

# Candesartan cilexetil prevents diet-induced insulin resistance via peroxisome proliferator-activated receptor- $\gamma$ activation in an obese rat model

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**Abstract.** Angiotensin II type 1 receptor (AT1R) blockers (ARBs) have been shown to reduce the incidence of type 2 diabetes mellitus; however, the underlying molecular mechanism is unknown. Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) is the central regulator of insulin and glucose metabolism, which improves insulin sensitivity. Whether candesartan cilexetil, as a prodrug of the AT1R blocker candesartan, has PPAR $\gamma$ -activating properties remains to be elucidated. The aim of the present study was to investigate the effects of oral administration of candesartan cilexetil on glucose tolerance and the actions of PPAR $\gamma$  on liver and adipose tissue in the insulin-resistant obese rat induced by high-fat diet. Animals treated with candesartan cilexetil showed an improved glucose tolerance after oral glucose challenge. Whole-body insulin sensitivity was evaluated using the hyperinsulinemic-euglycemic clamp technique. During high-fat feeding in high-fat diet (HF) rats, the glucose infusion rate (GIR) was 52.3% lower than that in normal chow (NC) rats. However, the GIR was significantly enhanced following candesartan cilexetil treatment. Angiotensin II receptor antagonism also resulted in significant increases in PPAR $\gamma$  protein expression in adipose and liver tissue. These results indicate that PPAR $\gamma$  activation by candesartan cilexetil may provide novel therapeutic options in the treatment of patients with metabolic syndrome.

## Introduction

Angiotensin II type 1 receptor blockers (ARBs) are widely and safely used as an alternative to angiotensin converting enzyme inhibitors for the treatment of hypertension and

hypertension-related cardiovascular diseases without inducing cough (1). In addition to their beneficial effects against hypertension, ARBs have been found to exhibit metabolic actions. In several clinical trials, the incidence of new-onset type 2 diabetes was significantly lower in hypertensive subjects treated with ARBs than in those treated with other hypertensive therapies, which suggests potential antidiabetic effects of angiotensin receptor blockade (2-4). In addition, ARBs have been found to improve insulin sensitivity in a 3T3-L1 cell model of insulin resistance (5). The underlying mechanisms of the insulin-sensitizing/antidiabetic effect of ARBs remain widely unknown.

The peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) is a clinically validated target for the treatment of insulin resistance and functions as a transcription factor that regulates the gene expression involved in carbohydrate and lipid metabolism, thereby ameliorating type 2 diabetes (6). A study conducted by Benson *et al* (7) demonstrated that telmisartan exhibited selective PPAR $\gamma$ -modulating activity when tested at concentrations typically achieved in plasma with conventional oral dosing. The other clinically approved ARBs that were tested had little or no effect on PPAR $\gamma$  (7,8), although the oral administration of an extremely high dose of irbesartan (50 mg/kg) was able to cause some activation of the receptor (9).

Therefore, the present study was conducted to investigate whether candesartan cilexetil exerts protective effects on glucose and lipid metabolism and increases PPAR $\gamma$  levels in adipose and liver tissues and thus improves insulin sensitivity in a rat model of diet-induced obesity.

## Materials and methods

**Chemicals.** Affinity-purified rabbit anti-PPAR $\gamma$  polyclonal antibody was purchased from Abcam Inc. (Cambridge, MA, USA; cat. no. ab19481). Human insulin (Novolin<sup>TM</sup> R) was from Novo Nordisk (Copenhagen, Denmark). Candesartan cilexetil was provided by Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). Rabbit anti- $\beta$ -actin polyclonal antibody was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA; cat. no. sc-1616). Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) was obtained from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China; cat. no. ZB-2301).

