# Liver-Specific Peroxisome Proliferator–Activated Receptor α Target Gene Regulation by the Angiotensin Type 1 Receptor Blocker Telmisartan

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**OBJECTIVE—**The angiotensin type 1 receptor blocker (ARB) and peroxisome proliferator–activated receptor (PPAR) γ modulator telmisartan has been recently demonstrated to reduce plasma triglycerides in nondiabetic and diabetic hypertensive patients. The present study investigates the molecular mechanisms of telmisartans hypolipidemic actions, in particular its effect on the PPARα pathway.

**RESEARCH DESIGN AND METHODS—**Regulation of PPARα target genes by telmisartan was studied by real-time PCR and Western immunoblotting in vitro and in vivo in liver/skeletal muscle of mice with diet-induced obesity. Activation of the PPARα ligand binding domain (LBD) was investigated using transactivation assays.

**RESULTS—**Telmisartan significantly induced the PPARα target genes carnitine palmitoyl transferase 1A (CPT1A) in human HepG2 cells and acyl-CoA synthetase long-chain family member 1 (ACSL1) in murine AML12 cells in the micromolar range. Telmisartan-induced CPT1A stimulation was markedly reduced after small interfering RNA–mediated knockdown of PPARα. Telmisartan consistently activated the PPARα-LBD as a partial PPARα agonist. Despite high in vitro concentrations required for PPARα activation, telmisartan (3 mg · kg$^{-1}$ · day$^{-1}$) potently increased ACSL1 and CPT1A expression in liver from diet-induced obese mice associated with a marked decrease of hepatic and serum triglycerides. Muscular CPT1B expression was not affected. Tissue specificity of telmisartan-induced PPARα target gene induction may be the result of previously reported high hepatic concentrations of telmisartan.

**CONCLUSIONS—**The present study identifies the ARB/PPARγ modulator telmisartan as a partial PPARα agonist. As a result of its particular pharmacokinetic profile, PPARα activation by telmisartan seems to be restricted to the liver. Hepatic PPARα activation may provide an explanation for telmisartan's antidyslipidemic actions observed in recent clinical trials. ***Diabetes* 57: 1405–1413, 2008**

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ACSL1, acyl-CoA synthetase long-chain family member 1; ALT, alanine aminotransferase; ARB, angiotensin type 1 receptor blocker; AST, aspartate aminotransferase; CPT1A, carnitine palmitoyl transferase 1A; hPPARα, human peroxisome proliferator–activated receptor α; LBD, ligand binding domain; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator–activated receptor; siRNA, small interfering RNA.



Angiotensin type 1 receptor blockers (ARBs) are commonly used in the treatment of hypertension and related cardiovascular and organ damage (1). Recently, a distinct subgroup of ARBs has been identified as partial agonists for the peroxisome proliferator–activated receptor (PPAR) γ with selective PPARγ modulating properties (2–4). In contrast to full glitazone agonists, PPARγ-activating ARBs exert selective recruitment of nuclear cofactors resulting in in vivo insulin sensitization in the absence of weight gain in obese insulin-resistant mice (3). Among the ARBs, telmisartan has been shown to be the most potent PPARγ modulator (3,4). Based on these in vitro results and data from animal experiments, a number of clinical studies (5–9) have been conducted in which the metabolic actions of the PPARγ-activating ARB telmisartan have been intensively investigated. When compared with ARBs that do not exert PPARγ-activating properties, telmisartan not only improves insulin sensitivity but also induces beneficial actions on serum lipid levels such as a reduction of serum triglycerides. (5,10,11)

PPARs are ligand-activated transcription factors belonging to the superfamily of nuclear receptors. PPARγ is abundantly expressed in adipose tissue and a major regulator of insulin and glucose metabolism (12). In contrast, PPARα is highly expressed in tissues displaying a high metabolic rate of fatty acids, such as the liver and skeletal muscle (13). PPARα modulates intracellular lipid metabolism by transcriptional regulation of genes involved in fatty acid uptake, mitochondrial fatty acid oxidation, and triglyceride catabolism (13,14). Natural PPARα ligands comprise mono- and polyunsaturated fatty acids as well as eicosanoids (15). In addition, PPARα is also the molecular target of lipid-lowering fibrates such as gemfibrozil, bezafibrate, clofibrate, and fenofibrate. These substances are used to treat dyslipidemia and cardiovascular disease. (13,15)

It has been reported that certain PPARγ activators such as pioglitazone are also able to activate PPARα. Furthermore, it has been proposed that the positive actions of

pioglitazone on diabetic dyslipidemia might at least in part be mediated by its PPARα-activating abilities (16,17). To understand the underlying mechanism of telmisartan's lipid-lowering actions we studied the effect of telmisartan on major PPARα target genes involved in fatty acid oxidation in the human hepatoma cell line HepG2, the murine hepatic cell line AML12, and in liver/skeletal muscle of diet-induced obese mice treated with telmisartan. Furthermore, activation of the PPARα ligand binding domain (LBD) and regulation of PPARα protein/mRNA expression by telmisartan was studied.

The present study demonstrates that telmisartan induces the PPARα target gene carnitine palmitoyl transferase 1 (CPT1A) in HepG2 cells and acyl-CoA synthetase long-chain family member 1 (ACSL1) in AML12 cells. Consistently, telmisartan acts as a partial PPARα agonist in PPARα transactivation assays and induces PPARα expression. High-fat diet–fed mice treated with telmisartan showed a pronounced induction of hepatic ACSL1 and CPT1A, which was associated with a significant decrease of hepatic and serum triglycerides. Interestingly, CPT1B expression in skeletal muscle was not affected by telmisartan. Tissue specificity of telmisartan-induced PPARα target gene induction may result from high hepatic telmisartan concentrations that have been documented in rodents during early preclinical studies (18).

In summary, the present study identifies the ARB telmisartan as a partial PPARα agonist. Based on its specific pharmacokinetic profile, PPARα activation by telmisartan appears to be liver specific. Hepatic induction of PPARα target genes involved in mitochondrial fatty acid oxidation might contribute to the antidyslipidemic actions of telmisartan.

