Deletion of the Angiotensin Type 2 Receptor (AT2*R*) Reduces Adipose Cell Size and Protects From Diet-Induced Obesity and Insulin Resistance

Laurent Yvan-Charvet,[1] Patrick Even,[2] May Bloch-Faure,[3] Michèle Guerre-Millo,[1] Naima Moustaid-Moussa,[4] Pascal Ferre,[1] and Annie Quignard-Boulange[1]

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 (AT2*R*) mediates the trophic action of Ang II on adipocyte differentiation and lipogenesis. We used AT2$R^{y/-}$ mice to delineate a potential role of AT2*R* in adipose tissue development and metabolism. AT2$R^{y/-}$ 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 (α, δ), and carnitine palmitoyl transferase-1, was increased in AT2*R*-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 AT2*R*-deficient mice than in wild-type mice, irrespective of the diet. This suggests that AT2*R*-dependent signaling exerts a direct or indirect negative control on lipid utilization in muscles. These data support the idea that AT2*R*-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

From [1]Unit 465, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France; [2]UMR914, Institut National de la Recherche Agronomique, INAPG, Paris, France; [3]Unit 367, INSERM, Paris, France; and the [4]Department of Nutrition and Agricultural Experiment Station, University of Tennessee, Knoxville, Tennessee.

Address correspondence and reprint requests to Annie Quignard-Boulange, INSERM U465, 15 rue de l'Ecole de Médecine, 75270 Paris Cedex 06, France. E-mail: quignard@bhdc.jussieu.fr.

Received for publication 27 September 2004 and accepted in revised form 2 January 2005.

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–biding protein-1C; UCP, uncoupling protein; WAT, white adipose tissue; WT, wild-type.

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 (AT1*R*) and type 2 (AT2*R*). Several studies using specific antagonists suggest that the major vascular functions of Ang II are primarily mediated by the AT1*R*. By contrast, the physiological functions of AT2*R* have not been clearly defined. Nevertheless, in adipose cells, recent in vitro studies have

implicated AT2*R* in the lipogenic effect of Ang II (16). AT2*R* 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 AT2*R* mediates the effects of Ang II on adipose tissue development in vivo. Whole-body glucose disposal and components of energy expenditure were performed using AT2$R^{y/-}$ mice (21) exposed to a nutritional challenge known to induce obesity and insulin resistance in rodents. We show that, in the absence of AT2*R*, mice develop normal adipose mass in spite of adipocyte hypotrophy because of an increased number of adipose cells. In addition, AT2$R^{y/-}$ 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

AT2*R*-deficient mouse strains. The generation of the AT2*R* gene–targeted mouse has been previously described (21). Male C57BL/6 AT2$R^{y/-}$ and wild-type (WT) littermates were obtained by crossing male WT and female AT2$R^{+/-}$ 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 AT2$R^{y/-}$ 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 (Vo_2), carbon dioxide production (VCO_2), and respiratory quotient (RQ: ratio of VCO_2 to Vo_2), 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 $\times 10^{-3}$ 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 [U-^{14}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 (AT1*R* antagonist) or 1 μmol/l PD 123319 (AT2*R* antagonist). After 2 h, the generated $^{14}CO_2$ and the ^{14}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 NAPDH oxidized per minute (mU) per 10^6 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@bhdc.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 AT2*R* gene disruption on growth and adipose tissue development. On an LFD, AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice compared with WT mice (Fig. 1*C*, 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 AT2$R^{y/-}$ 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. 2*A*). 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 AT2$R^{y/-}$ mice (Fig. 2*B* and *C*). By contrast, the mRNA of adiponectin and interleukin-6 were unchanged by the deletion of AT2*R* (Fig. 2*D*).

Lack of effect of an AT1*R* antagonist on adipocyte glucose metabolism. In adipose tissue, AT1*R* expression was clearly higher than AT2*R*, and the level of its mRNA was not altered in epididymal adipose tissue of AT2$R^{y/-}$ mice, suggesting that AT2*R* deficiency did not promote a compensatory increase in AT1*R* expression (Fig. 3*A*). Further, the rates of [U-^{14}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