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**Animals and treatment.** Male Wistar rats were obtained at 6-7 weeks of age from Vital River Laboratories Co., Ltd. (Beijing, China). The rats were housed two per cage at 22 $\pm$ 2°C and a relative humidity of 40-70% under a 12-h light/dark cycle with *ad libitum* access to food and tap water until the start of experiments. Starting at 8-9 weeks of age, the animals were randomly distributed into three groups: Normal chow group (NC group, n=15), high-fat diet group (HF group, n=15) and high-fat diet with daily candesartan cilexetil treatment group (HF + C group, n=15). The normal chow diet consisted of (as a percentage of total kcal) 17% fat, 63% carbohydrate and 10% protein, and a high-fat diet consisted of 27% fat, 53% carbohydrate and 20% protein. Rats in both the HF group and the HF + C group were initially fed a high-fat diet for 4 weeks, and then respectively received 8 mg/kg/day of either saline (vehicle) or candesartan cilexetil by gavage for 28 consecutive days, during which the high-fat diet was continued.

Blood was drawn from the retro-orbital venous plexus, and blood glucose (BG) was immediately measured with a Bayer Ascensia Breeze<sup>TM</sup> glucometer (Bayer HealthCare LLC, Mishawaka, IN, USA), after which the blood was collected in a gel tube containing a clotting accelerator and allowed to clot prior to centrifugation for 10 min at 3,000  $\times$  g. Subsequently, serum was collected and frozen at -80°C. Serum lipids and lipoproteins were determined using Rat Triglyceride, Cholesterol, Low Density Lipoprotein and High Density Lipoprotein kits (cat. nos. R6635, R6955, R6953 and R6952, respectively; TSZ ELISA, Waltham, MA, USA), according to the manufacturer's protocol. In addition, serum levels of insulin (Insulin RIA kit; Linco Research, Inc., St. Charles, MO, USA) and angiotensin II [ANG II enzyme-linked immunosorbent assay (ELISA) kit; RapidBio, West Hills, CA, USA] were determined. Insulin sensitivity index (ISI), a surrogate index of insulin sensitivity, was calculated as follows:  $ISI = 1/(FPG \times FINS)$ , where FPG is fasting plasma glucose (expressed in mmol/l) and FINS is fasting insulin (expressed in mU/l) (10). All experimental procedures and protocols conformed to guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996). The present study was approved by the Laboratory Animal Welfare and Ethics Committee of Chinese PLA General Hospital (Beijing, China).

**Oral glucose tolerance tests.** At the end of the 4-week treatment period, the rats were fasted overnight prior to the oral glucose tolerance test (OGTT). Animals underwent feeding with 2 g/kg body weight glucose by gavage. Blood was drawn from a cut at the tip of the tail at 0, 30, 60 and 120 min after the glucose feeding, as previously described (11). Plasma glucose concentrations were determined. The area under the curve (AUC) for glucose during the OGTT was calculated using the trapezoid method (12).

**Hyperinsulinemic-euglycemic clamp analysis.** At the end of the 4-week treatment period, conscious rats received local anesthesia using 2% lidocaine hydrochloride (Abbot Laboratories, North Chicago, IL, USA) on the tail root. The tail artery and vein were catheterized for blood sampling and infusion, respectively. Basic BG and basic insulin were measured. Regular human insulin (Novolin<sup>TM</sup> R) was infused intravenously at a

rate of 0.25 U/kg·h. Hyperinsulinemic-euglycemic clamp analysis was performed as described previously (13). Briefly, blood specimens (30  $\mu$ l) were obtained from the tail arterial catheter at 5-min intervals for measuring plasma glucose levels by the glucose oxidase method using the Bayer Ascensia Breeze<sup>TM</sup> glucometer. Based on these values, 10% glucose solution was variably infused to maintain normal glucose levels. A higher glucose infusion rate indicated a higher insulin sensitivity of the peripheral tissue.