## RESEARCH DESIGN AND METHODS

**Cell culture.** HepG2, AML12, and COS7 cells were cultured following the manufacturer's guidelines. Cells were serum deprived for 16 h before stimulation with the vehicle (DMSO) or different effectors.

**Quantitative real-time PCR.** Real-time PCR was performed as previously described using an ABI 7000 and Stratagene 3000 MXP PCR cycler with either the SybrGreen or FAM-TAMRA detection system (3). Primers and probes are shown in the online appendix (available at http://dx.doi.org/10.2337/db07-0839).

**Transfection and luciferase assay.** Transient transfection and luciferase assays were performed as previously described (3). Cos7 cells were transfected with the use of Lipofectamine 2000 (Invitrogen), with pGal4-hPPAR (human PPARα) αDEF (hPPARα LBD fused to Gal4 DBD [DNA binding domain]) and pGal5-TK-pGL3, and 10 ng pRL-CMV (Renilla-Cytomegalovirus), a renilla luciferase control reporter vector. After 4 h, transfection medium was replaced by 10% fetal bovine serum DMEM plus the indicated ARBs, Wy14.643, fenofibric acid, or vehicle (DMSO), and luciferase activity was measured after 24 h.

**Western immunoblotting.** Electrophoresis and blotting following protein isolation from murine liver tissue was performed as previously described (3). Blots were incubated with an anti-PPARα (Sigma) or anti-ACSL1 (kindly provided by Rosalind A. Coleman, Chapel Hill, NC) antibody.

**Gene silencing with small interfering RNA.** The small interfering RNA (siRNA) targeting human PPARα was purchased from Dharmacon, and two sequences (D-003434–01 and D-003434–02) were used simultaneously according to the manufacturer's instructions. The siRNA negative control from Dharmacon (D-001810–01) was used to test nonspecific effects on gene expression. Overnight-starved HepG2 cells were transfected using HiPerfect (Qiagen), according to the manufacturer's instructions, in 24-well plates containing $10^5$ cells/well with 5 nmol/l siRNA/well (each sequence 2.5 nmol/l). Thirty minutes after the start of transfection, cells were treated for 48 h with telmisartan (50 μmol/l; 0.5% serum) before RNA analysis.

**Animals.** Male C57BL/6J mice were treated as previously described (3). Mice aged 4–5 weeks, were purchased from Harlan Winkelmann (Borchen, Germany). All mice were housed in a temperature-controlled (25°C) facility with a 12-h light/dark cycle. Mice were fed with a high-fat diet (60% kcal from fat) for 16 weeks, followed by randomization to either a vehicle-treated ($n = 6$) (0.5% Tween 80/$H_2O$), a telmisartan-treated ($n = 6$) (3 mg · kg$^{-1}$ · day$^{-1}$), or a pioglitazone-treated ($n = 6$) (10 mg · kg$^{-1}$ · day$^{-1}$) group. Telmisartan was provided by Boehringer Ingelheim, and piogliatzone was extracted from tablets. Animals were treated by oral gavage for 10 weeks. Before and after treatment, blood samples were collected from overnight-fasted animals by retroorbital venous puncture under isoflurane anesthesia for analysis of serum triglycerides (enzymatic-colorimetric test; Cypress Diagnostics). After the experiment, animals were killed and organs were dissected. All animal procedures were in accordance with institutional guidelines and were approved.

Triglyceride content in liver was measured as described previously (19). Briefly, tissues were homogenized in liquid nitrogen and treated with ice-cold chloroform/methanol/water mixture (2:1:0.8) for 2 min. After centrifugation, the aqueous layer was removed and the chloroform layer was decanted. The mixture was incubated at 70°C for chloroform clearance, and the residues were dissolved in isopropanol and assessed for the triglyceride content using an enzymatic-colorimetric test (Cypress Diagnostics), according to the manufacturer's instructions. For immunohistochemical studies, organs were fixed in 4% formalin, embedded in paraffin, and stained with hematoxylin/eosin.

**Statistical analysis.** ANOVA, followed by multiple comparison testing or *t* test, was performed for statistical analysis as appropriate. Statistical significance was designated at $P < 0.05$. Values are expressed as means ± SE or as indicated.

## RESULTS

**Telmisartan induces PPARα target gene expression in human and murine hepatocytes.** To evaluate whether telmisartan regulates "classical" PPARα target genes, mRNA expression of CPT1A, the rate-limiting enzyme of fatty acid oxidation, in HepG2 cells was examined. Telmisartan induced CPT1A mRNA expression after 48 h in a dose-dependent manner starting at 10 μmol/l (1.5 ± 0.2–fold vs. vehicle-treated cells, $P < 0.05$) and reaching a maximum at 50 μmol/l telmisartan (2.84 ± 0.63–fold vs. vehicle-treated cells, $P < 0.001$ vs. control) (Fig. 1*A* and *B*). Treatment of HepG2 cells with a classical PPARα agonist Wy-14643 for 24 h resulted in a 6.6 ± 1.6–fold induction of CPT1A mRNA (100 μmol/l; $P < 0.05$ vs. vehicle-treated cells; data not shown).

To prove that telmisartan mediates its actions via PPARα activation, HepG2 cells were transfected with PPARα-specific siRNA. After siRNA treatment of HepG2 cells, PPARα mRNA expression was significantly reduced (Fig. 1*C*, *small graph*). This siRNA knockdown of PPARα resulted in a significant reduction of telmisartan-induced CPT1A mRNA expression compared with control siRNA, indicating a PPARα-dependent mechanism of action (Fig. 1*C*).

To determine whether PPARα target gene regulation by telmisartan was gene or species specific, we next studied the expression of ACSL1, the key player in lipid biosynthesis and fatty acid degradation, in the murine hepatic cell line AML12. ACSL1 mRNA was markedly induced by telmisartan (Fig. 1*D*). In contrast to CPT1A induction in HepG2 cells, maximum ACSL1 mRNA upregulation in AML12 cells was already achieved at 10 μmol/l 2.4 ± 0.1–fold after 48 h incubation with telmisartan 10 μmol/l ($P < 0.001$ vs. vehicle) (Fig. 1*D*). Wy-14643 (10 μmol/l) resulted in a 1.46 ± 0.12–fold ($P < 0.01$ vs. vehicle), and fenofibrate (100 μmol/l) in a 1.55 ± 0.38–fold, induction of ACSL1 mRNA ($P < 0.05$ vs. vehicle) (Fig. 1*D*). The present data demonstrate that telmisartan induces the PPARα target genes CPT1A in HepG2 cells and ACSL1 in AML12 cells.