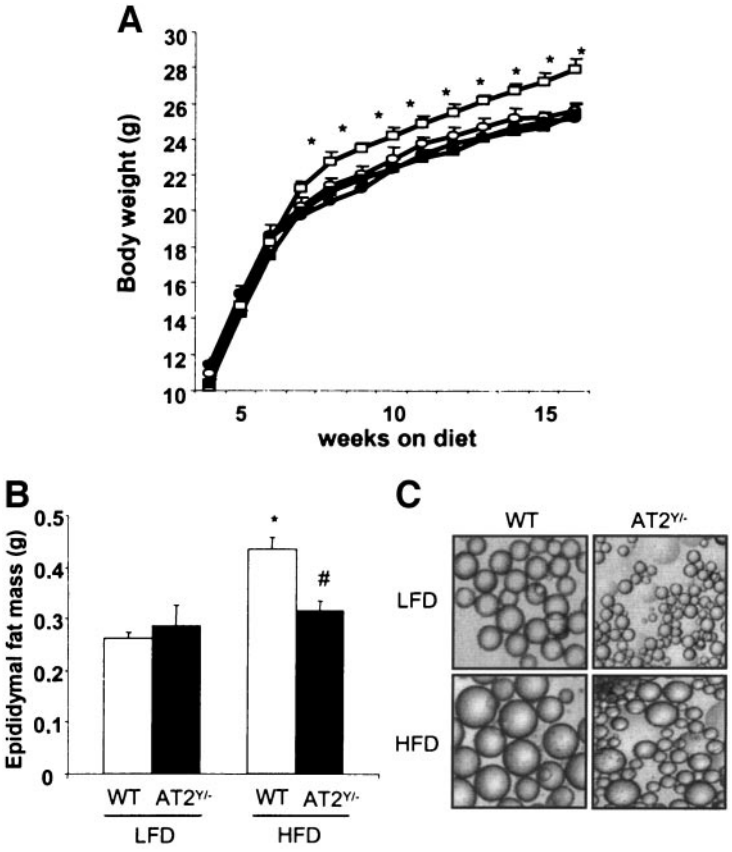

FIG. 1. Body growth and fat accumulation in WT and AT2$R^{y/-}$ mice fed an LFD or HFD. *A*: Body weight gain from weaning to 16-week-old WT mice fed an LFD (○) or HFD (□) and AT2$R^{y/-}$ mice fed an LFD (●) or HFD (■). *$P < 0.05$, WT vs. AT2$R^{y/-}$ in HFD-fed mice. *B*: Epididymal fat mass of WT (□) and AT2$R^{y/-}$ (■) mice fed an LFD or HFD. Data are means ± SE ($n = 6$ per group). *$P < 0.01$, HFD vs. LFD; #$P < 0.01$, AT2$R^{y/-}$ vs. WT. *C*: Micrographs of isolated epididymal adipose cells of WT and AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice were unchanged in the presence of Ang II alone or combined with the AT1*R* antagonist (Fig. 3*B* and *C*).

AT2*R* 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. 1*A*). By contrast, AT2$R^{y/-}$ 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 AT2*R* (Fig. 1*B*). Consistent with the maintenance of adipose tissue mass, AT2$R^{y/-}$ 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 AT2*R* was maintained on an HFD (Table 1). High-fat feeding induced a large decrease in FAS activity regardless of the genotype. Nevertheless, AT2$R^{y/-}$ 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 AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice (Fig. 2*D*).

AT2*R* 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 AT2$R^{y/-}$ mice, respectively; NS). However, AT2*R* 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 AT2$R^{y/-}$, respectively; $P < 0.05$).

AT2*R* deletion influences energy balance. In low fat–fed mice, neither food intake nor total energy expenditure significantly differed between genotypes (Table 2, Fig. 4*A*), as reflected by their similar body weights. However, AT2$R^{y/-}$ 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 AT2*R* deficiency on epididymal adipose tissue cellularity, FAS activity, and plasma leptin

	WT LFD	AT2$R^{y/-}$ LFD	WT HFD	AT2$R^{y/-}$ HFD
Fat cell number ($\times 10^6$)	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/10^6 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 ($P < 0.05$).