**Western blot analysis.** Rats were anesthetized following an overnight fast, and within 10-15 min the abdominal cavity was opened. The perirenal and epididymal adipose tissue, the liver and the heart were removed, snap frozen in liquid nitrogen, weighed and stored at -80°C until processed. Epididymal fat sample was subjected to homogenization as previously described (14). Following centrifugation at 4°C for 20 min at 12,000  $\times$  g, the resultant supernatants were used for immunoblotting. Total protein concentration was determined using the bicinchoninic acid method (Pierce BCA Protein Assay kit; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Blots were incubated overnight at 4°C with rabbit anti-PPAR $\gamma$  (1:400 dilution) and rabbit anti- $\beta$ -actin (1:200 dilution) polyclonal antibodies, followed by incubation for 1 h at room temperature with peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1:5,000 dilution). The blots were washed and then developed using an ECL Plus immunoblotting detection kit (Applygen Technologies Inc., Beijing, China). The protein bands were visualized by exposure of the membranes to Kodak X-ray film (Kodak, Rochester, NY, USA). Band intensities were scanned using a densitometer (Model GS-710; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and quantified by Quantity One software (Bio-Rad Laboratories, Inc.).

**Immunohistochemistry.** Five-micrometer sections of 4% paraformaldehyde-fixed paraffin-embedded liver tissues were deparaffinized and hydrated. Endogenous peroxidase activity was then blocked with 3% H<sub>2</sub>O<sub>2</sub> in phosphate-buffered saline for 10 min, and the tissues were processed for heat-induced antigen retrieval in 0.01 mol/l citrate buffer (pH 6.0) for 10 min. Primary rabbit-anti-PPAR $\gamma$  polyclonal antibody was incubated at a dilution of 1/50-1/100 for 30 min at room temperature before application to tissue sections. A two-step immunohistological detection kit (cat. no. PV-6001; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.), including a goat anti-rabbit IgG-HRP antibody, and a diaminobenzidine (DAB) chromogen (DAB kit; Sigma-Aldrich, St. Louis, MO, USA), were used to visualize specific binding. Five randomly selected high power fields in the centrilobular or periportal areas were examined randomly per section under a light microscope (Olympus CX31; Olympus Corporation, Tokyo, Japan). The number of PPAR $\gamma$  positive hepatocytes was counted in each section. The labeling indices, expressed as the percentage of positive hepatocytes of the total hepatocytes, were calculated.

**Statistical analysis.** Data are expressed as the mean  $\pm$  standard deviation. Comparisons between groups were performed by one-way analysis of variance.  $P < 0.05$  was considered to indicate a statistically significant difference.

Table I. Effect of candesartan treatment on final body weight, visceral organ weights and various biochemical parameters in the three experimental groups.

Characteristic	NC group	HF group	HF + C group
Body weight, g	461.33±36.48	518.40±28.30 <sup>a</sup>	478.33±34.79 <sup>b</sup>
Perirenal fat weight, g	13.54±3.21	26.89±4.81 <sup>a</sup>	20.50±4.09 <sup>a,c</sup>
Epididymal fat weight, g	7.64±1.72	11.50±1.95 <sup>a</sup>	9.61±1.83 <sup>a,b</sup>
Heart weight, g	1.34±0.07	1.29±0.13	1.09±0.11 <sup>a,c</sup>
Liver weight, g	13.04±1.63	17.86±1.76 <sup>a</sup>	15.72±1.70 <sup>a,c</sup>
Kidney weight, g	2.46±0.24	2.28±0.17	2.25±0.18
Triglyceride, mmol/l	1.82±0.67	1.27±0.24	1.30±0.37
Total cholesterol, mmol/l	1.62±0.54	2.01±0.26 <sup>d</sup>	1.37±0.39 <sup>b</sup>
Low-density lipoprotein cholesterol, mmol/l	0.07±0.03	0.72±0.15 <sup>a</sup>	0.62±0.23 <sup>a</sup>
High-density lipoprotein cholesterol, mmol/l	0.75±0.13	0.6±0.06 <sup>a</sup>	0.68±0.11
Serum angiotensin II, pg/ml	43.73±5.63	51.88±9.70 <sup>d</sup>	60.82±10.76 <sup>c,d</sup>
Fasting blood glucose, mmol/l	6.18±0.73	6.38±0.66	5.76±0.92
Fasting serum insulin, mU/l	1.57±0.98	4.76±2.75 <sup>d</sup>	3.03±1.37 <sup>c,d</sup>
Insulin sensitivity index, x10 <sup>-3</sup>	98.76±16.72	29.37±8.95 <sup>d</sup>	57.93±11.83 <sup>c,d</sup>
Hyperinsulinemic euglycemic clamp study, glucose infusion rate, mg/kg/min	28.3±5.4	13.5±3.9 <sup>a</sup>	22.4±5.1 <sup>b</sup>

Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. Data are presented as the mean ± standard deviation (n=15 per group). <sup>a</sup>P<0.01 vs. the NC group; <sup>b</sup>P<0.01 vs. the HF group; <sup>c</sup>P<0.05 vs. the HF group; <sup>d</sup>P<0.05 vs. the NC group.

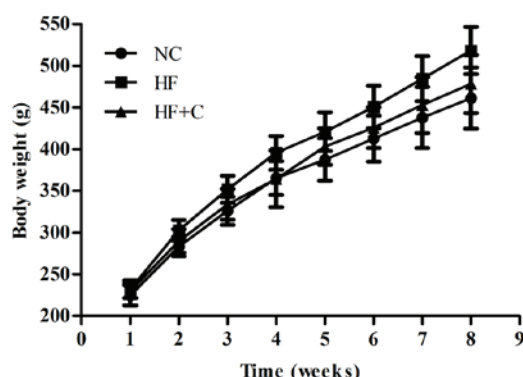


Figure 1. Changes in the body weight of rats. Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. Data are presented as the mean ± standard deviation (n=15).

## Results

**Characteristics of animals studied.** The initial body weights were similar in the groups of 15 rats (data not shown). Although the rats in the HF group had significantly higher body weights than those in the NC group from the second week, body weights were lower in the HF + C group than in the HF group from the third week of treatment until the end of the experiment (Fig. 1). The perirenal fat weight, epididymal fat weight and liver weight of the HF + C group were significantly lower than those of the HF group, but higher than those of the NC group (Table I). Although final heart weights and

kidney weights of rats in the HF group were slightly, but not significantly, lower than those for rats in the NC group, heart weights were significantly ( $P<0.05$ ) higher than those for the HF + C group (Table I).

**Serum lipid profile and angiotensin II concentration.** Although there was no statistically significant difference in serum triglyceride levels among all three groups, animals treated with 8 mg/kg candesartan cilexetil displayed significant reductions in serum cholesterol ( $1.37\pm0.39$  mmol/l in the HF + C group vs.  $2.01\pm0.26$  mmol/l in the HF group;  $P<0.01$ ) (Table I). Serum angiotensin II levels in the HF group were significantly higher than those in controls ( $P<0.05$ ); furthermore, the increase in angiotensin II levels in the HF + C group was significantly more pronounced than that in the HF group ( $P<0.05$ ) due to angiotensin II type 1 (AT1) receptor blockade (Table I).

**Glucose and insulin concentrations.** Fasting blood glucose levels were comparable in the NC and HF groups. Candesartan cilexetil showed a tendency to lower the blood glucose levels in the high-fat-fed rats, but this effect did not reach statistical significance ( $P>0.05$ ; Table I). The fasting insulin levels in the HF group were significantly higher compared with those in the NC group ( $P<0.05$ ; Table I). However, animals receiving candesartan cilexetil displayed significant reductions in insulin levels (36%;  $P<0.05$ ) compared with those in the HF group. As an indirect marker of peripheral insulin action, the ISI was calculated for each animal. As shown in Table I, the ISI of rats in the HF group was substantially lower compared with that of rats in the NC group, but the ISI of rats in the HF + C group

