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PPAR-α activator fenofibrate increases renal CYP-derived eicosanoid synthesis and improves endothelial dilator function in obese Zucker rats

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Zhao, Xueying, Jeffrey E. Quigley, Jianghe Yuan, Mong-Heng Wang, Yiqing Zhou, and John D. Imig. PPAR-α activator fenofibrate increases renal CYP-derived eicosanoid synthesis and improves endothelial dilator function in obese Zucker rats. Am J Physiol Heart Circ Physiol 290: H2187–H2195, 2006. First published February 24, 2006; doi:10.1152/ajpheart.00937.2005.—Previous studies have shown that the synthesis of renal cytochrome P-450 (CYP)-derived eicosanoids is downregulated in genetic or high-fat diet-induced obese rats. Experiments were designed to determine whether fenofibrate, a peroxisome proliferator-activated receptor (PPAR)-α agonist, would induce renal eicosanoid synthesis and improve endothelial function in obese Zucker rats. Administration of fenofibrate (150 mg·kg⁻¹·day⁻¹ for 4 wk) significantly reduced plasma insulin, triglyceride, and total cholesterol levels in obese Zucker rats. CYP2C11 and CYP2C23 proteins were downregulated in renal vessels of obese Zucker rats. Consequently, renal vascular epoxyenase activity decreased by 15% in obese Zucker rats compared with lean controls. Chronic fenofibrate treatment significantly increased renal cortical and vascular CYP2C11 and CYP2C23 protein levels in obese Zucker rats, whereas it had no effect on epoxyenase protein and activity in lean Zucker rats. Renal cortical and vascular epoxyenase activities were consequently increased by 54% and 18%, respectively, in fenofibrate-treated obese rats. In addition, acetylcholine (1 μM)-induced vasodilation was significantly reduced in obese Zucker kidneys (37% ± 11%) compared with lean controls (67% ± 9%). Chronic fenofibrate administration increased afferent arteriolar responses to 1 μM of acetylcholine in obese Zucker rats (69% ± 4%). Inhibition of the epoxyenase pathway with 6-(2-propargyloxyphenyl)hexanoic acid attenuated afferent arteriolar diameter responses to acetylcholine to a greater extent in lean compared with obese Zucker rats. These results demonstrate that the PPAR-α agonist fenofibrate increased renal CYP-derived eicosanoids and restored endothelial dilator function in obese Zucker rats.

kidney; cytochrome P-450; metabolic syndrome; renal vessels; peroxisome proliferator-activated receptor-α;

OBESITY is a major risk factor for the development of hypertension and type 2 diabetes. Endothelial dysfunction is associated with obesity-related hypertension and diabetes. Because of a nonfunctional leptin receptor gene, the obese Zucker rat develops obesity and metabolic syndrome. The obese Zucker rat has been widely used to investigate changes in responsiveness of the vasculature to vasoconstrictor and vasodilator hormones in an attempt to more clearly characterize the vascular dysfunction associated with obesity. Nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) are the main contributors to endothelium-dependent relaxation. A number of studies have reported that NO-dependent relaxation to acetylcholine is impaired in skeletal muscle arterioles (10, 11), small mesenteric arteries (23, 43, 45), and renal microvasculature (17) of obese Zucker rats. Our recent studies suggest that NO-independent dilation is also impaired in mesenteric arterioles of obese Zucker rats (48). In addition, the impairment of the endothelium-dependent dilation of the mesenteric arterial bed seen in streptozotocin-induced diabetic rats has been demonstrated to be largely due to a defective vascular response to EDHF (26, 42). However, the mechanisms responsible for the impaired endothelium-dependent dilation in the renal preglomerular vasculature of obese animals remain unclear.

Epoxyeicosatrienoic acids (EETs), the cytochrome P-450 (CYP) epoxyenase metabolites, have been identified as EDHFs in the kidney. 11,12- and 14,15-EETs are potent vasodilators, whereas their metabolic breakdown product, dihydroxyeicosatrienoic acids (DHETs), are devoid of renal vascular activity (9, 20, 35). CYP2C enzymes are the main epoxyenase isoforms responsible for EET production in the rat kidney (18, 44, 50). Recent studies demonstrate that renal CYP enzyme and CYP-derived eicosanoid synthesis are downregulated in rats with high-fat diet-induced hypertension (39). In addition, CYP2C11 and CYP2C23 protein expression is also significantly decreased in renal microvessels of obese Zucker rats (8). Decreased renal CYP enzymes and CYP-derived eicosanoid synthesis may contribute to endothelial dysfunction in obesity-related hypertension and diabetes.

