Fenofibrate and Pioglitazone Do Not Ameliorate the Altered Vascular Reactivity in Aorta of Isoproterenol-treated Rats

Lívia Emy Fukuda, Ana Paula Couto Davel, MSc,* Sidney Verissimo-Filho,† Lucia Rossetti Lopes, PhD,† Victoria Cachofeiro, PhD,‡ Vicente Lahera, PhD,‡ and Luciana Venturini Rossoni, MD, PhD**

Abstract: Chronic stimulation of β-adrenoceptors with isoproterenol induces alteration of vascular reactivity and increases local pro-inflammatory cytokines. We investigated whether fenofibrate and pioglitazone, PPAR-α and –γ agonists, respectively, improve the changes in vascular reactivity induced by isoproterenol. Wistar rats received isoproterenol (0.3 $mg \cdot kg^{-1} \cdot day^{-1}$, SC) or vehicle (CT) plus fenofibrate (α, 100 $mg \cdot kg^{-1} \cdot day^{-1}$, PO), pioglitazone (γ, 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$, PO), or water for 7 days. In aortas, isoproterenol treatment enhanced the maximal response (Rmax) to phenylephrine (10^{-10} to 10^{-4} M) compared to CT as previously demonstrated. The effects of endothelium removal (E–) or L-NAME incubation (100 μM) on the phenylephrine response were smaller in isoproterenol-treated animals compared to CT while superoxide dismutase (SOD, 150 U/mL) significantly reduced the Rmax to phenylephrine to CT levels. Neither fenofibrate nor pioglitazone changed the effects induced by isoproterenol in aorta. E–, L-NAME, or SOD effects were similar between CTα and CT. However, pioglitazone per se increased Rmax to phenylephrine (CT: 59 ± 4 versus CTγ: 72 ± 5 % of contraction to KCl). E– or L-NAME effects were reduced in CTγ compared to CT, and SOD normalized the altered reactivity to phenylephrine in the CTγ group. In conclusion, neither fenofibrate nor pioglitazone ameliorates the altered vascular reactivity present in aorta from isoproterenol-treated rats. Moreover, pioglitazone per se induced endothelial dysfunction and increased phenylephrine-induced contraction in aorta.

Key Words: isoproterenol, aorta, endothelium, PPAR, fenofibrate, pioglitazone

(*J Cardiovasc Pharmacol*™ 2008;52:413–421)

INTRODUCTION

Peroxisome proliferator-activated receptors (PPAR) are a family of 3 nuclear hormone receptors, α, β/δ, and γ, that are members of the steriod receptor superfamily.[1] Although PPAR-α is stimulated by natural ligands, such as fatty acids and eicosanoids, and by synthetic ligands, such as the lipid-lowering fibrates, activators of PPAR-γ are the insulin sensitizers known as thiazolidinediones or glitazones, including troglitazone, pioglitazone, and rosiglitazone.[2]

Until recently, the function of these receptors has concerned their ability to regulate energy balance.[2] In addition, expression of PPAR-α and -γ has been described in the cardiovascular system.[3,4] In vascular smooth muscle and endothelial cells, the activation of these nuclear receptors can suppress the production of proinflammatory cytokines and inhibit proliferation and cellular migration. Therefore, their activation seems to be associated with an antiinflammatory and antiatherosclerotic role.[5,6] However, the contribution of these compounds in modulating vascular function remains unclear, as studies describe beneficial,[7,8] innocuous,[9] and prejudicial[10] effects of PPAR agonists in the cardiovascular system.

Proinflammatory cytokines have been related to an upregulation of adhesion molecules on endothelial cells, activation of macrophages, smooth muscle cell proliferation, and vascular dysfunction.[11–13] Moreover, in cardiovascular diseases such as hypertension, atherosclerosis, and heart failure, studies demonstrate an increased production of proinflammatory cytokines in blood vessels, which could result in vascular damage.[14,15]

The prolonged stimulation of β-adrenergic receptors with isoproterenol, a non-selective β-adrenergic agonist, is a well-known experimental model of heart "injury" in rats.[16,17] In addition, Davel et al[18] describe an increased vasoconstrictor response to phenylephrine and serotonin in the aorta of isoproterenol-treated rats. This change in vascular reactivity

Received for publication March 17, 2008; accepted August 14, 2008.

From *Department of Physiological and Biophysical Sciences and †Department of Pharmacology, Institute of Biomedical Science, University of Sao Paulo, São Paulo, Brazil; and ‡Department of Physiology, Facultad de Medicina, Universidad Complutense, Madrid, Spain.

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Livia E. Fukuda was supported by a Scientific Initiation Fellowship award from FAPESP and Ana Paula Davel was supported by a PhD Fellowship award from CNPq. Luciana V. Rossoni is research fellow from CNPq.

The authors state that they have no financial interest in the products mentioned within this article.

