Telmisartan Prevents Weight Gain and Obesity Through Activation of Peroxisome Proliferator-Activated Receptor-\( \delta \)-Dependent Pathways
Hongbo He, Dachun Yang, Liqun Ma, Zhidan Luo, Shuangtao Ma, Xiaoli Feng, Tingbing Cao, Zhencheng Yan, Daoyan Liu, Martin Tepel and Zhiming Zhu

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Telmisartan Prevents Weight Gain and Obesity Through Activation of Peroxisome Proliferator-Activated Receptor-δ–Dependent Pathways

Hongbo He, Dachun Yang, Liqun Ma, Zhidan Luo, Shuangtao Ma, Xiaoli Feng, Tingbing Cao, Zhencheng Yan, Daoyan Liu, Martin Tepel, Zhiming Zhu

Abstract—Telmisartan shows antihypertensive and several pleiotropic effects that interact with metabolic pathways. In the present study we tested the hypothesis that telmisartan prevents adipogenesis in vitro and weight gain in vivo through activation of peroxisome proliferator-activated receptor (PPAR)-δ–dependent pathways in several tissues. In vitro, telmisartan significantly upregulated PPAR-δ expression in 3T3-L1 preadipocytes in a time- and dose-dependent manner. Other than enhancing PPAR-δ expression by 68.2±17.3% and PPAR-δ activity by 102.0±9.0%, telmisartan also upregulated PPAR-γ expression, whereas neither candesartan nor losartan affected PPAR-δ expression. In vivo, long-term administration of telmisartan significantly reduced visceral fat and prevented high-fat diet-induced obesity in wild-type mice and hypertensive rats but not in PPAR-δ knockout mice. Administration of telmisartan did not influence food intake in mice. Telmisartan influenced several lipolytic and energy uncoupling related proteins (UCPs) and enhanced phosphorylated protein kinase A and hormone sensitive lipase but reduced perilipin expression and finally inhibited adipogenesis in 3T3-L1 preadipocytes. Telmisartan-associated reduction of adipogenesis in preadipocytes was significantly blocked after PPAR-δ gene knockout. Chronic telmisartan treatment upregulated the expressions of protein kinase A, hormone-sensitive lipase, and uncoupling protein 1 but reduced perilipin expression in adipose tissue and increased uncoupling protein 2 and 3 expression in skeletal muscle in wild-type mice but not in PPAR-δ knockout mice. We conclude that telmisartan prevents adipogenesis and weight gain through activation of PPAR-δ–dependent lipolytic pathways and energy uncoupling in several tissues. (Hypertension. 2010;55:869-879.)

Key Words: peroxisome proliferator-activated receptor-δ • telmisartan • obesity • hormone sensitive lipase • adipogenesis • energy uncoupling

Abdominal obesity, which increases cardiometabolic risks, is often associated with hypertension.1 Reduction of high blood pressure significantly lowers cardiovascular mortality.2 Evaluation of antihypertensive drugs for their beneficial effects on weight gain may improve clinical management of obese patients with hypertension.

Angiotensin II receptor blockers (ARBs) are commonly used to lower blood pressure.2 Other than their antihypertensive effects, several clinical trials show that ARB can prevent the onset of type 2 diabetes mellitus.3,4 Recently, clinical and experimental studies have shown that ARBs have effects on weight gain and obesity,5–15 which indicate that ARB could be beneficial for the management of obesity related hypertension. Previous studies suggested that several pleiotropic effects of ARBs include upregulation of uncoupling protein (UCP) 1 and angiotensin II receptor type 1 expression, activation of peroxisome proliferator activated receptor (PPAR)-γ, increase of adiponectin, and promotion of caloric expenditure.5,7,10,14–20 However, the precise mechanisms responsible for the effect of ARBs on fat metabolism remain unresolved. Recent studies suggested that activation of PPAR-δ could prevent high-fat diet-induced obesity in rodents.21–23 In addition, obesity is characterized by increased fat storage and reduced lipolysis in adipose tissue and energy uncoupling.24–27 It is unknown whether ARBs may affect PPAR-δ, lipolytic pathways, and energy uncoupling, such as protein kinase A (PKA); hormone-sensitive lipase (HSL); perilipin, an essential lipid droplet-associated protein; and UCPs.5,24–28 In the present study we tested the hypothesis that telmisartan may prevent adipogenesis in vitro and weight gain in vivo through activation of PPAR-δ–dependent lipolytic pathways and energy uncoupling from rodent models.