Fenofibrate, a peroxisome proliferator-activated receptor-α (PPAR-α) activator, has been reported to retard angiographic progression of coronary atherosclerosis in diabetic patients (37) and improve the microcirculation in patients with hyperlipidemia (25). One recent report indicates that fenofibrate strongly induces renal CYP2C23 activity and protects from ANG II-induced renal injury (29). Therefore, in the current study, we examined whether the PPAR-α activator fenofibrate is able to increase CYP-derived eicosanoid synthesis and
restore the endothelium-dependent dilation in the pregglomerular vasculature of obese Zucker rats.

MATERIALS AND METHODS

Animals. Fifteen- to 16-wk-old male lean (337 ± 11 g) and obese (554 ± 12 g) Zucker rats (Charles River Laboratories, Wilmington, MA) were divided into four experimental groups: untreated lean control (LZR), untreated obese control (OZR), and fenofibrate (150 mg·kg⁻¹·day⁻¹ orally)-treated lean (F-LZR) and obese Zucker rats (F-OZR). Animals were treated for 4 wk and kidney tissue was harvested and frozen in liquid nitrogen for hydroxylase and epoxygenase activity, mRNA, or protein evaluation. Blood was collected for measuring plasma insulin, total cholesterol, and triglyceride levels. Animals were treated for 4 wk and kidney tissue was frozen in liquid N₂ and kept at -80°C in a freezer until assayed for arachidonic acid metabolism in renal cortex and microvessels. Renal cortex homogenate (500 μg) isolated from control, obese control, and F-OZR were incubated with [1-14C]arachidonic acid (0.4 μCi, 7 nmol) and NADPH (1 mmol/l, pH 7.4) containing 10 mmol/l MgCl₂ for 30 min at 37°C. The reaction was terminated by acidification to pH 3.5–4.0 with 2 mol/l formic acid, and arachidonic acid metabolites were extracted with ethyl acetate. The ethyl acetate was evaporated under nitrogen, and the metabolites were exposed to X-ray film (Hyperfilm-ECL, Amersham). Band intensity was measured densitometrically, and the values were normalized to β-actin internal controls. Values are expressed as relative densitometric units (du).

Preparation of renal microvessels. Renal microvessels were isolated according to a method described previously (50). Briefly, rats were anesthetized with an injection of pentobarbital sodium (50 mg/kg ip). The kidneys were infused with a physiological salt solution, and the renal microvessels were separated from the rest of the cortex with the aid of sequential sieving, a digestion period, and collection under a stereomicroscope. Renal microvessels were quickly frozen in liquid N₂ and kept at -80°C in a freezer until assayed for protein levels.

Immunoblot analysis of CYP4A, CYP2C23, CYP2C11, or endothelial nitric oxide synthase protein. Renal microvessels or kidney cortex was harvested and processed as previously described (21). Samples were separated by electrophoresis on a 10% stacking Tris-glycine gel, and proteins were transferred electrophoretically to a nitrocellulose membrane. The primary antibodies used were rabbit anti-goat CYP4A polyclonal antibody (1:2,000, Abcam), rabbit anti-rat CYP2C11 polyclonal antibody (1:2,000), rabbit CYP2C23 polyclonal antibody (1:5,000, from Dr. J. Capdevila, Vanderbilt University, Nashville, TN), and mouse anti-human endothelial nitric oxide synthase (eNOS) polyclonal antibody (1:500, Transduction Laboratories). The blots were then washed in PBS-0.3% Tween-20 and incubated with the second antibody (goat anti-rabbit 1:100,000 or goat anti-mouse 1:2,000) conjugated to horseradish peroxidase for 90 min at room temperature and washed. Detection was accomplished by enhanced chemiluminescence Western blotting (ECL, Amersham), and blots were exposed to X-ray film (Hyperfilm-ECL, Amersham). Band intensity was measured densitometrically, and the values were normalized to β-actin internal controls. Values are expressed as relative densitometric units (du).