**Telmisartan activates the PPARα LBD and acts like a partial PPARα agonist.** In order to prove whether the induction of hepatic PPARα target genes by telmisartan resulted from a direct activation of PPARα, we examined its ability to directly activate the PPARα LBD by using a

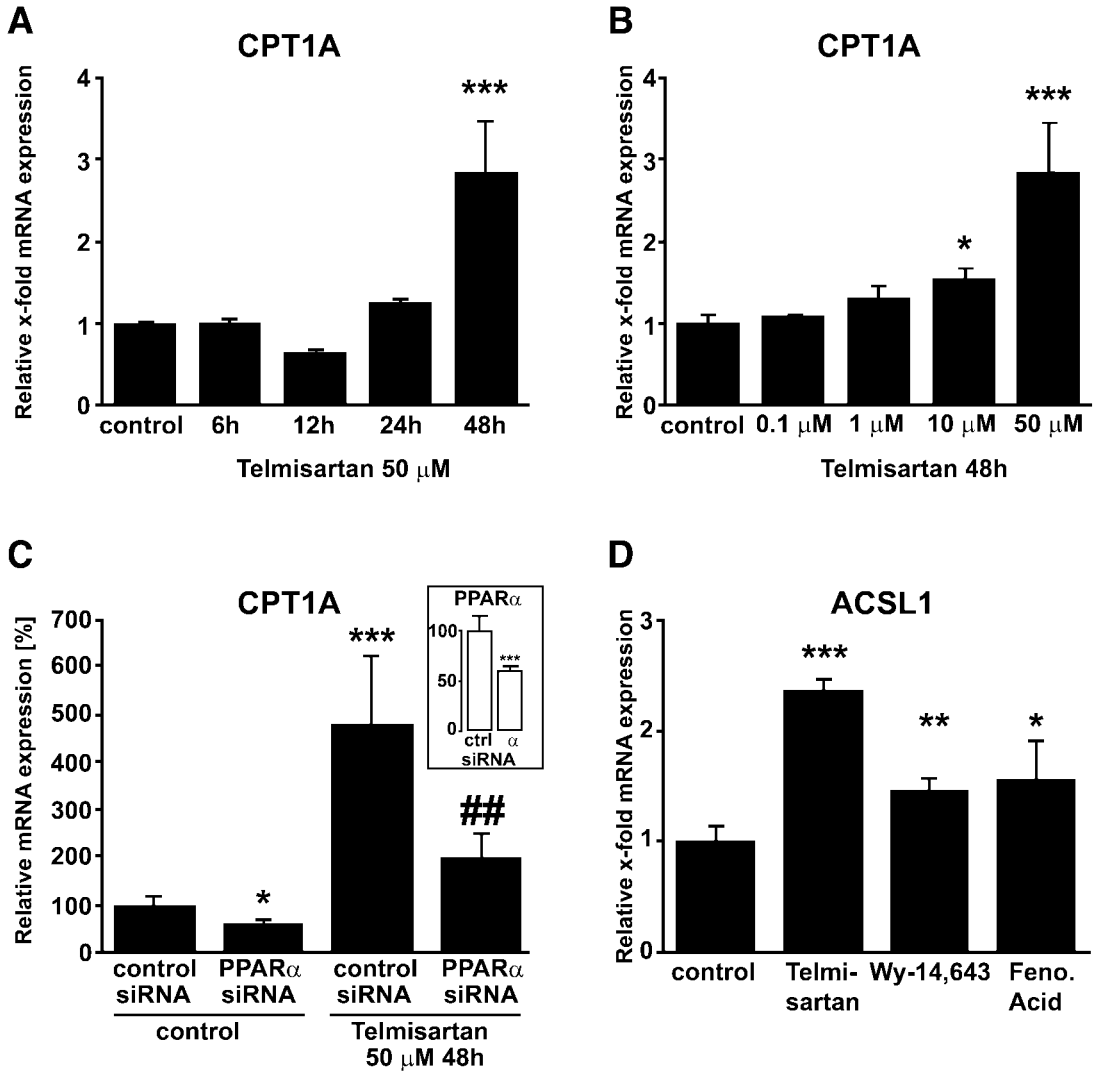


**FIG. 1. Telmisartan induces PPARα target gene expression in human and murine hepatocytes. *A*: HepG2 cells were serum deprived for 16 h and incubated with 50 μmol/l telmisartan. mRNA expression of CPT1A was determined by real-time PCR after the indicated time points. ***$P < 0.001$ vs. vehicle control. *B*: mRNA expression of CPT1A in HepG2 cells with incubation of telmisartan (0.1, 1, 10, and 50 μmol/l) for 48 h. *$P < 0.05$; ***$P < 0.001$ vs. vehicle control. *C*: HepG2 cells were serum deprived for 16 h and transfected with PPARα siRNA or control siRNA followed by incubation with 50 μmol/l telmisartan for 48 h. mRNA expression of CPT1A was determined by real-time PCR. *$P < 0.05$ and ***$P < 0.001$ vs. vehicle control with control siRNA; ##$P < 0.01$ vs. telmisartan-treated cells with control siRNA (small graph: PPARα mRNA expression in HepG2 cells with control/PPARα siRNA. ***$P < 0.001$ vs. control siRNA; data are shown as % of PPARα mRNA expression in control siRNA-transfected cells.) *D*: Murine AML12 cells were incubated with telmisartan (10 μmol/l), Wy-14643 (10 μmol/l), or fenofibric acid (100 μmol/l). After 48 h of incubation ACSL1 mRNA was determined. ***$P < 0.001$; ** $P < 0.01$; *$P < 0.05$ vs. vehicle control. Expression was normalized to 18S expression. Experiments were repeated four times and results are presented as x-fold induction over vehicle-treated cells. Means ± SE is shown.**

chimeric Gal4-DBD-hPPARα-LBD fusion protein on a Gal4-dependent luciferase reporter. Telmisartan induced the activation of the PPARα LBD in a concentration-dependent manner, reaching a maximum at 50 μmol/l (Fig. 2), with 22.5% of the maximum response induced by the reference PPARα agonist Wy-14643 identifying telmisartan as a partial PPARα agonist. No activation of the PPARα LBD was achieved with the ARBs irbesartan or losartan (Fig. 2). The computed EC50 values for activation are as follows: telmisartan EC50, 21.8 μmol/l; fenofibric acid EC50, 18.2 μmol/l; and Wy-14643 EC50, 6.4 μmol/l. Here, we identify telmisartan as a partial PPARα agonist inducing activation in the micromolar range.