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Long-Term Administration of Telmisartan In Vivo

Mice or SHRs were assigned to the different experimental procedures: (1) mice were fed with normal chow (control) and chow mixed into telmisartan 5 mg/kg daily (telmisartan) for 28 weeks; (2) mice were fed with normal chow or a high-fat diet for 24 weeks, then telmisartan (5 mg/kg daily) was given additionally by oral gavage for 4 weeks; and (3) SHRs on a normal chow or high-fat diet were treated with 5 mg/kg daily telmisartan for 16 weeks. Systolic blood
pressure of SHRs was measured by tail cuff, and body weight was quantified weekly. At the end of treatment, the animals were fasted for 12 hours before being euthanized by decapitation. Adipose tissues were assayed. Visceral fat weight was determined via dissection of epididymal, retroperitoneal, and mesenteric fat depots. Representative cross-sections of adipose tissue were analyzed for cell size according to standard techniques, and the rest was frozen and stored at −70°C until further processing.

Biochemical Parameters, Histological Analysis, and Immunohistochemistry
For details, refer to the online Data Supplement, available at http://hyper.ahajournals.org. The immunohistochemical procedure was described in our previous study.²¹

Immunoblotting
Immunoblots of PPAR-δ, PKA, phosphorylated PKA (p-PKA), HSL, perilipin, UCP-1, UCP-2, UCP-3, palmitoyltransferase-1 (CPT1), cytochrome c oxidase (COX2), and β-actin in tissue were performed using standard techniques. For details, refer to the online Data Supplement.

Preadipocyte Culture and Adipocyte Differentiation Assay
The procedures of preadipocyte culture and adipocyte differentiation assay were described in our previous studies.²¹,³⁰ For details, refer to the online Data Supplement. Other experiments were performed in isolated preadipocyte cells from visceral adipose tissue of C57BL/6J or PPAR-δ knockout mice in the presence of the 10 μmol/L of telmisartan, 10 μmol/L of candesartan, 10 μmol/L of losartan, and 10 μmol/L of GW0742.

Overexpression of PPAR-δ in 3T3-L1 Preadipocytes
The recombinant adenoviral vectors containing rat PPAR-δ were generated as described.²¹ For details, refer to the online Data Supplement.

Transfection and Luciferase Assay
PPAR-δ activity was determined by transactivation assays in 3T3-L1 preadipocytes and angiotensin II receptor type 1 knockout PC12W cells (Cell Bank, Chinese Academy of Sciences). Cells were plated in 48-well dishes and transfected by use of the Lipofectamine LTX Reagent (Invitrogen), delivering 150 ng of the full-length coding region of rat PPAR-δ expression plasmid pAdTrack-cytomegalovirus-PPAR-δ, 300 ng of luciferase reporter plasmid tk-peroxisome proliferator response element (PPRE) ×3-luc, and 150 ng of pRL-TK renilla luciferase reporter vector as an internal control. After 6 hours, transfection medium was replaced by 10% FBS/DMEM and cells were treated with varying concentrations of the telmisartan and PPAR-δ agonist OG 0742 and incubated for an additional 24 hours. Cells were assayed for luciferase and renilla activity using the Dual-Glo Luciferase Assay System (Promega) and the Varioskan Flash Type 3001 (Thermo Electron Corporation). All of the treatments were performed in triplicate and normalized for renilla activity.

Statistics
Data are expressed as mean ± SEM. Statistical significance of differences between mean values was assessed by the Student t test or 1-way ANOVA with a Bonferroni multiple comparison post hoc test, as appropriate. Two-sided P values <0.05 were considered to indicate statistical significance.
Results

Telmisartan Activates PPAR-δ in 3T3-L1 Preadipocytes

First, we examined whether telmisartan affects PPAR-δ protein expression. Compared with control, administration of telmisartan (10 μmol/L), but neither candesartan (10 μmol/L) nor losartan (10 μmol/L), significantly increased PPAR-δ expression in 3T3-L1 preadipocytes. Telmisartan significantly upregulated PPAR-δ expression by 68.2±17.3% (P<0.05; Figure 1A).