Real-time PCR. Total RNA was prepared from isolated kidney cortex by using ultra-pure TRIzol reagent according to the manufacturer’s instructions (GIBCO-BRL, Grand Island, NY). Reverse transcription was then performed on equal amounts of total RNA (2 μg) by using random hexanucleotide primers to produce a cDNA library for each sample. Real-time PCR reactions were run on an iCycler iQ Real-Time PCR Detection System by using iQ Supermix, which is optimized for real-time PCR applications (Bio-Rad Laboratories, Hercules, CA). TaqMan probes (Roche Molecular Systems) and oligonucleotide primers were designed from the published cDNA sequences for CYP2C23 and GAPDH by using Beacon Designer software (Premier Biosoft International, Palo Alto, CA). Each sample was run in triplicate, and the comparative threshold cycle (Cₜ) method was used to quantify fold increase (2⁻ΔΔCₜ) compared with controls. Probes and primer sequences used were as follows: CYP2C23 probe 5'-FAM-AGG CCA GAC AAC CAG CAC CAC-BHQ-3'; CYP2C23 forward 5'-TGG CTG TCT GTG GGT CTA ACT-3'; CYP2C23 reverse 5'-AAC CAC AGC TTC AAT TCA AT-3'; GAPDH probe 5'-FAM-ACC CCA CAT ACT CAG CAC CAG CA -BHQ-3'; GAPDH forward 5'-CAC GGC AAC TGG AAC GCC-3'; GAPDH reverse 5'-GGT GGT GAA GAC GCC AGT A-3'.

Activities of arachidonic acid metabolism in renal cortex and microvessels. Renal cortex homogenate (500 μg) isolated from control, obese control, and F-OZR were incubated with [1-14C]arachidonic acid (0.4 μCi, 7 nmol) and NADPH (1 mmol/l, pH 7.4) containing 10 mmol/l MgCl₂ for 30 min at 37°C. The reaction was terminated by acidification to pH 3.5–4.0 with 2 mol/l formic acid, and arachidonic acid metabolites were extracted with ethyl acetate. The ethyl acetate was evaporated under nitrogen, and the metabolites were resuspended in 50 μl of methanol and injected onto the HPLC column. The activity of the formation of the specific activity of the added [1-14C]arachidonic acid and was expressed as picomoles per milligram of protein per minute.

Rat kidney cortex homogenate (500 μg) was incubated with 0.1% Tween 80 in a 100 mmol/l MgCl₂ and 1 mmol/l EDTA for 15 min on ice. This step results in the permeabilization of the tissue and ensures free access of exogenous arachidonic acid and NADPH to CYP enzymes located in the endoplasmic reticulum. Renal microvessels were washed three times with buffer, spun down by centrifugation, and incubated with [1-14C]-arachidonic acid (50 μCi/μmol, 30 μmol/l final concentration) in 500 μl of potassium phosphate buffer containing 1 mmol/l NADPH in a shaking bath for 60 min at 37°C as described previously (39). The reactions were terminated by acidification to pH 4.0 with 2 mol/l formic acid, and renal microvessels were homogenized. Extraction and HPLC analysis were carried out as described (39).

Renal microvascular responses. In vitro perfused juxtaglomerular nephron preparation has been described previously (49). Briefly, after pentobarbital anesthesia (50 mg/kg ip) and midline laparotomy, the right renal artery was cannulated through the superior mesenteric artery, and the kidney was immediately perfused with a Tyrode solution containing 6% albumin and a mixture of l-amino acids. After the microdissection procedures were completed, renal artery perfusion pressure was set to 100 mmHg. The tissue surface was continuously superfused with a Tyrode solution containing 1% albumin. After a 30-min equilibration period, an afferent arteriole was chosen for study.

The afferent arteriole was exposed to increasing concentrations of acetylcholine (0.1–1 μM) after preconstriction with phenylephrine (1 μM) to ~50% of resting diameter. Afferent arteriolar responses to acetylcholine were assessed in the absence or presence of the selective epoxygenase inhibitor 6-[2-propargyloxyphenyl]hexanoic acid (PPOH, 50 μM), NOS inhibitor LNAME (100 μM, Sigma), and cyclooxygenase (COX) inhibitor indomethacin (10 μM, Sigma). The inhibitors were dissolved in 75% ethanol and added to the perfusate and superfusate to yield a final ethanol vehicle concentration of <0.05% (vol/vol). Inhibitors were added to the perfusate and superfusate for 30 min to ensure complete tissue blockade. After the 30-min period, the dose–diameter relationship of the pregglomerular vasculature was determined. Sodium nitroprusside (10⁻⁵ M) was used to assess endothelium-independent vasodilation. Diameter changes were monitored for 3 min at each concentration. Steady-state diameter to acetylcholine was attained by the end of the second minute, and the average diameter at the third minute of each treatment period was used for statistical analysis.