**Telmisartan induces hepatic PPARα target gene expression in diet-induced obese mice.** It has been shown previously that after a single oral administration of telmisartan at a dose of 1 mg/kg in rats, telmisartan prominently concentrates in the liver (tissue distribution of $^{14}$C-telmisartan 4 h after application: liver, 10,673.06 ± 1,274.93 ng eq/ml; plasma, 218.85 ± 6.08 ng eq/ml; and skeletal muscle, 17.55 ± 1.18 ng eq/ml), implicating that the high concentrations required for PPARα activation and target gene regulation observed in vitro might be achieved in vivo (18). To demonstrate that hepatic PPARα activation by telmisartan occurs in vivo and translates into metabolic changes, high-fat diet–fed obese mice were treated with telmisartan 3 mg · kg$^{-1}$ · day$^{-1}$ or vehicle for 10 weeks and hepatic/muscular PPARα target gene expression, hepatic triglyceride accumulation, and serum triglyceride levels were determined.

ACSL1 protein expression in liver tissue was markedly increased in telmisartan-treated mice compared with the vehicle-treated animals (Fig. 3*A*). Accordingly, relative ACSL1 mRNA expression increased 2.5 ± 0.3–fold in livers of telmisartan-treated animals ($P < 0.01$ vs. vehicle) (Fig. 3*B*). In consonance, telmisartan treatment led to a 3.2 ±

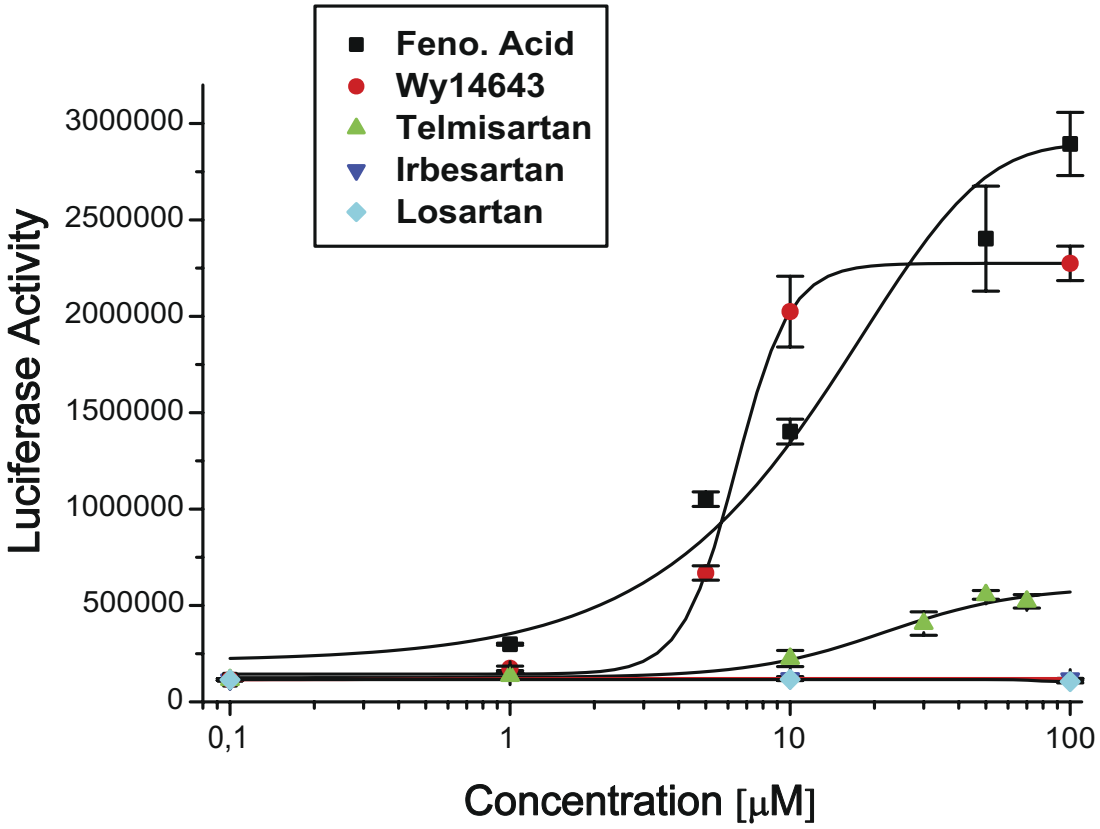


**FIG. 2. Telmisartan activates the PPARα LBD and acts like a partial PPARα agonist. COS7 cells were transiently transfected with the pGal4-h PPARα LBD and pGal5-Tk-pGL3 reporter followed by stimulation with the ARBs, fenofibric acid (Feno Acid), and Wy-14643 (Wy14643) as indicated. Firefly luciferase activity was measured after 24 h and normalized with activity of cotransfected renilla luciferase. Experiments were repeated three times. Results are presented as means ± SD.**

0.4–fold increase of hepatic CPT1A mRNA compared with vehicle-treated mice ($P < 0.01$ vs. vehicle) (Fig. 3*B*). Interestingly, telmisartan had no effect on PPARα target gene expression in skeletal muscle (Fig. 3*C*). Together, these results indicate that telmisartan markedly induces hepatic PPARα target genes involved in fatty acid catabolism in obese mice.