Figure 1B shows the dose-dependent effect of telmisartan on PPAR-δ in 3T3-L1 preadipocytes. We observed that telmisartan concentrations >1 μmol/L significantly increased the PPAR-δ expression in these cells. As indicated in Figure 1C, the effect of...
Telmisartan Prevents Weight Gain Through PPAR-δ

Overexpression or Activation Reduces Adipogenesis

Next, we determined whether PPAR-δ overexpression or activation might play a role in adipogenesis. We examined the effect of overexpression or activation of PPAR-δ on the adipogenesis in vitro. Oil red O staining for detection of adipocyte differentiation was investigated. PPAR-δ overexpression reduced lipid droplets in the cytoplasm from cultured 3T3-L1 preadipocytes (Figure 2A). PPAR-δ overexpression was validated by immunoblotting, as shown in Figure 2B. Administration of GW0742 and telmisartan markedly attenuated adipogenesis (Figure 2C, top) and intracellular triglyceride (TG) levels (GW0742 0.74±0.02 mmol/L and telmisartan 0.69±0.02 mmol/L versus control 0.89±0.03 mmol/L; n=6 for each group; P<0.01) in primary cultured visceral preadipocytes from wild-type mice (Figure 2D), whereas GW0742 and telmisartan failed to influence adipogenesis (Figure 2C, middle) and intracellular TG levels (Figure 2E) in cultured visceral preadipocytes from PPAR-δ knockout mice (GW0742 0.90±0.01 mmol/L and telmisartan 0.96±0.04 mmol/L versus control 0.89±0.07 mmol/L; n=6 for each group; P>0.05). As shown in Figure 2C (bottom), after transfecting PPAR-δ null preadipocytes with the recombinant adenoviral PPAR-δ vector, the inhibitory effects of telmisartan and GW0742 on adipogenesis were rescued compared with control conditions, supporting the importance of PPAR-δ for telmisartan-dependent effects.

Telmisartan Prevents Weight Gain in Mice Through Activation of PPAR-δ

We further studied whether telmisartan affects body weight and adipose tissue in vivo through PPAR-δ. PPAR-δ gene knockout mice and wild-type mice on a normal chow diet were adminis-
treated with telmisartan (5 mg/kg per day) for 28 weeks. Baseline characteristics of wild-type and PPAR-δ knockout mice were included in the online Supplemental Data (Table S1).

Telmisartan significantly reduced body weight, weight of adipose tissue, and adipocyte size in wild-type mice (Figure 3A through 3C). Telmisartan significantly reduced visceral fat from 3.0±0.4 to 1.2±0.2 g (P<0.01; Figure 3B). In contrast, administration of telmisartan for 28 weeks did not influence body weight, adipose tissue weight, and adipocyte size in PPAR-δ gene knockout mice (Figure 3D through 3F). Ectopic fat deposition could be observed in liver from wild-type mice on a high-fat diet (Figure S1).

Plasma TG levels and free fatty acid (FFA) levels were significantly lower in wild-type mice treated with telmisartan than in untreated mice (TG: 1.07±0.09 mmol/L versus 1.69±0.16 mmol/L, P<0.01; FFA: 0.55±0.06 mmol/L versus 0.79±0.09 mmol/L, P<0.05; n=8 to 10). In contrast, plasma TG and FFA levels were not significantly different in
PPAR-δ gene knockout mice treated with telmisartan and untreated knockout mice (TG: 1.58±0.19 mmol/L versus 1.66±0.18 mmol/L, \( P > 0.05 \); FFA: 0.78±0.23 mmol/L versus 0.63±0.12 mmol/L, \( P > 0.05 \); \( n = 5 \)). These findings suggest that long-term administration of telmisartan prevents weight gain and reduces the size of adipocytes and weight of adipose tissue by affecting PPAR-δ in mice.

**Telmisartan Prevents High-Fat Diet-Induced Obesity Through PPAR-δ**

As shown in Figure 4A, body weight was significantly higher in wild-type mice on a high-fat diet compared with mice on a normal chow diet. Then telmisartan was added after 24 weeks of a high-fat diet. In these mice, additional administration of telmisartan (5 mg/kg daily) for 1 month significantly reduced body weight in mice despite a continuous high-fat diet but had little effect on body weight in mice on a normal chow diet (Figure 4A). PPAR-δ expression was significantly higher in visceral and subcutaneous fat in wild-type mice treated with telmisartan compared with control mice (Figure 4B and 4C). Furthermore, a similar effect of telmisartan on PPAR-δ expression was also observed in skeletal muscle (Figure 4D). These findings support the idea that telmisartan affects PPAR-δ in several tissues.