Statistics. All data are presented as means ± SE. The significance of differences between groups for the afferent arteriolar diameter data
were evaluated with an ANOVA for repeated measures followed by a Duncan multiple range post hoc test. The PCR and Western blot data were analyzed by unpaired t-test. A value of *P < 0.05 was considered statistically significant.

RESULTS

Fenofibrate lowers plasma insulin, cholesterol and triglyceride levels, and body weight gain. Body weight data for age-matched lean and obese Zucker rats are presented in Fig. 1. As expected, at 15 and 20 wk the obese Zucker rats were 57% and 54% heavier than lean rats. Fenofibrate treatment significantly reduced weekly weight gain and final total body weight of obese animals (F-OZR 583 ± 11 g vs. OZR 650 ± 30 g). Consistent with the previous studies, blood glucose levels were significantly increased in obese Zucker rats (OZR 171 ± 28 mg/dl vs. LZR 92 ± 6 mg/dl). Chronic fenofibrate treatment did not lower blood glucose in obese Zucker rats (187 ± 26 mg/dl). Plasma insulin significantly increased in obese rats (3.8 ± 0.6 µg/l) compared with lean animals (1.3 ± 0.4 µg/l) (Table 1). Fenofibrate decreased plasma cholesterol and triglyceride levels in obese Zucker rats (Table 1). In addition, systolic blood pressure was not changed in F-OZR (139 ± 3 mmHg) compared with untreated OZR (139 ± 4 mmHg).

Fenofibrate induces renal cortical and vascular CYP2C and CYP4A protein expression. Figure 2 shows representative Western blots for CYP2C11, CYP2C23, and CYP4A protein in kidney cortex of Zucker rats. Although renal cortical CYP2C and CYP4A protein is not different between lean and obese Zucker rats, fenofibrate significantly increased CYP2C23 and CYP4A protein expression in kidney cortex of obese Zucker rats (Fig. 2). Consistent with the previous study (8), CYP2C23 and CYP2C11 proteins were decreased in renal microvessels of OZR compared with lean controls (Fig. 3). Fenofibrate admin-

Table 1. Serum metabolic data from lean, obese, and fenofibrate-treated obese Zucker rats at 20–22 wk of age

<table>
<thead>
<tr>
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<th>LZR (n = 6)</th>
<th>OZR (n = 5)</th>
<th>F-OZR (n = 5)</th>
</tr>
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<tbody>
<tr>
<td>Insulin, µg/l</td>
<td>1.3±0.4</td>
<td>3.8±0.6*</td>
<td>1.9±0.3†</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>49±8</td>
<td>230±81*</td>
<td>133±33</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>37±11</td>
<td>704±204*</td>
<td>396±90</td>
</tr>
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Values are mean ± SE. LZR, lean Zucker rats; OZR, obese Zucker rats; F-OZR, fenofibrate-treated obese Zucker rats. *P < 0.05 vs. LZR (lean control). †P < 0.05 vs. OZR (obese control).
Fig. 2. Effect of fenofibrate on renal cortical cytochrome P-450 (CYP) enzyme protein expression. Representative Western blots show CYP2C23, CYP2C11, and CYP4A bands (~51 kDa) in kidney cortex isolated from LZR (lean control), F-LZR, OZR (obese control), and F-OZR. Densitometric evaluations of protein levels (10 g/lane) were obtained from 4 different animals. Data are expressed as means ± SE. *P < 0.05 vs. untreated OZR.