Previously, telmisartan has been characterized as a partial PPARγ agonist (4). To evaluate the role of hepatic PPARγ activation in the actions of telmisartan, mRNA expression of the PPARγ target gene CD36 and PPARγ2 was analyzed in liver tissue from telmisartan-treated mice (Fig. 3*D*). Hepatic CD36 and PPARγ2 mRNA levels were not significantly regulated by telmisartan treatment, suggesting the absence of major hepatic PPARγ activation by telmisartan (Fig. 3*D*). Furthermore, we studied the effect of the full PPARγ agonist pioglitazone in livers from high-fat diet–fed mice. Mice treated with pioglitazone (10 $mg \cdot kg^{-1} \cdot day^{-1}$) for 10 weeks exhibited no regulation of hepatic CPT1A mRNA expression, whereas the PPARγ target gene CD36 was significantly induced by 2.8 ± 0.3–fold compared with vehicle-treated high-fat diet–fed mice ($P < 0.01$ vs. vehicle-treated high-fat diet–fed mice) (Fig. 3*E*).

**Telmisartan reduces hepatic and serum triglycerides in diet-induced obese mice.** Increased expression of hepatic CPT1A and ACSL1, and subsequent higher rates of hepatic fatty acid oxidation in telmisartan-treated animals, should result in decreased accumulation of triglycerides in liver and serum. Telmisartan treatment prominently reduced liver triglyceride content in obese mice (29.7 ± 11.7μmol/g wet wt) when compared with vehicle-treated mice (79.5 ± 17.4 μmol/g wet wt, $P < 0.005$) (Fig. 4*A* and *B*). In accordance, serum triglycerides in high-fat diet–fed mice declined from 122.3 ± 28.4 mg/dl before treatment to 53.9 ± 4.5 mg/dl after telmisartan treatment ($P < 0.005$) (Fig. 4*C*), implicating that hepatic PPARα gene induction by telmisartan translates into a lowering of systemic and local triglyceride level.

In humans, aspartate aminotransferase (AST)-to-alanine aminotrasferase (ALT) ratios are indicative for the extent and etiology of liver damage (20). Whereas mild liver disease like nonalcoholic fatty liver disease and uncomplicated virus hepatitis are affiliated with ratios <1, severe liver disease like chronic hepatitis, liver cirrhosis, and alcoholic fatty liver disease result in increased ratios >1 (20). First, we compared AST-to-ALT ratios in mice on a low-fat diet (10% kcal from fat) with high-fat diet–fed animals. The mean AST-to-ALT ratio in low-fat diet–fed control mice was 1.1 ± 0.1, which was significantly decreased by high-fat diet to 0.5 ± 0.1 ($P < 0.05$). In line with the reduction of liver steatosis, telmisartan treatment restored impaired the AST-to-ALT ratio to normal levels (1.1 ± 0.3), indicating that reduction in hepatic triglycerides by telmisartan also improved high-fat diet–mediated deterioration of liver function.

**Telmisartan induces PPARα expression in vivo and in vitro.** To explore additional mechanisms of PPARα activation by telmisartan, which might be additive to LBD-dependent activation, we investigated the regulation of PPARα by telmisartan. Liver PPARα protein expression markedly increased with telmisartan treatment (Fig.