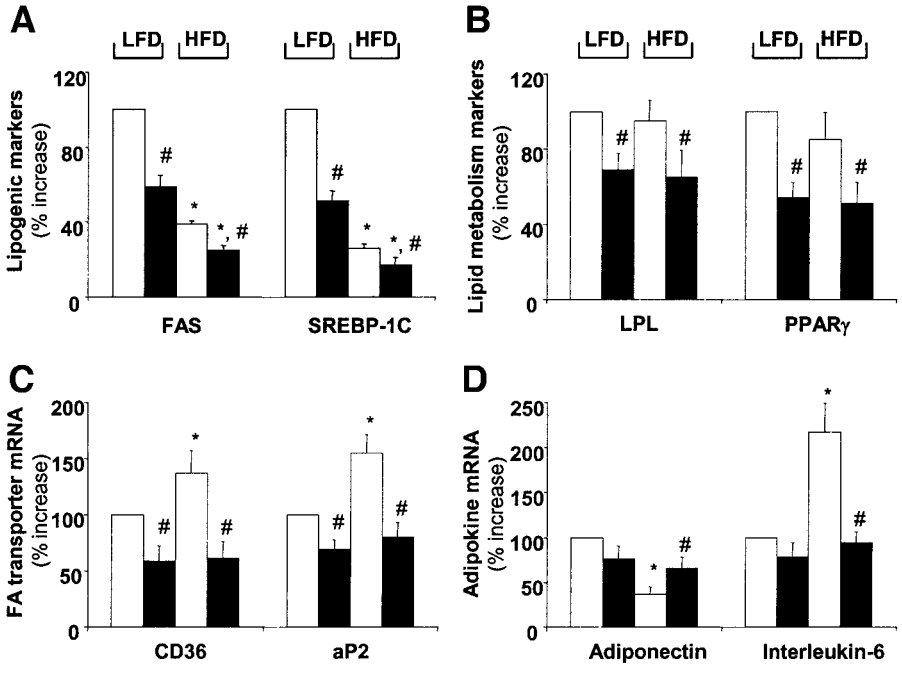

FIG. 2. Epididymal adipose tissue gene expression. Expression of mRNA in WT (□) and AT2$R^{y/-}$ (■) mice fed an LFD or HFD. *A*: FAS and SREBP-1C; *B*: LPL and PPAR-γ; *C*: CD36 and aP2; *D*: adiponectin and interleukin-6. Values were normalized to ribosomal 18S amount and expressed as percent over LFD-fed WT mice. Results are means ± SE (n = 6 per group). #P < 0.05, AT2$R^{y/-}$ vs. WT; *P < 0.05, HFD vs. LFD.

oxidation (Fig. 4*B*, Table 2). On an HFD, AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice under high-fat feeding (Table 2). On an HFD, AT2$R^{y/-}$ mice also displayed a decreased RQ that specifically occurred during the dark phase at variance with mice fed an LFD (Fig. 4*D*). In addition, total energy expenditure was significantly increased in AT2$R^{y/-}$ mice (Table 2, Fig. 4*C*). These data indicate that decreased food intake and increased total energy expenditure accounted for resistance to an HFD-induced weight gain in AT2$R^{y/-}$ mice.

AT2*R* 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 AT2$R^{y/-}$ mice. A coordinated upregulation of CPT-1, uncoupling protein (UCP)-2, UCP-3, and CD36 mRNAs (two- to fourfold) was observed in LFD-fed AT2$R^{y/-}$ 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 AT2$R^{y/-}$ compared with WT mice (Fig. 5*D*). 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 AT2$R^{y/-}$ mice, with the exception of UCP-2 and CD36 mRNA, which were unchanged (Fig. 5). The increase in lipid oxidation gene expression due to AT2*R* deletion was maintained under high-fat feeding except for CD36. Muscle PPAR-α mRNA was increased fourfold in WT and twofold in AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice than in WT high-fat–fed mice (Fig. 5*D*). In agreement with the higher CPT-1 mRNA levels, CPT activity was significantly increased in muscles from AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice (twofold increase).

AT2*R* gene deletion increases insulin sensitivity and prevents HFD-induced insulin resistance. On an LFD, AT2$R^{y/-}$ mice presented similar glycemia (2.01 ± 0.12 vs. 1.89 ± 0.09 g/l in WT and AT2$R^{y/-}$, respectively; NS) and insulinemia (46.4 ± 6.3 vs. 43.3 ± 5.7 μUI/ml in WT and AT2$R^{y/-}$, 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 AT2$R^{y/-}$ mice compared with WT mice (Fig. 6*A*, $P < 0.01$ 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 AT2*R* (Fig. 6*B*). This was confirmed by an insulin resistance index significantly lower in AT2$R^{y/-}$ mice compared with WT mice (Fig. 6*C*). 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 AT2$R^{y/-}$, respectively; NS). However, a significant increase in insulinemia was observed only in WT mice, resulting in higher insulin levels in WT mice compared with AT2$R^{y/-}$ mice (61.6 ± 2.6 vs. 51.5 ± 3.9 μUI/ml in WT and AT2$R^{y/-}$, 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 AT2$R^{y/-}$ mice maintaining a higher glucose