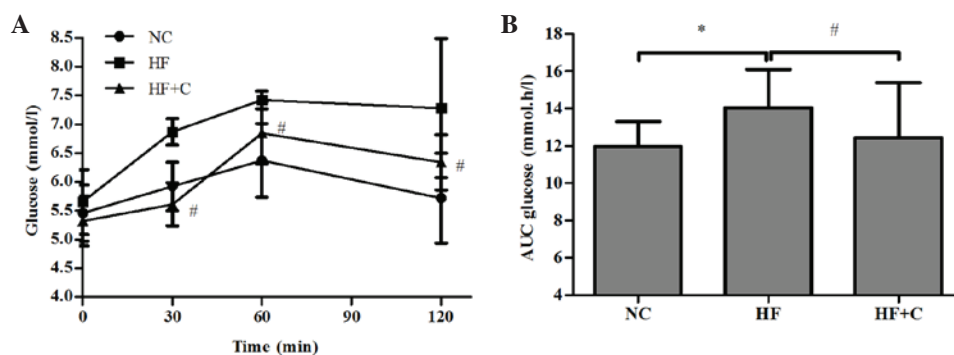


Figure 2. Effect of candesartan cilexetil on (A) glucose responses during the OGTT and (B) glucose AUC. Compared with the HF group, candesartan treatment resulted in lower glucose values and decreased AUC values implying improved insulin resistance. Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. Data are presented as the mean  $\pm$  standard deviation (n=15). \*P<0.05 vs. the NC group; #P<0.05 vs. the HF group. OGTT, oral glucose tolerance test; AUC, area under the curve.

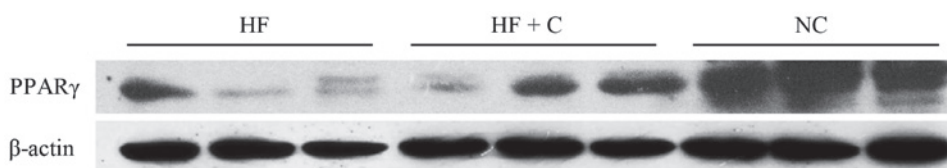


Figure 3. Western blots showing the effect of candesartan cilexetil on the protein expression of PPAR $\gamma$  in white adipose tissue. Candesartan cilexetil treatment together with high-fat diet induced a clear increase in PPAR $\gamma$  compared with that in HF rats treated with saline. Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. PPAR $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ .

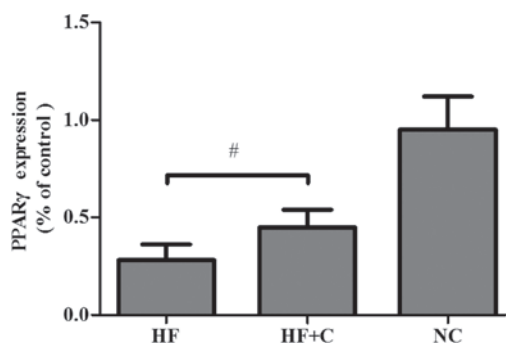


Figure 4. Quantification of the amount of PPAR $\gamma$  protein in white adipose tissue. Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. PPAR $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ . Data are presented as the mean  $\pm$  standard deviation (n=5). #P<0.05 vs. the HF group.

was significantly higher compared with that of the animals in the HF group (P<0.05).

Glucose response during an OGTT in the candesartan cilexetil treatment group is shown in Fig. 2A. Compared with the HF group, treatment with 8 mg/kg candesartan cilexetil did not cause significantly lower glucose values at 0 min, whereas treatment resulted in lower glucose values (18, 7 and 13% lower, respectively; all P<0.05) at the 30, 90 and 120 min time points. The AUC values for glucose are presented in Fig. 2B; a reduction in this value reflects an increase in insulin sensitivity (15). A significantly higher AUC for glucose was calculated during the OGTT for the rats in the HF group compared with the NC group ( $14.05 \pm 2.05$  vs.  $11.96 \pm 1.35$  mmol·h/l; P<0.05). Animals in the HF + C group had a lower glucose AUC than those in the HF group

( $12.44 \pm 2.95$  vs.  $14.05 \pm 2.05$  mmol·h/l; P<0.05) during the OGTT.