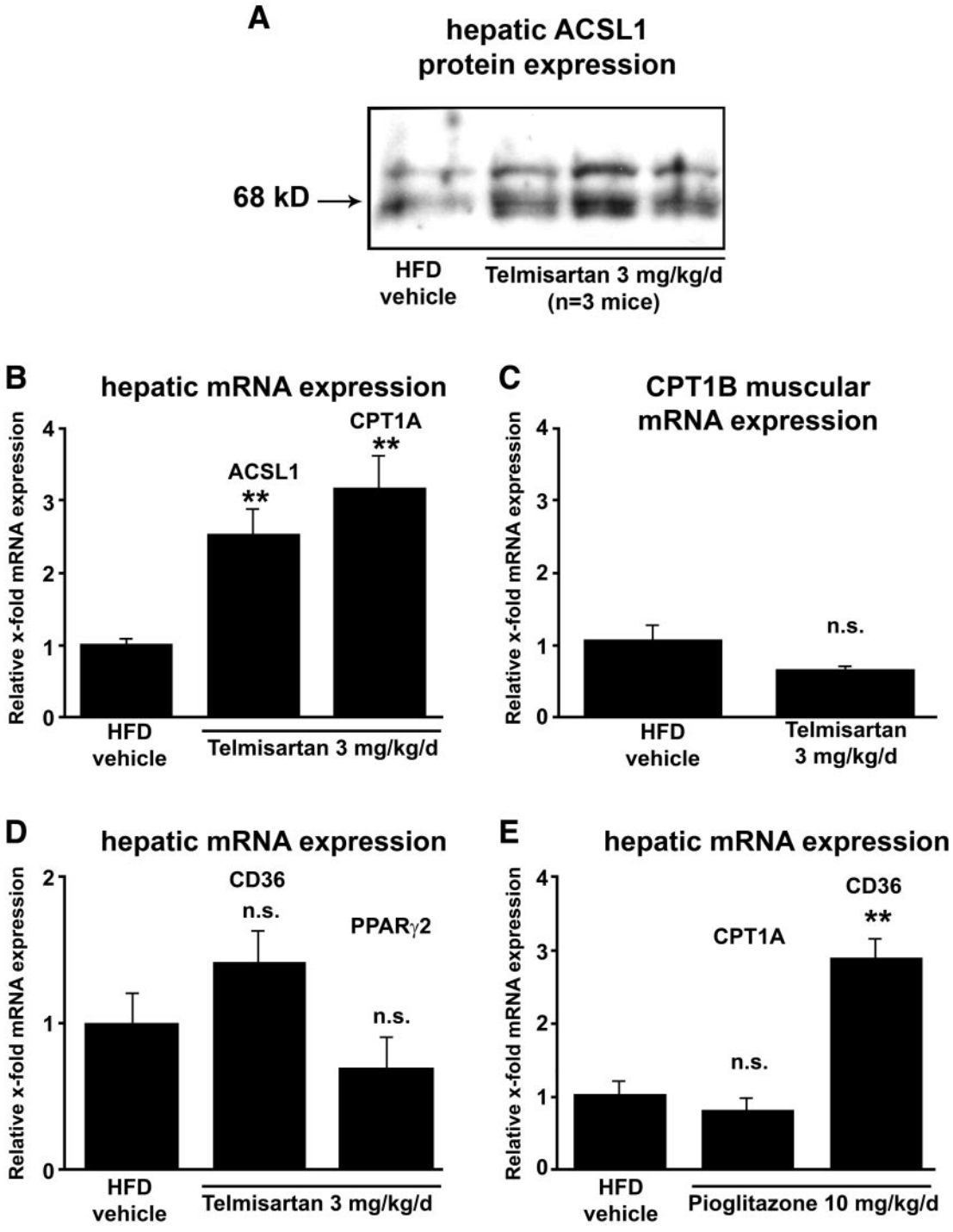


**FIG. 3. Telmisartan induces hepatic PPARα target gene expression in diet-induced obese mice. C57BL/6J mice were fed a high-fat diet (HFD) (60% kcal from fat) for 16 weeks followed by 10 weeks treatment with vehicle, telmisartan (3 mg · kg$^{-1}$ · day$^{-1}$), or pioglitazone (10 mg · kg$^{-1}$ · day$^{-1}$). At the end of the treatment period, animals were dissected and organs were shock frozen in liquid nitrogen. *A*: Hepatic ACSL1 protein expression. A representative Western immunoblot is shown. *B*: Relative hepatic mRNA expression of ACSL1 and CPT1A in telmisartan-treated animals. ***P* < 0.01 vs. HFD vehicle. *C*: mRNA expression of CPT1B in skeletal muscle tissue of telmisartan-treated animals. n.s., statistically not significant versus HFD vehicle animals. *D*: Relative hepatic mRNA expression of CD36 and PPARγ2 in telmisartan-treated animals. n.s., statistically not significant versus HFD vehicle animals. *E*: Relative hepatic mRNA expression of CPT1A and CD36 in pioglitazone-treated animals. n.s., statistically not significant versus HFD vehicle animals. ***P* < 0.01 vs. HFD vehicle. Expression was normalized to 18S expression. Experiments were repeated four times and results are presented as x-fold induction over vehicle-treated cells. Mean ± SE is shown.**

5*A*). In accordance, hepatic PPARα mRNA was upregulated 1.9 ± 0.2–fold in the telmisartan-treated animals compared with vehicle-treated mice ($P < 0.01$) (Fig. 5*B*). Moreover, PPARα mRNA induction by telmisartan was observed in HepG2 cells, with a maximum induction of 3.4 ± 0.4–fold ($P < 0.05$ vs. vehicle) after 48 h at high concentrations of telmisartan (50 μmol/l) (Fig. 5*C*). The ARB eprosartan had no effect on PPARα mRNA expression (Fig. 5*C*). These data show that, in addition to LBD activation, telmisartan is capable of inducing

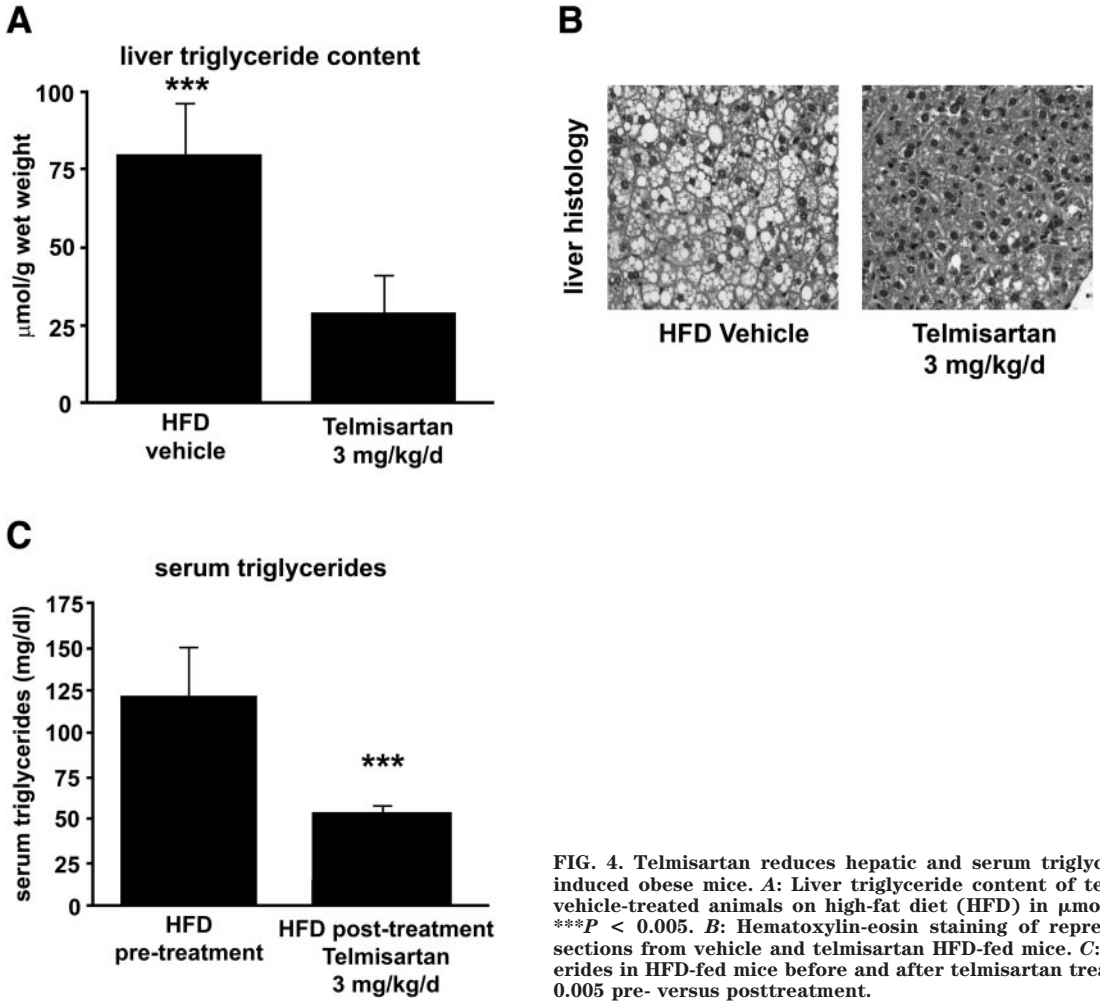


**FIG. 4. Telmisartan reduces hepatic and serum triglycerides in diet-induced obese mice. *A*: Liver triglyceride content of telmisartan- and vehicle-treated animals on high-fat diet (HFD) in μmol/g wet weight. ***$P < 0.005$. *B*: Hematoxylin-eosin staining of representative liver sections from vehicle and telmisartan HFD-fed mice. *C*: Serum triglycerides in HFD-fed mice before and after telmisartan treatment. ***$P < 0.005$ pre- versus posttreatment.**

PPARα expression, which might contribute to the observed target gene regulation.