Fig. 3. Effect of fenofibrate on CYP enzyme protein expression in renal microvessels. Western blots show that fenofibrate treatment increased CYP2C11, CYP2C23, and CYP4A protein levels in renal microvessels of obese Zucker rats. Densitometric evaluations of protein levels (20 g/lane) were obtained from 4 different animals. Data are expressed as means ± SE. *P < 0.05 vs. LZR. #P < 0.05 vs untreated OZR.
Interestingly, chronic fenofibrate treatment restored afferent arteriolar responses to 1 μM of acetylcholine in F-OZR rats (69 ± 4%) but had no effect on acetylcholine-induced dilation in lean rats. These data suggest that PPAR-α activation improves endothelial-dependent dilation in obese Zucker rats. Sodium nitroprusside was further used to investigate the endothelium-independent vasodilation in lean and obese Zucker rats. Afferent arteriolar responses to sodium nitroprusside (10⁻⁵ M) were not significantly altered in obese (70 ± 11%, n = 6) compared with lean Zucker rats (64 ± 9%, n = 5).

To determine the role of epoxygenase metabolites in the beneficial effect of fenofibrate on vascular function in obese rats, we further observed the afferent arteriolar responses to acetylcholine in the presence of epoxygenase inhibition or NOS and COX inhibition. Preincubation with NOS and indomethacin produced a similar and slight decrease in afferent arteriolar diameters in the four groups, whereas PPOH pretreatment did not change the afferent arteriolar baseline diameter. In the presence of NOS and COX inhibitors, the response to 1 μM acetylcholine was significantly reduced in obese Zucker rats (17 ± 15%) compared with lean controls (56 ± 17%). Fenofibrate restored dilation in obese kidneys (57 ± 19%), indicating an impaired NO- and COX-independent component in obese Zucker rats. Therefore, PPOH was used to inhibit the production of renal CYP-derived eicosanoids. In the presence of PPOH, 1 μM of acetylcholine evoked a similar vasodilation in the preglomerular vasculatures in the four groups (Fig. 6B), suggesting the involvement of epoxygenase metabolites in the improvement of endothelial dilator function in obese Zucker rats. However, the afferent arteriolar responses to 0.1 μM of acetylcholine were similar between OZR and F-OZR rats and were lower than lean Zucker rats. These data suggest a possible role of eicosanoid-independent component in the beneficial effect of fenofibrate in obese Zucker rats.

**DISCUSSION**

Many epidemiological studies have shown that there is a positive correlation between obesity and endothelial dysfunction in humans. The presence of endothelial dysfunction can be regarded as a clinical syndrome that is associated with and predicts an increased rate of adverse cardiovascular events (2, 27, 46, 47). The obese Zucker rat is an animal model of obesity and metabolic syndrome. Endothelium-dependent relaxation is impaired in skeletal muscle arterioles (10, 11), small mesenteric arteries (23, 43, 45), and renal arterioles (17) in obese Zucker rats. In the current study, we demonstrate that endothelium-dependent acetylcholine-induced dilation was attenuated in renal preglomerular vasculature of obese Zucker rats. Furthermore, decreased renal vascular epoxygenase enzyme and activity are associated with the impaired vascular function in obese Zucker rats. Consistent with previous studies, renal vascular CYP2C protein and epoxygenase activity were lower in obese Zucker rats. Treatment with fenofibrate, a PPAR-α agonist, increased renal epoxygenase and hydroxylase enzyme protein expression, as well as enzyme activity in obese Zucker rats. In addition, fenofibrate restored afferent arteriolar responses to acetylcholine in kidneys of obese Zucker rats. These results suggest that endothelium-dependent EDHF-mediated dilation is impaired in renal preglomerular vasculature of obese Zucker rats, and induction of CYP-derived eicosanoid synthesis by fenofibrate improves renal vasodilator function in obese Zucker rats.

Endothelium-dependent relaxation is mainly attributed to the release of PGI₂, NO, and EDHF. In resistance arteries, EDHF is abundant and may compensate for the loss in NO production. One recent study showed that NO and prostaglandin-mediated dilation is decreased in mesenteric arteries incubated for 20 h in culture medium at 37°C, while EDHF served as a backup system that preserved the capability of this
vessel to respond to vasodilators (3). However, previous studies also show that EDHF-mediated dilation in response to acetylcholine is attenuated in vascular beds of spontaneously hypertensive rats (1, 13, 24), deoxycorticosterone acetate-salt hypertensive rats (28), and salt-sensitive hypertensive patients (12). In addition, our recent studies indicate that NO-independent dilation is attenuated in the mesenteric arteries of obese Zucker rats (48). In the present study, we further observed decreased afferent arteriolar responses to acetylcholine in the absence or presence of NO and COX inhibitors in obese Zucker rats, suggesting an impaired NO- and COX-independent, EDHF-mediated vasodilation in the preglomerular vasculature of obese Zucker rats.

