we measured the triglyceride content in skeletal muscle from mice of both genotypes. Table 3 shows that muscle triglyceride content was similar in mice from both genotypes when maintained on an LFD. However, whereas high-fat feeding led to a fivefold increase in muscle triglyceride content in WT mice, this triglyceride accumulation was partially prevented in AT2R<sup>-/-</sup> mice (twofold increase).

**AT2R gene deletion increases insulin sensitivity and prevents HFD-induced insulin resistance.** On an LFD, AT2R<sup>-/-</sup> mice presented similar glycemia (2.01 ± 0.12 vs. 1.89 ± 0.09 g/l in WT and AT2R<sup>-/-</sup>, respectively; NS) and insulinemia (46.4 ± 6.3 vs. 43.3 ± 5.7 μUI/ml in WT and AT2R<sup>-/-</sup>, respectively; NS) compared with WT mice. However, intraperitoneal injection of a bolus of glucose resulted in a more rapid clearance of glucose from the peripheral circulation in AT2R<sup>-/-</sup> mice compared with WT mice (Fig. 6A, *P* < 0.01 across all time points between 15 and 90 min after injection). Because the insulin response curve was similar in both genotypes, this suggests an increase in insulin sensitivity in mice lacking AT2R (Fig. 6B). This was confirmed by an insulin resistance index significantly lower in AT2R<sup>-/-</sup> mice compared with WT mice (Fig. 6C). Blood glucose levels were unchanged by an HFD in both genotypes (2.05 ± 0.08 vs. 2.02 ± 0.12 g/l in WT and AT2R<sup>-/-</sup>, respectively; NS). However, a significant increase in insulinemia was observed only in WT mice, resulting in higher insulin levels in WT mice compared with AT2R<sup>-/-</sup> mice (61.6 ± 2.6 vs. 51.5 ± 3.9 μUI/ml in WT and AT2R<sup>-/-</sup>, respectively; *P* < 0.05). In response to an HFD, glucose tolerance test curves demonstrated a decrease in whole-body glucose uptake regardless of the genotype, with AT2R<sup>-/-</sup> mice maintaining a higher glucose



**FIG. 3.** Expression and influence of AT1R in epididymal adipose tissue from AT2R<sup>-/-</sup> mice. **A:** Expression of AT1R and AT2R mRNA in WT and AT2R<sup>-/-</sup> mice ( $n = 6$  per group). **B and C:** Effect of Ang II on [U-<sup>14</sup>C]glucose conversion into CO<sub>2</sub> and lipids in adipocytes ( $n = 4$  each conditions). □, WT mice; ■, AT2R<sup>-/-</sup> mice. # $P < 0.05$ , AT2R<sup>-/-</sup> vs. WT; \* $P < 0.05$  vs. basal condition within genotype.

clearance than WT mice (Fig. 6A,  $P < 0.05$  across last time points 90 and 120 min after injection). Moreover, insulin response was markedly increased in WT mice and unchanged in AT2R<sup>-/-</sup> mice. Accordingly, the insulin resistance index was 40% lower in AT2R<sup>-/-</sup> mice than in WT mice.

## DISCUSSION

The present study demonstrates that mice lacking AT2R display adipocyte hypotrophy and are protected against

**TABLE 2**  
Effect of AT2R deficiency on energy metabolism

	WT LFD	AT2R <sup>-/-</sup> LFD	WT HFD	AT2R <sup>-/-</sup> HFD
Food intake (kcal/day)	13.6 ± 1*	12.4 ± 1*	20.5 ± 1.4†	16.4 ± 1.3‡
Food intake (kcal/day/g <sup>0.75</sup> )	1.19 ± 0.09*	1.10 ± 0.09*	1.69 ± 0.11†	1.45 ± 0.11‡
Energy expenditure (W/kg <sup>0.75</sup> )	5.47 ± 0.11*	5.58 ± 0.09*	7.45 ± 0.14†	8.02 ± 0.21‡
Glucose oxidation (W/kg <sup>0.75</sup> )	4.73 ± 0.15*	4.57 ± 0.16*	4.83 ± 0.13*	4.78 ± 0.23*
Lipid oxidation (W/kg <sup>0.75</sup> )	0.74 ± 0.11*	1.01 ± 0.12†	2.62 ± 0.21‡	3.24 ± 0.25§

Data are means ± SE ( $n = 5$  per group). Data with different symbols (\*, †, ‡, §) are significantly different ( $P < 0.05$ ).