Reprints: Luciana V. Rossoni, MD, PhD, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas I, Universidade de São Paulo, Av. Professor Lineu Prestes, 1524, sala 225, Cep: 05508-900, São Paulo, SP, Brazil (e-mail: lrossoni@icb.usp.br).

was related to altered endothelial modulation of the response to phenylephrine, mostly due to increased superoxide anion generation and reduced bioavailability of nitric oxide. In addition, we recently demonstrated that isoproterenol-treatment for 7 days enhanced RNA and protein expression of local pro-inflammatory factors in rat aortas.[19] Thus, the aim of the present study was to investigate the effects of fenofibrate, a PPAR-α agonist, and pioglitazone, a PPAR-γ agonist, in the increased vasoconstrictor response to the α-adrenergic receptor agonist phenylephrine in aortic rings from 7-day isoproterenol-treated rats.

METHODS

Animals

Three-month-old male Wistar rats (270 to 370 g) were obtained from colonies maintained at the Animal Quarters of the Institute of Biomedical Sciences of the University of Sao Paulo. The animals were housed 4 to 6 per cage at a constant room temperature and light cycle (12:12 hours light:dark). Food and water were allowed ad libitum to all animals. Care and use of laboratory animals and all experiments were conducted in compliance with the guidelines for biomedical research as stated by the Brazilian Society of Experimental Biology.

A group of rats was treated for 7 days with the nonselective β-adrenergic agonist, isoproterenol (0.3 $mg \cdot kg^{-1} \cdot day^{-1}$, SC, suspended in 0.1 mL of soy oil), and control rats received the same volume of vehicle.[18,19]

Simultaneously, both control and isoproterenol-treated rats received fenofibrate, PPAR-α agonist, (100 $mg \cdot kg^{-1} \cdot day^{-1}$, PO)[20] or pioglitazone, PPAR-γ agonist, (2.5 $mg \cdot kg^{-1} \cdot day^{-1}$, PO).[21,22] Fenofibrate was suspended in water[23] while pioglitazone hydrochloride tablets (Actos, Abbott, Brazil) were transformed into dust and then suspended in carboximethylcelulosis 0.5%.[8,9] These suspensions were administered by gavage to the animals. Thus, rats were randomly assigned to one of the following groups: control (CT), isoproterenol (ISO), control + fenofibrate (CTα), isoproterenol + fenofibrate (ISOα), control + pioglitazone (CTγ), and isoproterenol + pioglitazone (ISOγ).

Vascular Reactivity

After 7 days, all animals were weighed, anaesthetized, and killed by exsanguination. The thoracic aorta was dissected and divided into rings (4 mm), as described previously.[24] To analyze the influence of the endothelium on vascular responses, the endothelial layer was mechanically removed in some experiments by rubbing the lumen with a needle.

The rings were mounted in an isolated organ bath system containing Krebs–Henseleit bicarbonate buffer (KHB). The buffer consisted of (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$-$2H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4$-$7H_2O$ 1.2, glucose 11, and EDTA 0.01. Thoracic aorta segments were subjected to tension of 1.0 g during a 45-minute equilibration period. Isometric tension was recorded by using an isometric force transducer (Letica TRI 210, Barcelona, Spain) connected to an acquisition system (Soft & Solution, SP, Brazil).

Experimental Protocols

After 45 minutes of stabilization, the viability of vascular smooth muscle was assessed using KCl (75 mM). The maximal contraction of each ring was determined after 30 minutes of exposure to KCl (75 mM). Endothelial integrity was then tested by the response to acetylcholine (ACh, 10 μM) in segments precontracted with phenylephrine at a concentration (~10 μM) producing 50 to 70% of the contraction induced by KCl (75 mM). Concentration-response curves to phenylephrine (10^{-10} to 10^{-3} M) were determined in thoracic aortic rings with (E+) and without (E–) endothelium. The endothelium was considered intact if the aortic ring relaxed more than 90% to 10 μM ACh, and endothelial denudation was confirmed by less than 10% relaxation.

To evaluate the influence of fenofibrate or pioglitazone treatments on the role of nitric oxide and superoxide anion in the contractile response to phenylephrine, aortic rings were incubated with either the nonselective nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM) or the superoxide anion scavenger superoxide dismutase (SOD, 150 U/mL). Drugs were added 30 minutes before the concentration-response curve to phenylephrine was determined.

Drugs

Phenylephrine hydrochloride, (-)-isoproterenol, acetylcholine chloride, fenofibrate, L-NAME dihydrochloride, and SOD (bovine erythrocyte) were purchased from Sigma (St Louis, MO). Pioglitazone was purchased from Abbott (Takeda Chemical Industries, Osaka, Japan; distributed by Abbott, Brazil).

Statistical Analyses

Data were expressed as mean ± SEM and analyzed by Student *t* test and one-way or two-way ANOVA plus Tukey post-hoc test at $P < 0.05$ significance level.