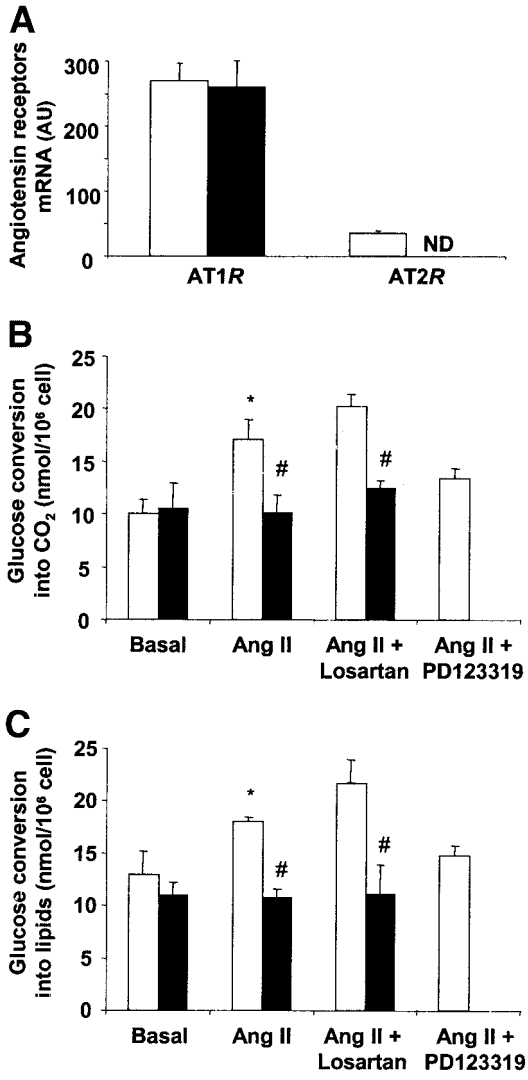

FIG. 3. Expression and influence of AT1*R* in epididymal adipose tissue from AT2$R^{y/-}$ mice. *A*: Expression of AT1*R* and AT2*R* mRNA in WT and AT2$R^{y/-}$ mice (n = 6 per group). *B* and *C*: Effect of Ang II on [U-^{14}C]glucose conversion into CO_2 and lipids in adipocytes (n = 4 each conditions). □, WT mice; ■, AT2$R^{y/-}$ mice. #P < 0.05, AT2$R^{y/-}$ vs. WT; *P < 0.05 vs. basal condition within genotype.

clearance than WT mice (Fig. 6*A*, $P < 0.05$ across last time points 90 and 120 min after injection). Moreover, insulin response was markedly increased in WT mice and unchanged in AT2$R^{y/-}$ mice. Accordingly, the insulin resistance index was 40% lower in AT2$R^{y/-}$ mice than in WT mice.

DISCUSSION

The present study demonstrates that mice lacking AT2*R* display adipocyte hypotrophy and are protected against 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 AT2$R^{y/-}$ mice display increased whole-body lipid oxidation, regardless of the diet, which could be related to an increase in β-oxidation within the muscle.

Implication of AT2*R* in adipose tissue development. Adipocyte hypotrophy exhibited by AT2$R^{y/-}$ mice was also manifest in transgenic mice that are deficient in AGT, the Ang II precursor (28). These observations argue for a role of AT2*R* in mediating Ang II effects on adiposity in vivo. The diminution of fat cell size observed in AT2$R^{y/-}$ 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 AT2*R* (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-γ, could also contribute to limiting fat deposition into adipocytes from AT2$R^{y/-}$ mice. Our findings support a role of AT2*R* 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 AT1*R* (29,30). Whether AT1*R* 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 AT2*R* that is not influenced by an AT1*R* blocker. This was confirmed by the lack of Ang II effect in adipocytes from AT2$R^{y/-}$ 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 AT1*R* plays in vivo, our data showing the similar level of expression in adipose tissue from WT and AT2$R^{y/-}$ mice do not support a specific role for AT1*R* in the impaired lipogenesis capacity displayed by AT2$R^{y/-}$ mice adipose tissue. In addition to adipocyte hypotrophy, mice lacking AT2*R* 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 AT2*R* (19). Thus, it is likely that the adipocyte hyperplasia shown in AT2$R^{y/-}$ mice is mediated by other mechanisms. One possibility is that, in vivo, Ang II could decrease the proliferation of preadipocytes through AT2*R* as previously demonstrated in smooth muscle or endothelial cell types (32,33). Thus, if Ang II through AT2*R* exerts an antiproliferative action on preadipocytes, this could account for the adipose tissue hyperplasia observed in the mice lacking AT2*R*.

AT2*R* deficiency and energy metabolism. Another in-

TABLE 2
Effect of AT2*R* deficiency on energy metabolism

	WT LFD	AT2$R^{y/-}$ LFD	WT HFD	AT2$R^{y/-}$ HFD
Food intake (kcal/day)	13.6 ± 1*	12.4 ± 1*	20.5 ± 1.4†	16.4 ± 1.3‡
Food intake (kcal/day/g$^{0.75}$)	1.19 ± 0.09*	1.10 ± 0.09*	1.69 ± 0.11†	1.45 ± 0.11‡
Energy expenditure (W/kg$^{0.75}$)	5.47 ± 0.11*	5.58 ± 0.09*	7.45 ± 0.14†	8.02 ± 0.21‡
Glucose oxidation (W/kg$^{0.75}$)	4.73 ± 0.15*	4.57 ± 0.16*	4.83 ± 0.13*	4.78 ± 0.23*
Lipid oxidation (W/kg$^{0.75}$)	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 ($P < 0.05$).