Whole-body insulin sensitivity was evaluated using the hyperinsulinemic-euglycemic clamp technique (Table I). During high-fat feeding in the HF group, the glucose infusion rate (GIR) was 52.3% lower than that in the NC group. However, the GIR was significantly enhanced following candesartan cilexetil treatment (by 65.9%; P<0.01).

**PPAR $\gamma$  expression in the epididymal adipose tissue and liver tissue.** To identify the potential activation of PPAR $\gamma$  brought about by candesartan cilexetil, protein levels of PPAR $\gamma$  in epididymal adipose tissue were assessed by western blotting. High-fat feeding significantly reduced the amount of PPAR $\gamma$  in the adipose tissue compared with that in rats of the NC group. Candesartan cilexetil treatment together with a high-fat diet caused a significant increase in PPAR $\gamma$  expression compared with that in rats fed a high-fat diet and treated with saline (Figs. 3 and 4).

Subsequent immunohistochemical analysis of the liver tissue is shown in Fig. 5. Periportal and centrilobular hepatocytes exhibited low positivity for PPAR $\gamma$  in the livers of rats in the HF group. However, levels of PPAR $\gamma$  expression were increased significantly (P<0.05) in the livers of rats treated with candesartan cilexetil (Figs. 5 and 6).

## Discussion

In order to study the influence of the renin-angiotensin system on adipose and liver tissue, diet-induced obese rats were treated with long-term administration of candesartan cilexetil. Absorbed candesartan cilexetil is completely



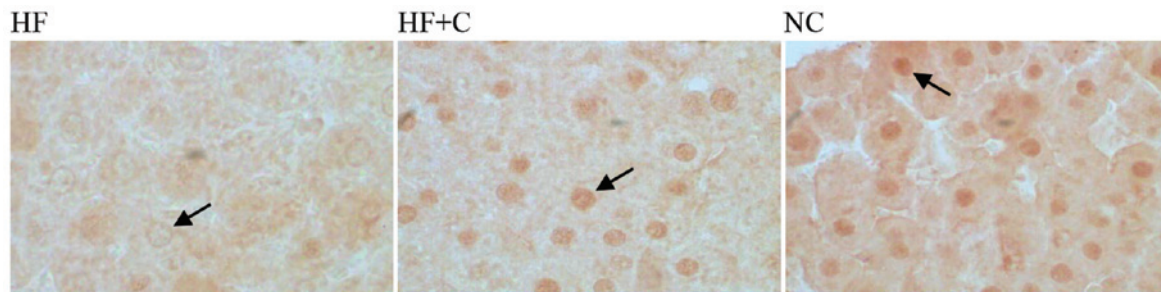


Figure 5. Immunostaining for PPAR $\gamma$  (brown in the nucleus) in the liver tissue (original magnification, x400). The arrows indicate hepatocytes. Levels of PPAR $\gamma$  expression were increased significantly in the livers of rats treated with candesartan cilexetil. Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. PPAR $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ .

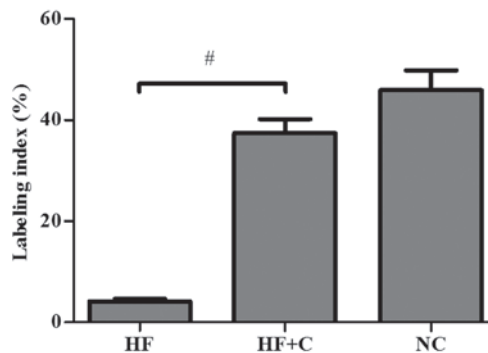


Figure 6. PPAR $\gamma$  labeling index of hepatocytes in the centrilobular and periportal areas. Rat groups were as follows: NC, normal chow; HF, high-fat diet; HF + C, high-fat diet with daily candesartan cilexetil treatment. PPAR $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$  (n=5 per section). #P<0.05.