HFD-induced obesity. This protection is linked to a synergic increase in energy expenditure and a decrease in food intake compared with WT mice. We also demonstrate that AT2R<sup>-/-</sup> mice display increased whole-body lipid oxidation, regardless of the diet, which could be related to an increase in  $\beta$ -oxidation within the muscle.

**Implication of AT2R in adipose tissue development.** Adipocyte hypotrophy exhibited by AT2R<sup>-/-</sup> mice was also manifest in transgenic mice that are deficient in AGT, the Ang II precursor (28). These observations argue for a role of AT2R in mediating Ang II effects on adiposity in vivo. The diminution of fat cell size observed in AT2R<sup>-/-</sup> mice is associated with a decrease in FAS activity, i.e., in fat synthesis capacity. This is in agreement with a previous report showing that Ang II is a lipogenic hormone that enhances adipocyte FAS gene transcription via AT2R (16,17). In addition, the decrease in a subset of genes involved in fatty acid uptake and storage as LPL, aP2, and CD36, along with PPAR- $\gamma$ , could also contribute to limiting fat deposition into adipocytes from AT2R<sup>-/-</sup> mice. Our findings support a role of AT2R in mediating the lipogenic effects of Ang II in vivo. In contrast, in vitro studies showed an inhibitory effect of Ang II on adipose conversion of human preadipocytes, which is mediated by AT1R (29,30). Whether AT1R signaling could be implicated in such a decrease in lipogenic pathway is questionable. We reported here that Ang II induced an increase in glucose metabolism mediated by AT2R that is not influenced by an AT1R blocker. This was confirmed by the lack of Ang II effect in adipocytes from AT2R<sup>-/-</sup> mice leading to a decrease in glucose metabolism in agreement with a lower insulin-stimulated glucose uptake displayed by adipose tissue from these mice (31). Moreover, whatever the role that AT1R plays in vivo, our data showing the similar level of expression in adipose tissue from WT and AT2R<sup>-/-</sup> mice do not support a specific role for AT1R in the impaired lipogenesis capacity displayed by AT2R<sup>-/-</sup> mice adipose tissue. In addition to adipocyte hypotrophy, mice lacking AT2R exhibit adipose tissue hyperplasia, suggesting that Ang II could inhibit differentiation of adipocyte precursors. However, Ang II has been previously shown in vitro to stimulate adipocyte differentiation through AT2R (19). Thus, it is likely that the adipocyte hyperplasia shown in AT2R<sup>-/-</sup> mice is mediated by other mechanisms. One possibility is that, in vivo, Ang II could decrease the proliferation of preadipocytes through AT2R as previously demonstrated in smooth muscle or endothelial cell types (32,33). Thus, if Ang II through AT2R exerts an anti-proliferative action on preadipocytes, this could account for the adipose tissue hyperplasia observed in the mice lacking AT2R.