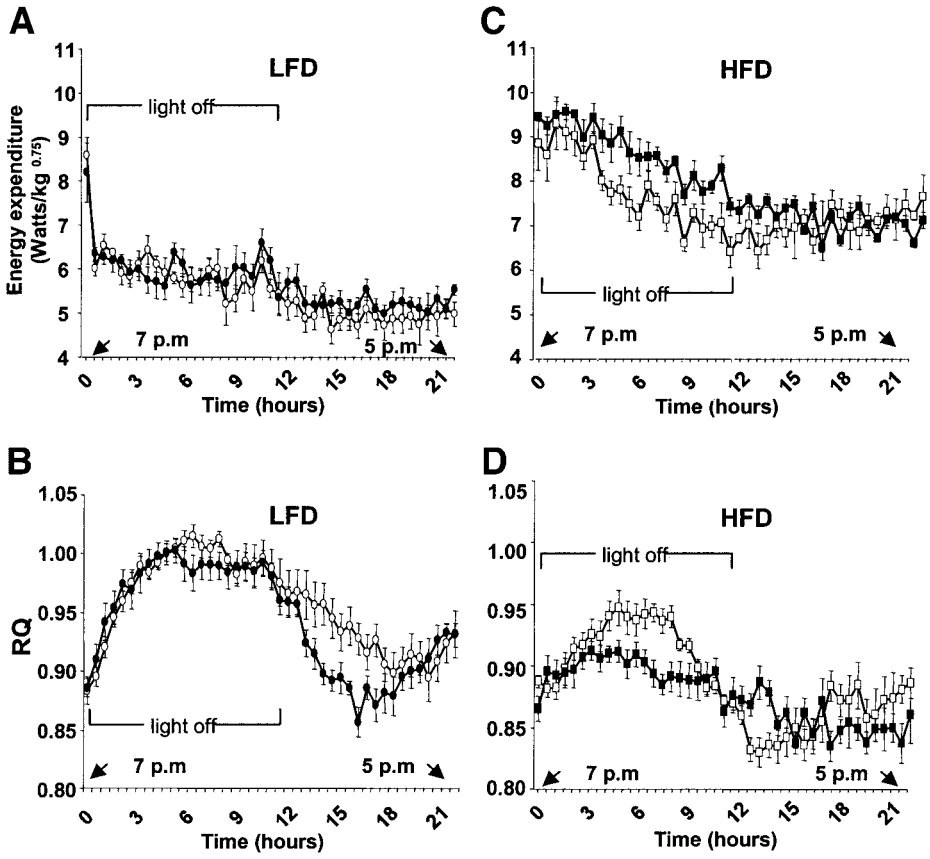

FIG. 4. Effects of AT2*R* deficiency on whole-body energy expenditure (EE) (*A* and *C*) and RQ (*B* and *D*) were measured by indirect calorimetry. WT (○) and AT2$R^{y/-}$ (■) mice fed an LFD or HFD (WT, □; AT2$R^{y/-}$, ■). Data are expressed as means ± SE (n = 5 per group). Comparisons show a significant 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.

teresting aspect of the phenotype of AT2$R^{y/-}$ mice is an increase in whole-body lipid oxidation. This observation is consistent with a higher β-oxidation capacity in muscle of AT2$R^{y/-}$ 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 AT2*R* could contribute to the control of lipid oxidation. Despite the presence of AT2*R* 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 AT2*R* on an LFD in the current study argues for a direct implication of this receptor to control muscle metabolism.

AT2*R* deficiency improves insulin sensitivity. Both adipose and muscular tissues, which display abnormal lipid metabolism, could be implicated in the better glucose tolerance exhibited by AT2$R^{y/-}$ 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 AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice was similar in skeletal muscles compared with WT mice. However, the fact that AT2$R^{y/-}$ 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 AT2*R* 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 AT2$R^{y/-}$ mice, which was also observed in AGT-deficient mice

TABLE 3
Effect of AT2*R* deficiency on indicators of skeletal muscle lipid metabolism

	WT LFD	AT2$R^{y/-}$ LFD	WT HFD	AT2$R^{y/-}$ 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).