**Telmisartan Prevents High-Fat Diet-Induced Obesity in SHRs**

Obesity is often associated with hypertension. We further asked whether the effect of telmisartan on obesity and PPAR-δ can also be observed in obese SHRs. SHRs on a normal chow diet and on a high-fat diet were treated by telmisartan (3 mg/kg per day) for 16 weeks. Body weight time dependently increased in SHRs. In contrast, the administration of telmisartan for 16 weeks significantly reduced body weight in SHRs (Figure 5A; each \( P < 0.01 \) compared with controls). Telmisartan also significantly lowered systolic blood pressure in SHRs on a normal chow diet (107±8 mm Hg versus 169±9 mm Hg; \( P < 0.01 \)) or on a high-fat diet (113±6 mm Hg versus 195±8 mm Hg; \( P < 0.01 \)). High-fat diet significantly increased weights of subcutaneous and visceral adipose tissue and the size of adipocytes in obese hypertensive rat. Compared with controls, the weight of adipose tissue and the size of adipocytes were significantly lower in SHRs treated with telmisartan (Figure 5B and 5C). As shown in Figure 5F, PPAR-δ expressions in visceral and subcutaneous fat were significantly higher in SHRs treated with telmisartan compared with controls (Figure 5D and 5E).

**Effect of Telmisartan on PPAR-δ-Dependent Lipolytic Pathway and Energy Uncoupling**

To evaluate whether the effects on telmisartan are mediated by interaction with lipolytic pathways and energy uncoupling, we measured the PKA, HSL, and perilipin in both adipose tissue and preadipocytes. Furthermore, several energy uncoupling–related molecules were investigated in skeletal muscle.

First, we showed p-PKA, HSL, and perilipin in adipose tissue by immunofluorescence (Figure 6A). Second, administration of the PPAR-δ agonist, GW0742, or telmisartan significantly increased the expression of p-PKA and HSL but reduced perilipin expression in cultured preadipocytes from wild-type mice. In contrast, GW0742 and telmisartan had no effect on p-PKA, HSL, and perilipin expression in cultured preadipocytes from PPAR-δ gene knockout mice (Figure 6B). Third, we further observed that administration of telmisartan increased the expression of p-PKA and HSL and reduced the perilipin expression in visceral adipose tissue from wild-type mice. In contrast, telmisartan did not affect the expression of p-PKA, HSL, or perilipin in visceral adipose tissue from PPAR-δ gene knockout mice (Figure 6C). Fourth, chronic administration of telmisartan increased the expression of UCP-1 in brown fat (Figure 6D), UCP-2, and UCP-3 in skeletal muscle (Figure 6E) but had no effect on CPT1 and COX2 in skeletal muscle from wild-type mice (Figure 6F).

As expected, telmisartan did not affect the expressions of energy uncoupling–related molecules mentioned above in adipose tissue and skeletal muscle from PPAR-δ gene knockout mice (Figure 6D through 6F).

**Discussion**

The major findings of this study are that telmisartan significantly upregulated PPAR-δ expression and activity in 3T3-L1 preadipocytes, activated PPAR-δ-dependent lipolytic pathway, and finally reduced adipogenesis in vitro. The effect of telmisartan on lipolysis was abolished after PPAR-δ gene knockout of preadipocytes. In vivo, long-term administration of telmisartan significantly reduced the rise of body weight and prevented high-fat diet-induced obesity in wild-type mice and hypertensive rats but not in PPAR-δ knockout mice. Telmisartan increased the expression of PPAR-δ and several lipolytic and energy uncoupling–related proteins, including PKA, HSL, and UCPs in adipose tissue and skeletal muscle from wild-type mice and SHRs. This effect of telmisartan could not be observed in PPAR-δ knockout mice.

Currently, it is unclear which drugs may be superior to manage obesity-related hypertension. However, antihypertensive drugs showing antiobese effects may be beneficial for obese subjects with hypertension. ARBs are generally used to lower blood pressure. Other than their antihypertensive effect, several ARBs have been shown to prevent the new-onset diabetes mellitus, which is supposed to be related to PPAR-γ. 