## DISCUSSION

The present data demonstrate that the ARB telmisartan induces PPARα target genes in human and murine hepatic cells and acts as a partial PPARα agonist in the higher micromolar range in vitro. As a result of its particular pharmacokinetic profile with high concentrations in liver, telmisartan potently induces hepatic PPARα target genes involved in fatty acid catabolism in obese mice, which was associated with a significant reduction of systemic and local triglyceride level.

Derosa et al. (5) could demonstrate that telmisartan, when compared with eprosartan, significantly reduced serum triglycerides in hypertensive type 2 diabetic patients by 24.8% compared with baseline, an effect that may not be fully explained by its AT1-blocking/PPARγ-modulating actions. Additional studies (10,11) in diabetic and nondiabetic hypertensive patients have confirmed significant lower plasma triglyceride levels after telmisartan treatment. In the present study, we identify telmisartan as a weak PPARα agonist in vitro. Furthermore, telmisartan treatment in vivo induced PPARα-regulated genes involved in hepatic fatty acid oxidation at relatively low doses, suggesting that hepatic PPARα activation by telmisartan might be clinically relevant. It is well known that treatment of hypertriglyceridemic patients with fibrates results in potent lowering of triglyceride levels, which is, at least in part, mediated via hepatic PPARα activation, subsequent induction of fatty acid oxidation in the liver, and decreased VLDL particle production and plasma triglycerides (21). In consonance with this, telmisartan also significantly reduced circulating triglyceride levels in our animal model, which is consistent with recent data observed in the leptin receptor–deficient Zucker rat treated with telmisartan (22). Together, these results underscore that PPARα activation in the liver may contribute to telmisartans action on dyslipidemia in patients.

Telmisartan has been recently characterized as a selective PPARγ modulator (3,4). Hepatic activation of PPARγ contributes to the actions of PPARγ agonists on lipid and glucose metabolism in mice (23–25). To characterize the relevance of hepatic PPARγ activation for telmisartan's effects, CD36 and PPARγ2 expression were investigated and compared with the full PPARγ agonist pioglitazone. Telmisartan did not regulate CD36 or PPARγ2 in livers from high-fat diet–fed mice, excluding a major role of hepatic PPARγ in telmisartan's action. In contrast, pioglitazone failed to induce CPT1A but stimulated CD36 mRNA expression in liver. These data are consistent with previous findings of distinct gene expression profiles in adipocytes treated with telmisartan or piogliatzone (3). In liver, telmisartan may mainly act as a partial PPARα agonist, whereas pioglitazone also activates PPARγ pathways.

Telmisartan reduced hepatic triglyceride content in high-fat diet–fed mice. Nonalcoholic steatohepatitis (NASH) frequently develops during obesity as a result of