**AT2R deficiency and energy metabolism.** Another in-

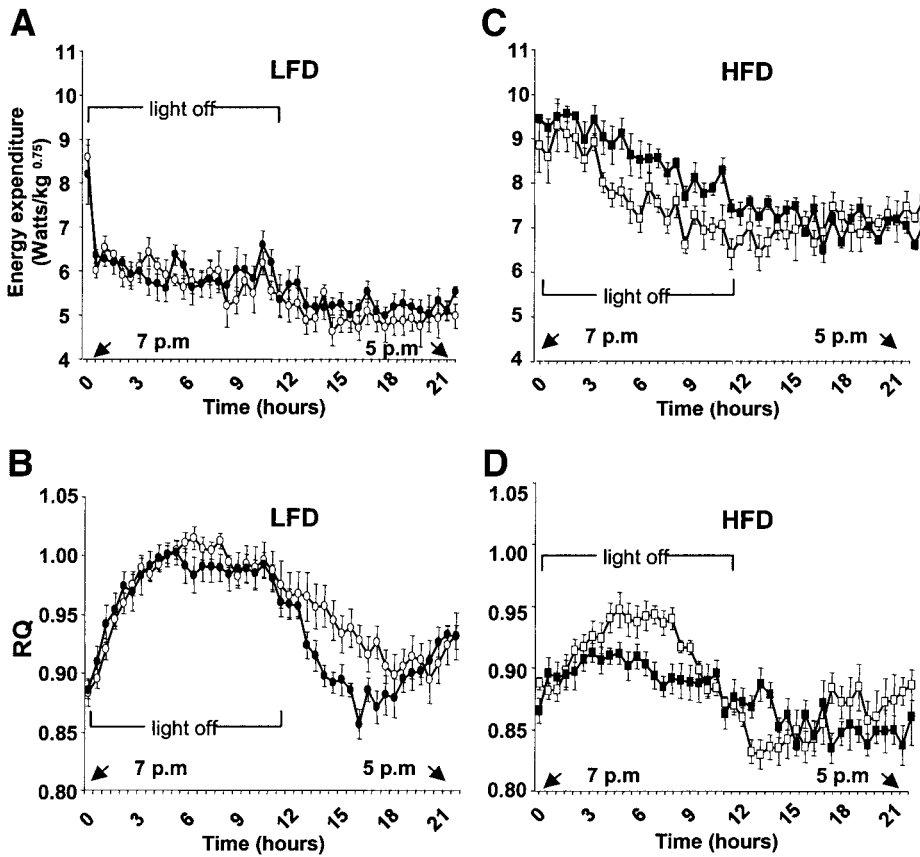


FIG. 4. Effects of AT2R deficiency on whole-body energy expenditure (EE) (A and C) and RQ (B and D) were measured by indirect calorimetry. WT (○) and AT2R<sup>-/-</sup> (■) mice fed an LFD or HFD (WT, □; AT2R<sup>-/-</sup>, ■). Data are expressed as means ± SE (n = 5 per group). Comparisons show a significant genotype effect (P < 0.05) on RQ values during the diurnal period (light on) in LFD-fed mice and on both RQ and EE values during the nocturnal period (light off) in HFD-fed mice.

interesting aspect of the phenotype of AT2R<sup>-/-</sup> mice is an increase in whole-body lipid oxidation. This observation is consistent with a higher β-oxidation capacity in muscle of AT2R<sup>-/-</sup> mice, as suggested by the increase in CPT activity and UCP expression. The coordinate increase of genes involved in the lipid oxidative pathway could be mediated by the higher expression levels of both PPAR-α and -δ transcriptional factors, which play an essential role in the upregulation of lipid oxidation in cardiac and skeletal muscles in vivo (34,35). This is the first evidence that AT2R could contribute to the control of lipid oxidation. Despite the presence of AT2R in muscle of WT mice (unpublished data), we cannot conclude whether excessive lipid oxidation can be directly attributed to the lack of this receptor in muscle. Indeed, it is well known that adipocytes secrete hormones such as leptin or adiponectin, which can in turn increase fatty acid oxidation (36,37). However, the fact that neither plasma leptin nor adipose tissue adiponectin expression was affected in mice lacking AT2R on an LFD in the current study argues for a direct implication of this receptor to control muscle metabolism.

**AT2R deficiency improves insulin sensitivity.** Both adipose and muscular tissues, which display abnormal lipid metabolism, could be implicated in the better glucose

tolerance exhibited by AT2R<sup>-/-</sup> mice when fed a standard diet. In adipose tissue, the possibility exists that decreased PPAR-γ expression improved insulin sensitivity, as demonstrated in PPAR-γ-deficient mice (38). Moreover, decreasing PPAR-γ activity by a specific antagonist was shown to improve insulin sensitivity, which is accompanied by adipocyte hypotrophy and hyperplasia as observed in AT2R<sup>-/-</sup> mice (39). There is also some experimental evidence for a beneficial effect of enhanced β-oxidation on insulin sensitivity in vitro (40,41) and in vivo (42). Studies related to the role of Ang II in insulin sensitivity are limited and contradictory (12,31,43,44). Recently, Shiuchi et al. (31) have reported that insulin-mediated glucose uptake in AT2R<sup>-/-</sup> mice was similar in skeletal muscles compared with WT mice. However, the fact that AT2R<sup>-/-</sup> mice display an increase in both whole-body lipid oxidation argues for a beneficial effect of β-oxidation in the control of glucose homeostasis.