EETs have been identified as EDHFs in the kidney, and 11,12- and 14,15-EETs are potent vasodilators (9, 20, 35). Many CYP enzymes can carry out the epoxidation of arachidonic acid, and several reports have suggested that CYP2C isoforms are primarily responsible for renal epoxygenase generation (18, 44, 50). There is evidence suggesting that the CYP epoxygenase pathway is involved in the pathogenesis of obesity and hypertension. An inability to increase renal epoxygenase in rats fed a high-salt diet results in ANG II salt-sensitive hypertension (44). The elevation in blood pressure and development of hypertension in the Dahl S rats fed a high-salt diet is also associated with an inability of these animals to increase renal EET production. In addition, one recent study indicates that renal CYP-derived eicosanoid synthesis is downregulated in rats with high-fat diet-induced hypertension (39). Consistent with these studies, we have reported that epoxygenase protein expression is downregulated, and this decreased expression is

![Graph](image_url_1)

**Fig. 5.** Effect of fenofibrate on arachidonic acid metabolites. 

- **A:** arachidonic acid metabolites formed by renal cortical homogenates isolated from untreated lean (LZR), untreated obese (OZR), and F-OZR. 
- **B:** epoxygenase and ω-hydroxylase metabolites in renal vessels freshly isolated from LZR, F-LZR, OZR, and F-OZR. ω-Hydroxylase activity was determined from the 20-HETE formation. Epoxygenase activity was determined from the sum of dihydroxy-eicosatetraenoic acid and epoxyeicosatrienoic acid formation. Results are means ± SE; n = 4–6. *P < 0.05 vs. lean animals; #P < 0.05 vs. untreated OZR.

![Graph](image_url_2)

**Fig. 6.** Afferent arteriolar responses to 0.1–10 µmol/L acetylcholine. 

- **A:** percentage changes of afferent arteriolar diameter in response to acetylcholine in kidneys of LZR (lean controls; basal diameter 27.2 ± 2.1 µm, n = 5), F-LZR (basal diameter 23.3 ± 2.0 µm, n = 6), untreated OZR (basal diameter 26.9 ± 1.8 µm, n = 6), and F-OZR (basal diameter 26.9 ± 1.4 µm, n = 7). 
- **B:** in the presence of 6-(2-propargyloxyphenyl)hexanoic acid, the percentage changes of afferent arteriolar diameter in response to acetylcholine in kidneys of LZR (basal diameter 27.2 ± 2.3 µm, n = 4), F-LZR (22.5 ± 3.8 µm, n = 4), OZR (basal diameter 26.0 ± 0.7 µm, n = 5), and F-OZR (basal diameter 27.7 ± 2.0 µm, n = 5). Values are means ± SE. *P < 0.05 vs. lean control.
associated with impaired NO-independent dilation in mesenteric arteries of obese Zucker rats (48). Interestingly, our studies also indicated that CYP2C11 and CYP2C23 protein levels and epoxygenase activity were significantly decreased in the renal microvessels of obese Zucker rats. Further studies with the selective epoxygenase inhibitor PPOH demonstrate that CYP-derived eicosanoid-mediated dilation was attenuated in the preglomerular vasculature of obese Zucker rats. Therefore, decreased renal vascular CYP2C enzyme may lead to decreased vascular EET production, which may contribute to the impaired dilation in afferent arterioles of obese Zucker rats.