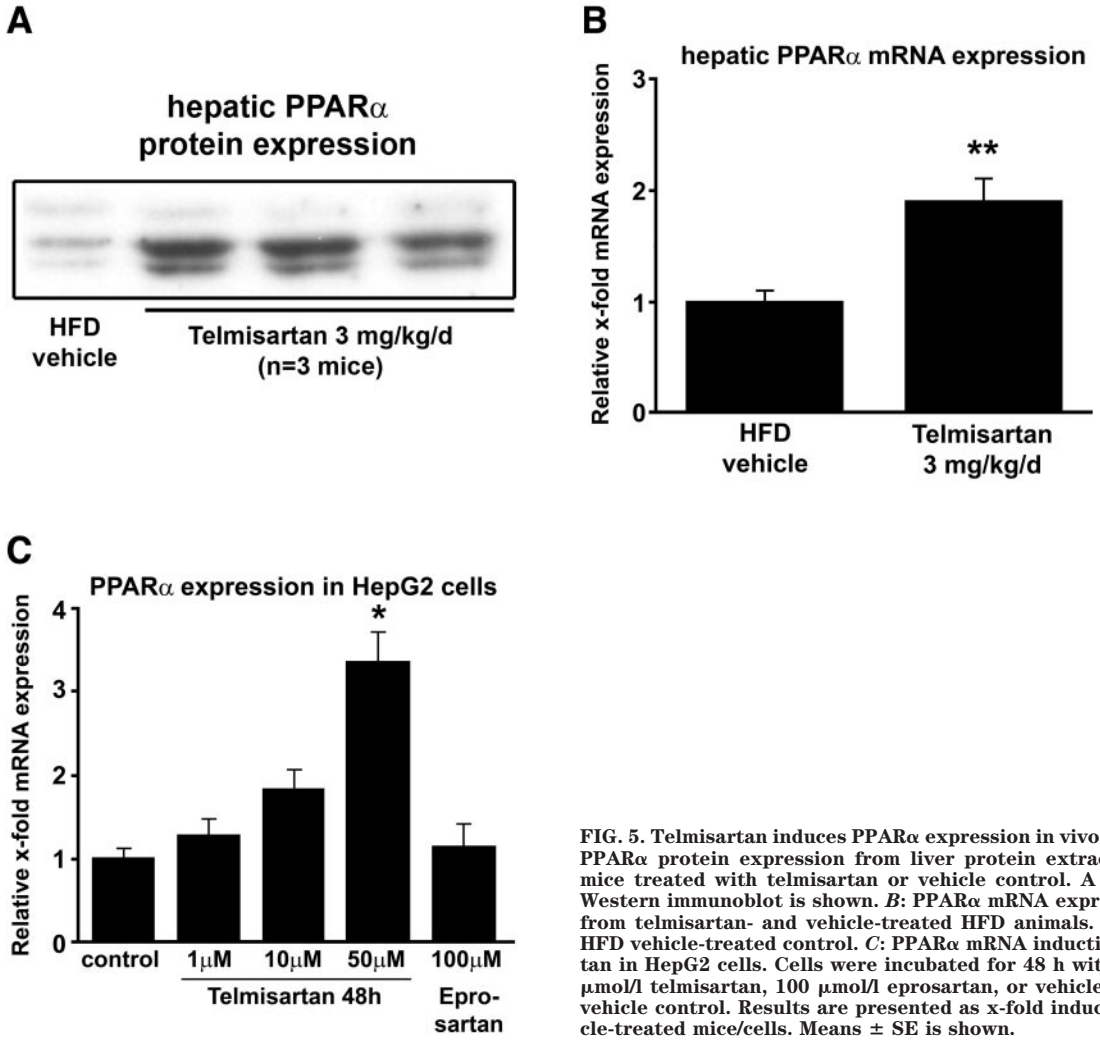


**FIG. 5. Telmisartan induces PPARα expression in vivo and in vitro. *A*: PPARα protein expression from liver protein extracts of HFD-fed mice treated with telmisartan or vehicle control. A representative Western immunoblot is shown. *B*: PPARα mRNA expression in livers from telmisartan- and vehicle-treated HFD animals. **$P < 0.01$ vs. HFD vehicle-treated control. *C*: PPARα mRNA induction by telmisartan in HepG2 cells. Cells were incubated for 48 h with 1, 10, and 50 μmol/l telmisartan, 100 μmol/l eprosartan, or vehicle. *$P < 0.05$ vs. vehicle control. Results are presented as x-fold induction over vehicle-treated mice/cells. Means ± SE is shown.**

insulin resistance and subsequent hepatic triglyceride overload (26). NASH is considered a hepatic component of the metabolic syndrome, leading to liver fibrosis and cirrhosis (26). Currently, no drug therapy has been established for the treatment of NASH. Recently, a number of small clinical trials (27–29) have demonstrated that the PPARγ agonists rosiglitazone and pioglitazone improve liver histology and aminotransferase levels in patients with NASH. PPARα activation by fibrates has been recently reported to reduce the development of NASH in different animal models (30,31). Therefore, combination of hepatic PPARα activation and systemic PPARγ modulation by telmisartan, together with the observed reduction of liver triglyceride content, may provide a new therapeutic option for the future treatment of NASH. Telmisartan's action on hepatic pathology has already been described by Sugimoto et al. (32). In rats fed a high-fat, high-carbohydrate diet, telmisartan, but not valsartan, significantly reduced hepatic triglyceride (32). Along this line, Fujita et al. (33) could show that telmisartan application to a rat model of NASH improved numerous pathological features of the disease, including liver steatosis, liver inflammation, and liver fibrosis, underlining the potential role of telmisartan for NASH therapy. The hepatic actions of telmisartan will gain clinical importance in light of previous data demonstrating an increased prevalence of fatty liver disease in hypertensive patients (34). The antihypertensive actions combined with hepatoprotective actions of telmisartan could be of added clinical value in these patients.

The pharmacokinetic profile of telmisartan seems to be highly important for its PPARα-activating properties. In preclinical studies with telmisartan, Shimasaki et al. (18) have shown that telmisartan prominently concentrates in the liver with ~40 times higher levels compared with plasma and skeletal muscle, which opens the possibility that the high concentrations required for PPARα activation and target gene regulation observed in vitro might be achieved in vivo in a tissue-specific manner. The high liver content of telmisartan is likely caused by binding of the compound to the glutathione-*S*-transferase type 1–1 (ligandin) protein, which is present at high concentrations in the liver (35). Hepatic storage may be further supported by the lipophilic characteristic of telmisartan. Compared with the liver, telmisartan concentrations in skeletal muscle are extremely low 4 h after administration, which makes it unlikely that concentrations required for muscular PPARα activation are reached (18). In accordance with the pharmacokinetic data, telmisartan did not affect PPARα target gene expression in skeletal muscle of obese mice, strongly supporting a tissue-specificity for telmisartan-induced PPARα activity.

The liver specificity of telmisartan-mediated PPARα activation does not only provide a mechanism for its beneficial effects on triglyceride levels but also plays a

major role for potential PPARα-mediated side effects. One of the most common toxic side effect during fibrate therapy are myopathies associated with myalgias, in particular in combination with statin therapy (36). Since muscular PPARα target genes are not activated by telmisartan, and telmisartan concentrations in skeletal muscle are minor, the occurrence of PPARα-mediated muscular side effects under telmisartan therapy are very unlikely. Moreover, the unique pharmacokinetic profile of telmisartan allows beneficial liver-specific PPARα activation in the absence of common PPARα-mediated side effects.

In addition to activation of the PPARα LBD, telmisartan-mediated induction of protein and mRNA expression of PPARα could be detected in vitro and in vivo. This data are in accordance with previously published studies, demonstrating a stabilization of the PPARα protein after ligand binding (37). Here, we identify that also PPARα mRNA expression is positively regulated by telmisartan, suggesting an additional transcriptional mechanism of ligand (telmisartan)-mediated receptor regulation. This is in line with previous reports demonstrating an induction of hepatic PPARα mRNA expression by fibrate treatment in rodents and human hepatocytes (38–40). Nevertheless, future studies are required to investigate whether PPARα mRNA regulation by telmisartan depends on telmisartan PPARα LBD interactions or whether this might be mediated via blockade of the AT1 receptor. As yet, it seems that telmisartan positively regulates the PPARα pathway by two different mechanisms: *1*) LBD activation and *2*) receptor upregulation.

In summary, the present study identifies the ARB/PPARγ modulator telmisartan as a partial PPARα agonist. As a result of its particular pharmacokinetic profile with high concentration in liver, PPARα activation by telmisartan seems to be restricted to the liver. Hepatic PPARα activation by telmisartan may provide an explanation for its antidyslipidemic actions observed in clinical trials and prevents simultaneously potential danger from systemic PPARα-mediated adverse effects. The multimodal mechanism of action of telmisartan, including AT1-receptor blockade/PPARγ modulation and hepatic PPARα activation, characterizes this compound as a therapeutic option for the treatment of patients suffering from multiple cardiometabolic disorders such as hypertension, glucose intolerance, and dyslipidemia.

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