Furthermore, Shimabukuro et al showed that the visceral fat area, determined by abdominal computed tomography scan, was...
reduced in hypertensive patients treated with telmisartan for 24 weeks, whereas treatment of patients withamlodipine did not affect abdominal fat. Chau et al^{11} reported that telmisartan treatment decreases visceral fat accumulation and improves serum levels of adiponectin and vascular inflammation markers in Japanese hypertensive patients.

In addition, a recent clinical trial showed that administration of irbesartan significantly reduced waist circumference in hypertensive patients with metabolic syndrome.^{9} Several experimental studies further demonstrated that mice and rats treated with different ARBs show reduced weight gain.^{11–14} Although telmisartan was reported to reduce the visceral fat, it did not influence body weight or body mass index either in the Ongoing Telmisartan Alone and in Combination With Ramipril Global Endpoint Trial or in the Prevention Regimen for Effectively Avoiding Second Strokes study.^{32,33} A recent study suggested that body mass index and waist circumference do not adequately mirror visceral fat accumulations in different racial/ethnic groups.^{34}

The effects of ARB on fat metabolism may be mediated by several pathways, including upregulation of UCP1 and angiotensin II type 2 receptor expression,^{5,15} activation of PPAR-γ,^{13,16–18} increase of adiponectin,^{13} and promotion of caloric expenditure.^{5,14} However, the underlying mechanisms are a matter of debate. For example, telmisartan is regarded as a partial agonist for PPAR-γ;^{10–18} however, PPAR-γ agonists such as rosiglitazone promote adipocyte differentiation and lead to weight gain.^{13,35} Furthermore, Clemenz et al^{16} recently identified the telmisartan as a partial PPAR-α agonist at least in the liver. In addition, Benson et al^{18} showed that telmisartan can also inhibit the proliferation of cells that lack angiotensin II receptors and cells treated with a PPAR-γ antagonist, suggesting that the antiproliferative effects of telmisartan may involve more than just angiotensin II receptor blockade or activation of PPAR-γ. Therefore, published data and our present findings indicate that the actions of telmisartan were not restricted to PPAR-γ alone, but telmisartan may affect different PPAR subtypes.

Now, the present study including PPAR-δ knockout in vitro and in vivo clearly demonstrates that the effects of telmisartan are mediated by its effect on PPAR-δ. The present findings support results by Wang et al^{20} showing that activation or overexpression of PPAR-δ can prevent high-fat–induced obesity and weight gain by promoting FFA oxidation in adipose tissue.

First, we demonstrated that telmisartan significantly upregulated PPAR-δ expression and activity in 3T3-L1 preadipocytes. Second, administration of telmisartan, the PPAR-δ agonist, GW0742, or PPAR-δ overexpression reduced accumulation of lipid droplets in cultured preadipocytes. Third, long-term administration of telmisartan increased PPAR-δ expression and reduced weight gain and high-fat–induced obesity in wild-type mice. Fourth, the inhibitory effect of telmisartan on weight gain could not be observed in PPAR-δ knockout mice. Telmisartan may affect PPAR-δ because of its high lipophilicity, which is required to obtain sufficient high penetration rates to bind to intracellular PPAR-δ.^{17,18} The region where telmisartan may bind to that transcription factor is unknown yet.