PPARs are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, and PPAR agonists have been used for treating metabolic syndrome. Treatment with PPAR-α agonist, fibrates, improves insulin sensitivity and glycemic control in obese mice and rats (15). Fenofibrate has been reported to retard angiographic progression of coronary atherosclerosis in diabetic patients (37) and to improve the microcirculation of patients with hyperlipidemia (25). Although direct activation of PPAR-α in arterial wall (12), correction of lipid abnormalities (4, 5, 30), and increasing the formation, availability, and action of NO (16, 34) have all been postulated, the mechanisms responsible for the beneficial effects of fenofibrate on vascular function has not been fully elucidated. More recent studies have demonstrated that treatment with the PPAR-α activator fenofibrate induced renal CYP2C23-dependent arachidonic acid-epoxygenase activity and protected double-transgenic rats (dTGRs) from hypertension and inflammatory end-organ damage (29). In the current study, we observed that fenofibrate upregulated CYP2C and CYP4A protein expression in the renal cortex and microvessels of obese Zucker rats. Consequently, renal epoxygenase activity was markedly increased in fenofibrate-treated obese animals. Real-time PCR analysis suggests that a posttranscriptional mechanism is involved in this regulation because CYP2C23 mRNA level was not changed in the kidneys of fenofibrate-treated obese rats. This is consistent with the previous finding that CYP2C23 mRNA levels were not changed in fenofibrate-treated dTGR kidneys (29). Our current study also shows that CYP4A protein expression was upregulated in kidney cortex and renal microvessels of fenofibrate-treated rats. However, renal vascular hydroxylase activity was not altered in fenofibrate-treated lean and obese rats, suggesting that 20-HETE may play a minor role in the beneficial effect of fenofibrate on vascular function in obese rats. Wang et al. (40) reported that the renal microvessels that highly express CYP4A2 readily metabolize arachidonic acid to 20-HETE and 11,12-DHET, the hydrolytic metabolite of 11,12-EET. Thus it is possible that CYP4A2 functions as both arachidonic acid ω-hydroxylase and 11,12-epoxygenase. In addition, it has been reported that CYP4A4 is able to hydroxylate EETs to hydroxy-EETs (HEETs), and HEETs function as high-affinity endogenous PPAR-α activators (6, 29). Therefore, the CYP hydroxylase pathway responsible for improving endothelial function in fenofibrate-treated obese Zucker rat remains to be elucidated.

Previous studies have indicated that NO-dependent relaxation to acetylcholine is impaired in skeletal muscle arterioles (10, 11), small mesenteric arteries (23, 43, 45), and renal microvasculature (17) of obese Zucker rats. There is clear evidence that PPAR-α ligands can modulate NO production in vascular tissues and macrophages (5, 32, 33). Newaz et al. (32) reported that PPAR-α activators amplify inducible NOS (iNOS) expression and increased renal NO production as measured by urinary excretion of nitrite/nitrate (32). It has been reported that fenofibrate is likely to improve endothelial function by restoring the impaired formation or efficacy of the endothelium-derived relaxing factor such as NO (16, 34). Our studies suggest that fenofibrate improves acetylcholine-induced dilation at lower doses partly via modulating vascular NOS and COX pathways. Considering that renal and vascular eNOS protein levels were not different between control and fenofibrate-treated obese rats, iNOS induction or decreased reactive oxygen species could contribute to the improvement of endothelial function by PPAR-α activation.

Our previous studies have shown that mean arteriolar blood pressure was mildly (10 mmHg) but significantly increased in obese Zucker rats (8). In the current study, we measured systolic blood pressure and found that fenofibrate did not change blood pressure in obese Zucker rats, suggesting a blood pressure-independent protective effect of fenofibrate on endothelial function. In agreement with previous reports (14, 30), we observed that fenofibrate reduced total body weight, plasma insulin, triglyceride, and total cholesterol, but did not decrease blood glucose levels in obese Zucker rats. Although PPAR-α activation has been shown to increase fatty acid catabolism in liver, decreasing skeletal muscle triglyceride content or decreasing production of cytokines associated with insulin resistance pathways (30, 41), the precise mechanism by which fenofibrate improves insulin resistance is not fully known yet. Previous studies with mice showed that PPAR-α activation both increases CYP4A expression and enhances hepatic lipid turnover; the latter effect removes fatty acids as substrate for lipid peroxidation and is sufficiently powerful to prevent the development of dietary steatohepatitis (22). Interestingly, in the current study, fenofibrate significantly increased renal CYP4A protein and activity in obese Zucker rats. Therefore, whether upregulation of CYP4A expression contributes to an improvement in the metabolite syndrome in this model remains to be elucidated.

Overall, this study demonstrates that fenofibrate induces CYP enzyme activity and improves endothelium-dependent dilator responses in afferent arterioles of obese Zucker rats. These findings suggest that the therapeutic improvement of PPAR-α agonist on endothelial function is in part because fibrates increase CYP-derived eicosanoid synthesis.

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