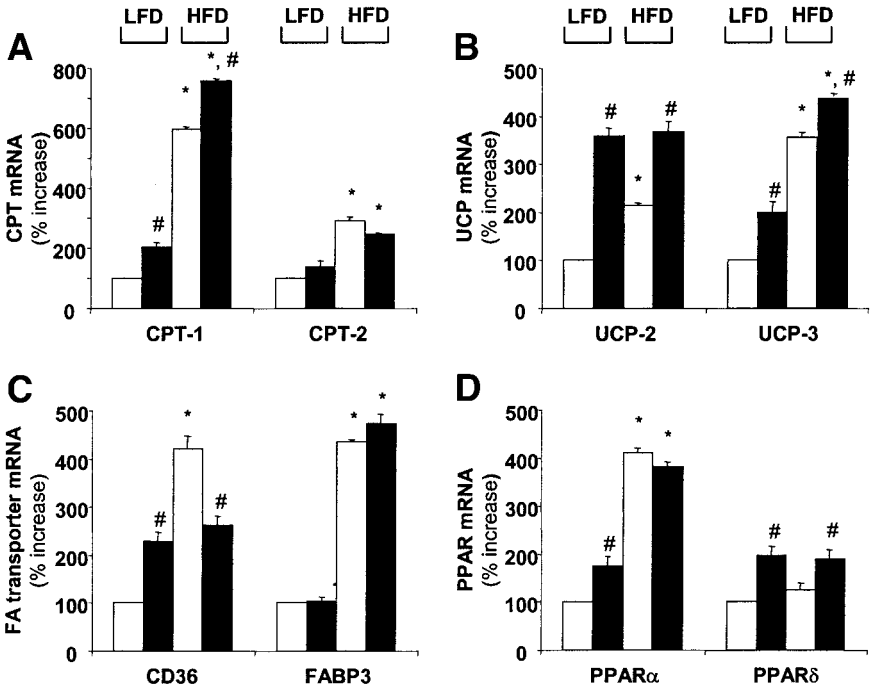

FIG. 5. Expression of skeletal muscle–derived genes. mRNA expression of mitochondrial CPT and UCP (*A* and *B*, respectively), fatty acid (FA) transporters (*C*), and transcriptional factors (*D*) in WT (□) and AT2$R^{y/-}$ (■) mice fed an LFD or HFD. Values were normalized to ribosomal 18S. Results are means ± 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 AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice than in control mice. This, together with a lower food intake, explains the resistance to obesity of the AT2$R^{y/-}$ mice. This protection can be related to the adipocyte phenotype. Indeed, hypotrophic adipocytes of AT2$R^{y/-}$ mice with a reduced expression of PPAR-γ could contribute to the prevention of obesity as previously reported (45). AT2$R^{y/-}$ 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 β-oxidation capacity. RQ profiles clearly indicate that during the feeding period, HFD-fed AT2$R^{y/-}$ mice maintain a higher lipid oxidation, suggesting that AT2$R^{y/-}$ 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 AT2$R^{y/-}$ mice, reflecting a mobilization of stored lipids. Altogether, these findings indicate that AT2$R^{y/-}$ mice use much more readily the available lipids than control mice. Divergent effects of AT2*R* 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 AT2$R^{y/-}$ 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 AT2$R^{y/-}$ 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 AT2*R* participates in the control of energy metabolism and in glucose homeostasis. Such a role of AT2*R* might be physiologically important in the control of adiposity and in obesity-related disorders.

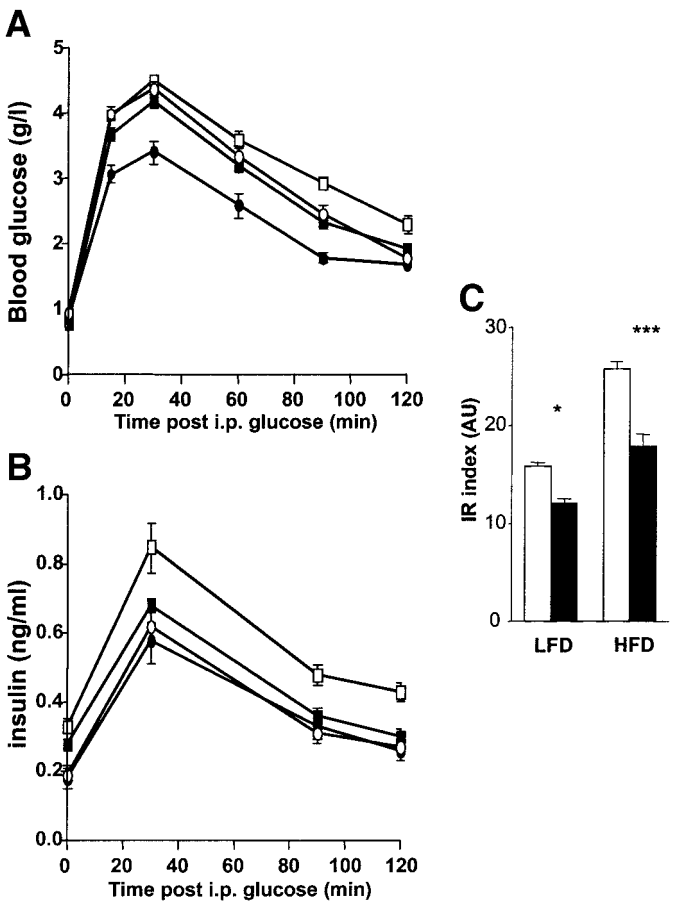

FIG. 6. Effect of high-fat feeding on glucose tolerance. Intraperitoneal glucose tolerance tests were performed in WT and AT2$R^{y/-}$ mice. Blood glucose concentrations (*A*) and insulin (*B*) were measured at the indicate points. Data for WT (○) and AT2$R^{y/-}$ (●) mice fed an LFD and after an HFD are shown (WT, □; AT2$R^{y/-}$, ■). The insulin resistance index (*C*) is the product of areas under glucose and insulin curves. Data are expressed as the mean ± SE (n = 6 per group). *$P < 0.05$, ***$P < 0.001$, AT2$R^{y/-}$ vs. WT mice.