metabolized to candesartan. The absolute bioavailability is relatively poor at 15% (candesartan cilexetil tablets) to 40% (candesartan cilexetil solution) (16), and the dose administered (8 mg/kg/day) was chosen because it had been demonstrated to be effective in a previous insulin-resistant obese rat model without any overt signs of toxicity (17). In the present study, it was demonstrated that high-fat feeding of male Wistar rats caused insulin resistance, increased plasma total cholesterol, low-density lipoprotein cholesterol, body weight and adiposity. Treatment with candesartan cilexetil for 4 weeks improved insulin sensitivity, and decreased plasma lipid levels and adiposity. The induction of PPAR $\gamma$  activity demonstrates new pleiotropic actions of candesartan cilexetil, providing a potential mechanism for its insulin-sensitizing effects. Clinical observation of a reduction in the development of diabetes mellitus with candesartan cilexetil is supported by the Candesartan in Heart-Failure Assessment of Reduction in Mortality and Morbidity (CHARM) Preserved trial (18), wherein the incidence of new-onset type 2 diabetes was significantly lower in subjects given candesartan cilexetil than in those given matching placebo, implicating an antidiabetic action of candesartan cilexetil. However, notably, in other trials involving candesartan cilexetil, the CHARM Alternative (19), the CHARM Added (20), and the Study on Cognition and Prognosis in the Elderly (SCOPE) (21), there was no benefit of candesartan cilexetil over placebo in the prevention of incident diabetes. Candesartan cilexetil also failed to show any effect on glucose, insulin or lipid levels in both the Candesartan

Role in Obesity and on Sympathetic System (CROSS) (22) and Antihypertensive Treatment and Lipid Profile in a North Sweden Efficacy Evaluation (ALPINE) (23).

As previously reported with AT1 receptor (AT1R) blocker therapy (24), the data obtained in the present study showed a marked postprandial 2 h glucose level reduction and higher GIR in rats in the HF + C group treated for 21 days with candesartan cilexetil. Fasting glucose levels were similar among the three groups, whereas fasting insulin levels in rats fed a high-fat diet were significantly higher than those in the chow-fed controls. The observation of normoglycemia with hyperinsulinemia indicates that the rats fed a high-fat diet were insulin resistant. Increased glucose levels and AUC of glucose were also observed following glucose loading in fat-fed rats. Despite high insulin levels, the AUC of glucose in the HF group was greater than that of HF + C group. However, the insulin levels of rats treated with candesartan cilexetil were found to be significantly lower compared with those of the HF control rats. These results indicate that although candesartan cilexetil does not affect insulin in normal conditions, under conditions of hyperinsulinemia, it increases insulin sensitivity for effective glucose disposal.

PPARs are ligand-activated transcription factors that have a number of pleiotropic effects (25). The  $\gamma$ -subtype of the receptor (PPAR $\gamma$ ) plays an important role in carbohydrate and lipid metabolism and is a therapeutic target in the treatment of insulin resistance, diabetes and metabolic syndrome (26). PPAR $\gamma$  is mainly expressed in adipocytes as well as hepatocytes and considered to be a key regulator of adipocyte differentiation (27). Thiazolidinediones typically function as full agonists of PPAR $\gamma$ , and improve insulin signaling and insulin sensitivity (28). Some ARBs such as telmisartan and irbesartan, are partial agonists of PPAR $\gamma$  and are able to induce its activity and adipocyte differentiation independent of their blocking properties. However, other ARBs have failed to show any effects on PPAR $\gamma$  activity or adipogenesis (29).