Obesity is characterized by increased fat storage in the form of TGs in adipose tissue. In addition, lipolysis has been found to be impaired in obesity.^{37} In humans, catecholamines and insulin are the most important hormones regulating adipocyte lipolysis. HSL is the predominant lipase effector of catecholamine-stimulated lipolysis in adipocytes.^{27} HSL-dependent lipolysis in response to catecholamines is mediated by PKA-dependent phosphorylation of perilipin, an essential lipid droplet–associated protein.^{25} It is believed that phosphorylation of perilipin is important for the translocation of HSL from the cytosol to the lipid droplet, a key event to stimulate lipolysis.^{24–27} In the present study, we show that either activation of PPAR-δ by GW0742 or by telmisartan significantly increased p-PKA and HSL but reduced perilipin expression in preadipocytes. Furthermore, in wild-type mice, long-term administration of telmisartan increased the expression of PPAR-δ, p-PKA, and HSL but reduced the expression of perilipin in visceral fat. By contrast, the effects of telmisartan on p-PKA and HSL were abolished in PPAR-δ knockout mice. These results indicate that activation of PPAR-δ stimulates the lipolytic pathway. Given the wide expression of PPAR-δ, we showed that the observed effects of telmisartan in adipose tissue could also be obtained in skeletal muscle. Our additional experiments showed that telmisartan upregulated UCP-1 expression in brown fat and expressions of UCP-2 and UCP-3 but had no effect on expressions of CPT1 and COX2 in skeletal muscle. These data supported the notion that the effect of telmisartan is not restricted to fat tissue but a common cellular pathway. In addition, telmisartan can improve insulin sensitivity, supporting the hypothesis that telmisartan may exert additional benefit in the management of obese patients with hypertension.^{38,39} The beneficial effects of telmisartan may be caused by its lipolysis and energy uncoupling, which is in agreement with recent studies.^{40,41} Benson et al^{18} and Schupp et al^{19} have shown that telmisartan dose-dependently induced fatty acid binding protein 2 expression that is an adipogenic marker through PPAR-γ activation. However, Janke et al^{16} reported that 1 μmol/L of telmisartan increased lipoprotein lipase expression that is one of the key genes involved in TG breakdown, and 10 μmol/L of telmisartan slightly reduced lipid content in preadipocytes through PPAR-γ activation. In the present study, we showed that 10 μmol/L of telmisartan significantly promoted lipolytic pathway and increased PPAR-δ expression and inhibited adipogenesis in 3T3-L1 preadipocytes through PPAR-δ activation. These somewhat discrepant findings reported in the literature indicate that several pathways may finally determine the effect of telmisartan on adipogenesis by interaction with PPAR-γ and PPAR-δ.

Perspectives

The present study gives experimental evidence that telmisartan prevents lipogenesis and weight gain through activation of PPAR-δ–dependent lipolytic pathways in adipose tissue and energy uncoupling in skeletal muscle. Our data point toward a
previously unrecognized role of telmisartan in fat metabolism and energy uncoupling, which may be relevant for the management of obese patients with hypertension. Additional benefit in the treatment of obese patients with hypertension using ARBs including telmisartan would be greatly appreciated and should be investigated in future clinical trials.

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Disclosures

None.

References


Online Supplement

Telmisartan prevents weight gain and obesity through activation of PPAR δ dependent pathways

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Short title: Telmisartan prevents weight gain through PPAR δ

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Materials and Methods

Biochemical parameters

Blood was obtained from the carotid artery, and then the serum was separated and
immol/L; immediately frozen at -70°C until assayed. Levels of serum glucose, triglycerides, and free fatty acids were measured using commol/Lercially available assay kits (Applygen Technologies Inc., China).

**Histological analysis**

Adipose tissue was fixed with 10% formalin and embedded in paraffin. Twenty-micron sections were cut and stained with hematoxylin and eosin for microscopic examination (Nikon, TE2000).

**Immunohistochemistry**

Fresh adipose tissue was fixed with 10% formalin for 10 minutes at room temperature, washed with phosphate buffered saline (PBS) and then permeabilized with PBS containing 0.5% Triton X-100 for 20 minutes at room temperature. Sections of adipose tissues were incubated with PBS containing with 5% bovine serum and 0.1% Triton X-100 for 1 hour at room temperature. Then adipose tissues were incubated with rabbit anti-p-PKAThr198, anti-PKA, HSL, perilipin, and PPAR δ antibodies (Santa Cruz, USA) over night at 4°C. Adipose tissue sections were washed and incubated with antibodies conjugated to a fluorescent probe FITC-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, USA), 1:200 for 30 minutes at room temperature. In the negative control experiments which were performed by omitting the primary antibody. After removing the unbound secondary antibodies by washing the preparations with PBS, imaging was performed using the fluorescence microscope (Nikon, TE2000).