ACKNOWLEDGMENTS

This work was supported by grants from the Fondation de France and INSERM.

We thank Bronwyn Hegarty and Jean-Charles Martin for scientific discussions. We acknowledge the help of Dr. Meneton, who provided us with AT2$R^{y/-}$ mice, originally supplied by Dr. Ichiki.

REFERENCES

1. Cassis LA, Saye JA, Peach MJ: Location and regulation of rat angiotensinogen messenger RNA. *Hypertension* 11:591–596, 1988
2. Hainault I, Nebout G, Turban S, Ardouin B, Ferre P, Quignard-Boulange A: Adipose tissue-specific increase in angiotensinogen expression and secretion in the obese (fa/fa) Zucker rat. *Am J Physiol* 282:E59–E66, 2002
3. Frederich RC, Kahn BB, Peach MJ, Flier JS: Tissue-specific nutritional regulation of angiotensinogen in adipose tissue. *Hypertension* 19:339–344, 1992
4. Jones BH, Standridge MK, Taylor JW, Moustaid N: Angiotensinogen gene expression in adipose tissue: analysis of obese models and hormonal and nutritional control. *Am J Physiol* 273:R236–R242, 1997
5. Engeli S, Gorzelniak K, Kreutz R, Runkel N, Distler A, Sharma AM: Co-expression of renin-angiotensin system genes in human adipose tissue. *J Hypertens* 17:555–560, 1999
6. Prat-Larquemin L, Oppert JM, Clement K, Hainault I, Basdevant A, Guy-Grand B, Quignard-Boulange A: Adipose angiotensinogen secretion, blood pressure, and AGT M235T polymorphism in obese patients. *Obes Res* 12:556–561, 2004
7. Yusuf S, Gerstein H, Hoogwerf B, Pogue J, Bosch J, Wolffenbuttel BH, Zinman B: Ramipril and the development of diabetes. *JAMA* 286:1882–1885, 2001
8. Berne C, Pollare T, Lithell H: Effects of antihypertensive treatment on insulin sensitivity with special reference to ACE inhibitors. *Diabetes Care* 14 (Suppl. 4):39–47, 1991
9. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP: Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels: a potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J Clin Invest* 100:2158–2169, 1997
10. Fukuda N, Satoh C, Hu WY, Nakayama M, Kishioka H, Kanmatsuse K: Endogenous angiotensin II suppresses insulin signaling in vascular smooth muscle cells from spontaneously hypertensive rats. *J Hypertens* 19:1651–1658, 2001
11. Velloso LA, Folli F, Sun XJ, White MF, Saad MJ, Kahn CR: Cross-talk between the insulin and angiotensin signaling systems. *Proc Natl Acad Sci U S A* 93:12490–12495, 1996
12. Ogihara T, Asano T, Ando K, Chiba Y, Sakoda H, Anai M, Shojima N, Ono H, Onishi Y, Fujishiro M, Katagiri H, Fukushima Y, Kikuchi M, Noguchi N, Aburatani H, Komuro I, Fujita T: Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. *Hypertension* 40:872–879, 2002
13. Schling P, Mallow H, Trindl A, Loffler G: Evidence for a local renin angiotensin system in primary cultured human preadipocytes. *Int J Obes Relat Metab Disord* 23:336–341, 1999
14. Karlsson C, Lindell K, Ottosson M, Sjostrom L, Carlsson B, Carlsson LMS: Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. *J Clin Endocrinol Metab* 83:3925–3929, 1998
15. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M: Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J* 15:2727–2729, 2001
16. Jones BH, Standridge MK, Moustaid N: Angiotensin II increases lipogenesis in 3T3–L1 and human adipose cells. *Endocrinology* 138:1512–1519, 1997
17. Kim S, Dugail I, Standridge M, Claycombe K, Chun J, Moustaid-Moussa N: Angiotensin II-responsive element is the insulin-responsive element in the adipocyte fatty acid synthase gene: role of adipocyte determination and differentiation factor 1/sterol-regulatory-element-binding protein 1c. *Biochem J* 357:899–904, 2001
18. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T: International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 52:415–472, 2000
19. Darimont C, Vassaux G, Ailhaud G, Negrel R: Differentiation of preadipose cells: role of protacyclin upon stimulation of adipose cells by angiotensin-II. *Endocrinology* 135:2030–2036, 1994
20. Saint-Marc P, Kozak LP, Ailhaud G, Darimont C, Negrel R: Angiotensin II as a trophic factor of white adipose tissue: stimulation of adipose cell formation. *Endocrinology* 142:487–492, 2001
21. Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BL, Inagami T: Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature* 377:748–750, 1995
22. Briquet-Laugier V, Dugail I, Ardouin B, Le Liepvre X, Lavau M, Quignard-Boulange A: Evidence for a sustained genetic effect on fat storage capacity in cultured adipose cells from Zucker rats. *Am J Physiol* 267:E439–E446, 1994
23. Even PC, Mokhtarian A, Pele A: Practical aspects of indirect calorimetry in laboratory animals. *Neurosci Biobehav Rev* 18:435–447, 1994