**The disruption of AT2R in mice protects against HFD-induced obesity and obesity-associated disorders.** Protection from weight gain can be explained by either a reduced food intake and/or increased energy expenditure. On an HFD, food intake is lower in AT2R<sup>-/-</sup> mice, which was also observed in AGT-deficient mice

TABLE 3  
Effect of AT2R deficiency on indicators of skeletal muscle lipid metabolism

	WT LFD	AT2R <sup>-/-</sup> LFD	WT HFD	AT2R <sup>-/-</sup> HFD
CPT activity (mU/mg protein)	4.78 ± 0.16*	6.17 ± 0.25†	7.03 ± 0.26‡	8.03 ± 0.39§
Triglyceride content (mg/g tissue)	19.2 ± 1.8*	18.8 ± 2.4*	92.8 ± 15.5†	45.7 ± 6.6‡

Data are means ± SE (n = 6 per group). Data with different symbols (\*, †, ‡, §) are significantly different (P < 0.05).

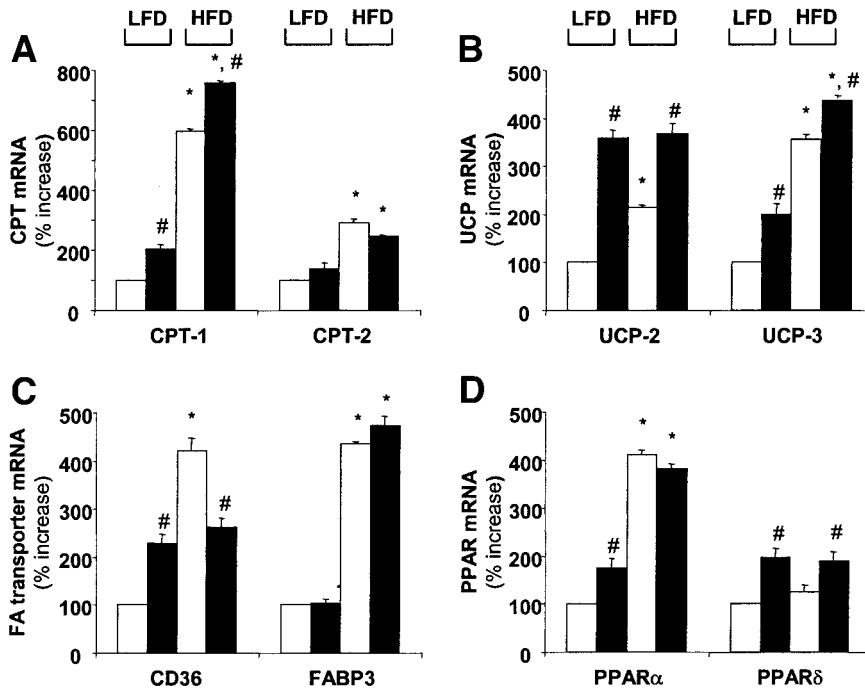


FIG. 5. Expression of skeletal muscle-derived genes. mRNA expression of mitochondrial CPT and UCP (A and B, respectively), fatty acid (FA) transporters (C), and transcriptional factors (D) in WT ( $\square$ ) and AT2R<sup>-/-</sup> ( $\blacksquare$ ) mice fed an LFD or HFD. Values were normalized to ribosomal 18S. Results are means  $\pm$  SE ( $n = 6$  per group). Differences were significant between genotype (# $P < 0.05$ ) or between diet (\* $P < 0.05$ ).