A major finding in the present study is that candesartan cilexetil has PPAR $\gamma$ -activating properties. The 8 mg/kg/day dose of candesartan cilexetil in rats is approximately twice the maximum recommended daily human dose of 32 mg on a mg/m<sup>2</sup> basis (this comparison assumes a human body weight of 50 kg). That the dosage required in obese rats was much higher than that recommended in humans may be due to inter-species differences or to the fact that obese rats have not only glucose and lipid metabolic disorders but also insulin resistance. The mechanism

by which candesartan cilexetil activates PPAR $\gamma$  remains to be precisely defined. However, its relatively high volume of distribution and lipophilicity, which have sufficiently high penetration rates to gain access to the PPAR $\gamma$ -retinoid X receptor complex within the cell nucleus, may be relevant. Once activated, the complex influences the expression of the key target gene glucose transporter-4 (GLUT-4) and increases glucose delivery to skeletal muscle by improving insulin sensitivity (30). Consistent with the latter hypothesis, we have observed that candesartan cilexetil shows other properties of a PPAR $\gamma$  activator, which can induce small increases in GLUT-4 protein expression in the skeletal muscle of obesity-associated rats (unpublished data). In addition, PPAR $\gamma$ -activating candesartan cilexetil promotes adipocyte differentiation, resulting in the redistribution of lipids from ectopic distribution to adipose tissue and may increase adiponectin levels in humans (31), thereby contributing to the weight loss and adiposity reduction of rats.

Obesity is one of the most common causes of insulin resistance. Adipose tissue, particularly in the visceral compartment, secretes various bioactive molecules that may directly contribute to the development of obesity-related diseases. It is acknowledged that adipose tissue is a complex and highly active metabolic and endocrine organ (32), producing proteins such as leptin, adiponectin and resistin (33). Several peptides of the renin-angiotensin system (34) are also produced in adipose tissue. Angiotensin II is a main final effector molecule of the renin-angiotensin system. The present study confirms that serum angiotensin II levels are elevated in diet-induced obese rats. This finding is consistent with earlier observations that circulating levels of angiotensinogen, renin and angiotensin II were increased in obese individuals (35). Systemic and local angiotensin II has been shown to inhibit substrate delivery and cross-talk between angiotensin and insulin receptor signaling pathways, which can be restored by AT1R antagonism (36). Beneficial effects of ARBs on impaired insulin signaling may represent an additional molecular mechanism for insulin sensitizing actions. Blockade of AT1 receptors leads to compensatory increases in angiotensin II levels and the subsequent increased activation of AT2 receptors (37). The AT2 receptor is now recognized as the counter-regulator of the AT1 receptor, exerting mostly beneficial actions (38). It has been reported that angiotensin II stimulation can decrease the expression of PPAR $\gamma$  in cardiac myofibroblasts (39), while PPAR $\gamma$  causes a downregulation of AT1R gene expression via a PPAR $\gamma$ -dependent mechanism in vascular smooth muscle cells (40). Therefore, PPAR $\gamma$  may also play a role in the regulation of angiotensin II action. Candesartan cilexetil, by virtue of its ability to block AT1Rs and to activate PPAR $\gamma$ , may not only inhibit angiotensin II-mediated pathways of insulin resistance, but also stimulate PPAR $\gamma$  pathways that help prevent resistance.

There are several limitations of the present study. First, the study did not demonstrate that candesartan cilexetil stimulates PPAR $\gamma$  activation independent of AT1R blocking actions in the absence of AT1R. Second, the effects of different ARBs with PPAR $\gamma$ -activating properties were not compared. The superiority of candesartan cilexetil for the enhancement of insulin sensitivity in association with improvement of insulin resistance requires further assessment.

In summary, the present study has demonstrated that long-term administration of candesartan cilexetil, a specific

ARB, to insulin-resistant obese rats elicited a great improvement of whole-body insulin sensitivity, at least in part because of PPAR $\gamma$  activation. This angiotensin II receptor antagonism also resulted in significant increases in PPAR $\gamma$  protein expression in adipose and liver tissue. PPAR $\gamma$  activation by candesartan cilexetil may provide novel therapeutic options in the treatment of patients with metabolic syndrome.

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