24. Mondon CE, Dolkas CB, Oyama J: Enhanced skeletal muscle insulin sensitivity in year-old rats adapted to hypergravity. *Am J Physiol* 240: E482–E488, 1981
25. Bieber LL, Abraham T, Helmrath T: A rapid spectrophotometric assay for carnitine palmitoyltransferase. *Anal Biochem* 50:509–518, 1972
26. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294–5299, 1979
27. Le Lay S, Lefrere I, Trautwein C, Dugail I, Krief S: Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3–L1 adipocytes: identification of CCAAT/enhancer-binding protein beta as an SREBP-1C target. *J Biol Chem* 277:35625–35634, 2002
28. Massiera F, Seydoux J, Geloen A, Quignard-Boulange A, Turban S, Saint-Marc P, Fukamizu A, Negrel R, Ailhaud G, Teboul M: Angiotensinogen-deficient mice exhibit impairment of diet-induced weight gain with alteration in adipose tissue development and increased locomotor activity. *Endocrinology* 142:5220–5225, 2001
29. Schling P, Loffler G: Effects of angiotensin II on adipose conversion and expression of genes of the renin-angiotensin system in human preadipocytes. *Horm Metab Res* 33:189–195, 2001
30. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM: Mature adipocytes inhibit in vitro differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes* 51:1699–1707, 2002
31. Shiuchi T, Iwai M, Li S, Wu L, Min LJ, Li JM, Okumura M, Cui TX, Horiuchi M: Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. *Hypertension* 43:1003–1010, 2004
32. Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horiuchi M, Pratt RE, Dzau VJ: The angiotensin II type 2 (AT2) receptor antagonizes the growth effects of the AT1 receptor: gain-of-function study using gene transfer. *Proc Natl Acad Sci U S A* 92:10663–10667, 1995
33. Stoll M, Steckelings UM, Paul M, Bottari SP, Metzger R, Unger T: The angiotensin AT2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. *J Clin Invest* 95:651–657, 1995
34. Leone TC, Weinheimer CJ, Kelly DP: A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci U S A* 96:7473–7478, 1999
35. Luquet S, Lopez-Soriano J, Holst D, Fredenrich A, Melki J, Rassoulzadegan M, Grimaldi PA: Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability. *FASEB J* 17:2299–2301, 2003
36. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415:339–343, 2002
37. Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang CC, Itani SI, Lodish HF, Ruderman NB: Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A* 99:16309–16313, 2002
38. Miles PD, Barak Y, He W, Evans RM, Olefsky JM: Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency. *J Clin Invest* 105:287–292, 2000
39. Rieusset J, Touri F, Michalik L, Escher P, Desvergne B, Niesor E, Wahli W: A new selective peroxisome proliferator-activated receptor gamma antagonist with antiobesity and antidiabetic activity. *Mol Endocrinol* 16:2628–2644, 2002
40. Perdomo G, Commerford SR, Richard AM, Adams SH, Corkey BE, O'Doherty RM, Brown NF: Increased beta-oxidation in muscle cells enhances insulin-stimulated glucose metabolism and protects against fatty acid-induced insulin resistance despite intramyocellular lipid accumulation. *J Biol Chem* 279:27177–27186, 2004
41. Huppertz C, Fischer BM, Kim YB, Kotani K, Vidal-Puig A, Slieker LJ, Sloop

KW, Lowell BB, Kahn BB: Uncoupling protein 3 (UCP3) stimulates glucose uptake in muscle cells through a phosphoinositide 3-kinase-dependent mechanism. *J Biol Chem* 276:12520–12529, 2001
42. Abu-Elheiga L, Oh W, Kordari P, Wakil SJ: Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. *Proc Natl Acad Sci U S A* 100:10207–10212, 2003
43. Townsend RR, DiPette DJ: Pressor doses of angiotensin II increase insulin-mediated glucose uptake in normotensive men. *Am J Physiol* 265: 362–E366, 1993
44. Buchanan TA, Thawani H, Kades W, Modrall JG, Weaver FA, Laurel C, Poppiti R, Xiang A, Hsueh W: Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. *J Clin Invest* 92:720–726, 1993
45. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T: PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 4:597–609, 1999
46. Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW: The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand* 178:373–383, 2003
47. Ichihara S, Senbonmatsu T, Price E Jr, Ichiki T, Gaffney FA, Inagami T: Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension. *Circulation* 104:346–351, 2001
48. Hein L, Barsh GS, Pratt RE, Dzau VJ, Kobilka BK: Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor in mice. *Nature* 377:744–747, 1995