(28). Energy expenditure is increased in both control and AT2R<sup>-/-</sup> mice as a direct result of the increased energy intake induced by an HFD. It is important to note, however, that energy expenditure is higher in AT2R<sup>-/-</sup> mice than in control mice. This, together with a lower food intake, explains the resistance to obesity of the AT2R<sup>-/-</sup> mice. This protection can be related to the adipocyte phenotype. Indeed, hypotrophic adipocytes of AT2R<sup>-/-</sup> mice with a reduced expression of PPAR- $\gamma$  could contribute to the prevention of obesity as previously reported (45). AT2R<sup>-/-</sup> mice also exhibit a reduced muscle triglyceride content, in response to an HFD, that potentially could protect against insulin resistance (46); this is likely the consequence of increased muscle  $\beta$ -oxidation capacity. RQ profiles clearly indicate that during the feeding period, HFD-fed AT2R<sup>-/-</sup> mice maintain a higher lipid oxidation, suggesting that AT2R<sup>-/-</sup> mice oxidize more of the ingested lipids than control mice. At variance, a higher lipid oxidation takes place during the postprandial period in LFD-fed AT2R<sup>-/-</sup> mice, reflecting a mobilization of stored lipids. Altogether, these findings indicate that AT2R<sup>-/-</sup> mice use much more readily the available lipids than control mice. Divergent effects of AT2R deficiency on blood pressure have been reported (21,47,48). In our studies, we did not find an elevation in blood pressure in LFD-fed AT2R<sup>-/-</sup> mice. Different factors such as diet, environmental stress, or age might explain this discrepancy. High-fat feeding provoked a relative hypertension only in WT mice. This is in support of the documented contribution of obesity to the onset of hypertension. Altogether, we demonstrated that the protection of AT2R<sup>-/-</sup> mice from the deleterious effects of an HFD could implicate concomitantly both adipose tissue and muscles.

In conclusion, our observations provide the first evidence that AT2R participates in the control of energy metabolism and in glucose homeostasis. Such a role of AT2R might be physiologically important in the control of adiposity and in obesity-related disorders.

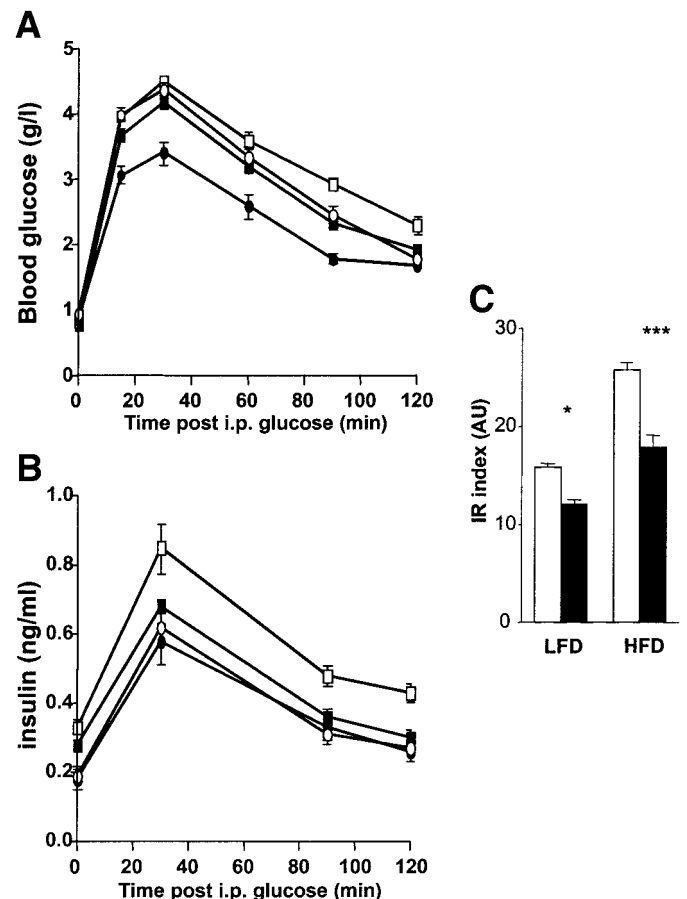


FIG. 6. Effect of high-fat feeding on glucose tolerance. Intraperitoneal glucose tolerance tests were performed in WT and AT2R<sup>-/-</sup> mice. Blood glucose concentrations (A) and insulin (B) were measured at the indicate points. Data for WT ( $\circ$ ) and AT2R<sup>-/-</sup> ( $\bullet$ ) mice fed an LFD and after an HFD are shown (WT,  $\square$ ; AT2R<sup>-/-</sup>,  $\blacksquare$ ). The insulin resistance index (C) is the product of areas under glucose and insulin curves. Data are expressed as the mean  $\pm$  SE ( $n = 6$  per group). \* $P < 0.05$ , \*\*\* $P < 0.001$ , AT2R<sup>-/-</sup> vs. WT mice.

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