**Immunoblotting**

Immunoblots of PPAR δ, PKA, p-PKA, HSL, perilipin, UCP 1, 2, 3, palmitoyltransferase-1 (CPT1), cytochrome c oxidase (COX2) and β-actin in tissue were performed using standard techniques. Primary antibodies (Santa Cruz, CA) were used for adipose tissue, skeletal muscle and 3T3-L1-preadipocytes cells. Tissue was homogenized in high-salt extraction buffer containing 50 mmol/L Tris, 1 ml NP40, 1 ml TritonX-100, 0.1 g sodium dodecyl sulfate, 150 mmol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid, 1 mmol/L ethylene glycol-bis(2-aminoethyl ether)-N,N’,N’-tetraacetic acid. Tissue was transferred to Eppendorf tubes, and homogenized by sonication for 5 seconds. The protein supernatant was separated by centrifugation, and protein concentrations were determined with Bio-Rad protein assay reagent (Bio-Rad, Hercules CA). Proteins were separated by using 10% SDS-polyacrylamide gel, transferred to polyvinylidene difluoride membranes at 90 mA overnight, and detected with the ECF kit (Amersham). After incubation with the secondary antibodies for 1 hour, the proteins were detected by enhanced chemiluminescence and quantified using a 5 Gel Doc 2000 Imager (Bio-Rad).

**Preadipocytes culture and adipocytes differentiation assay**

Adipose tissue was processed, including the careful removal of fibrous tissue and blood vessels, followed by collagenase digestion. After centrifugation, floating mature adipocytes were separated from the stromal-vascular cell pellet, which was subjected to additional filtrations (25 mm final pore size) to yield the preadipocytes fraction. Primary cultured murine preadipocytes and 3T3-L1 preadipocytes (Cell Bank,
Chinese Academy of Sciences, Shanghai, China) were cultured and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (HyClone) containing 100 µg/ml penicillin and 100 µg/ml streptomycin (Gibco) as previously described. The adipocytes differentiation assay was performed as described. Briefly, after reaching confluence 3T3-L1-preadipocytes were treated with vector alone for control, adenoviral vector containing rat PPAR δ. Cells were fixed and stained with oil red O (Sigma–Aldrich). Red staining shows lipid droplets in the cytoplasm indicating adipocytes differentiation. In additional experiments PPAR δ expression in 3T3-L1 preadipocytes cells in vitro was determined after stimulation with 3-isobutyl-1-methylxanthine (0.5 mmol/L), dexamethasone (1 µmol/L), and insulin (5 µg/ml). Other experiments were performed in isolated preadipocytes cells from visceral adipose tissue of C57BL/6J or PPAR δ knockout mice in the presence of the 10µmol/L telmisartan, 10µmol/L candesartan, 10 µmol/L losartan and 10 µmol/L GW0742.

The recombinant adenoviral vectors containing rat PPAR δ were generated as described. The cDNA encoding rat PPAR δ was amplified by RT-PCR using the isolated total RNA as the template from the white fat. Recombined shuttle plasmid pAdTrack-CMV-PPAR δ was constructed by linking the pAdTrack-CMV with PPAR δ and was sequenced. For generation of recombinant adenoviral plasmids, pAdTrack-CMV-PPAR δ was linearized with PmeI, and was transformed into electrocompetent Escherichia coli BJ5183 cells, which contained adenoviral backbone vectors (pAdEasy-1). Clones that had inserts were screened by restriction endonuclease digestions. Once confirmed, supercoiled plasmid DNA was transformed into LX10 cells for large-scale amplification. For production of adenoviruses in AD293 cells, a transfection mix was prepared by adding 4µg of PacI linearized plasmid DNA, purified by gel extraction, and 10µL of Lipofectamine 2000 (Invitrogen). Transfected cells were monitored for green fluorescent protein expression and collected 7–10 days after transfection by scraping cells off flasks and pelleting them along with any floating cells in the culture. After three cycles of freezing in a methanol dry ice bath and rapid thawing at 37°C, supernatant of viral lysate was used to infect AD293 cells in flasks. Three to four days later, viruses were harvested as described above. 10 MOI of viruses were infected into 3T3-Li preadipocyte. After 3hrs of infection, cells were induced to differentiate.

References


Supplemental table and figure

**Table S1. Baseline characteristics of wild type and PPAR δ knockout mice**

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<td>33.17±3.22</td>
<td>37.16±6.87</td>
</tr>
<tr>
<td>Food intake</td>
<td>4.33±0.04</td>
<td>4.56±0.06</td>
</tr>
<tr>
<td>(g/animal/day)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WT, wild-type mice; PPAR δ -/-, PPAR δ -/- mice; HR, heart rate; SBP, systolic blood pressure; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; Results are means ± SEM for 6-12 animals.

**Figure S1 Ectopic fat deposition in liver**
Figure S1. Histological examination of liver from mice on normal diet (ND) and on high fat diet (HD)