The maximal contractile response to phenylephrine (Rmax) was expressed as percentage of the maximal contraction induced by KCl 75 mM. The negative log of the agonist concentrations resulting in 50% of the maximum response (pD_2) was calculated from concentration–response curves by nonlinear regression analysis of the curve using computer-based fitting program Graphpad Prism (San Diego, CA).

RESULTS

Isoproterenol, PPAR agonists, or combined treatments did not significantly modify the body weight of the animals (CT: 340 ± 49 g, n = 20; ISO: 311 ± 25 g, n = 26; CTα: 333 ± 36 g, n = 13; ISOα: 294 ± 18 g, n =13; CTγ: 336 ± 43 g, n =13; ISOγ: 327 ± 26 g, n = 14; mean ± SD, $P > 0.05$, one-way ANOVA).

Vascular Contraction to KCl

Aortas from isoproterenol-treated rats showed reduced maximal contraction to KCl compared to aortas from the control group (CT: 1.82 ± 0.10 g, n = 17 versus ISO: 1.52 ± 0.09 g, n = 19; $P < 0.05$, *t* test) as previously described.[18] This pattern of response was not modified by treatment with

fenofibrate (CTα: 2.10 ± 0.23 g, n = 9 versus ISOα: 1.60 ± 0.11 g, n = 6; $P < 0.05$, *t* test) or pioglitazone (CTγ: 1.83 ± 0.10 g, n = 13 versus ISOγ: 1.50 ± 0.07 g, n = 14; $P < 0.05$, *t* test).

Endothelium-dependent Relaxation to ACh

To evaluate the endothelium-dependent relaxation response to ACh, aortic rings were precontracted with phenylephrine to a level reaching 50 to 70% of the maximal contraction to KCl (75 mM). As demonstrated in Table 1, the phenylephrine-induced plateau was not significantly different among the groups studied. In addition, there were no changes in ACh (10 μM)–induced relaxation between control and isoproterenol-treated rats (Table 1), as previously demonstrated.[18] Fenofibrate or pioglitazone did not modify the ACh-induced relaxation in aortas from either the control or isoproterenol-treated rats (Table 1).

Effects of Fenofibrate and Pioglitazone Treatment in the Vasoconstrictor Response to Phenylephrine in Aortas from Control and Isoproterenol-treated Rats

Aortic rings from isoproterenol-treated rats showed a greater maximal vasoconstrictor response (Rmax) to phenylephrine than the control group (Table 2, Figure 1A) without significant changes in pD_2 (Table 2). Neither fenofibrate (Figure 1B) nor pioglitazone (Figure 1C) treatment prevented the increased Rmax to phenylephrine induced by isoproterenol (Table 2).

In control rats, fenofibrate did not modify the Rmax or pD_2 to phenylephrine (Table 2). However, pioglitazone per se increased the Rmax to this α-adrenoceptor agonist in aorta from control animals (Table 2). Treatment with PPAR agonists did not alter the pD_2 to phenylephrine in any group studied (Table 2).

Effects of Fenofibrate and Pioglitazone on the Endothelial Modulation of the Vasoconstrictor Response to Phenylephrine in Aortas from Control and Isoproterenol-treated Rats

Removal of the endothelium enhanced the Rmax and pD_2 to phenylephrine in aortic rings from all groups studied (Figure 2 and Table 2).

In the absence of the endothelium, there were no differences between the control and isoproterenol-treated groups (Table 2, Figures 2A and 2B), indicating that aortas from the isoproterenol-treated group show impaired endothelial modulation of phenylephrine-induced contraction in comparison to controls. Neither fenofibrate (Figure 2D) nor pioglitazone (Figure 2F) was able to reverse this effect of isoproterenol treatment on the endothelial modulation of phenylephrine-induced contraction.

In control animals, fenofibrate treatment did not induce changes in endothelial modulation of the vasoconstrictor response to phenylephrine (Table 2, Figures 2A and 2C). However, pioglitazone impaired the endothelial modulation in comparison to nontreated control animals (Table 2, Figures 2A and 2E).

Involvement of NO and Superoxide Anion in the Vasoconstrictor Response to Phenylephrine in Aortic Rings

When the vascular rings were incubated with L-NAME, it was observed that, in a similar manner to endothelial removal, L-NAME enhanced both pD_2 and Rmax to phenylephrine in all groups studied (Table 2 and Figure 3). After L-NAME incubation, there were no differences in phenylephrine-induced contraction between aortas from control and isoproterenol-treated rats (Table 2, Figures 3A and 3B).

Neither fenofibrate nor pioglitazone was able to reverse the reduced effect of L-NAME on the contraction induced by phenylephrine in aortas of isoproterenol-treated animals (Figures 3D and 3F). Fenofibrate treatment did not significantly modify the influence of L-NAME in control animals (Table 2, Figures 3A and 3C). However, pioglitazone per se reduced the effect of L-NAME on phenylephrine-induced contraction (Table 2, Figures 3A and 3E).

SOD incubation had no effect in aortas from control animals (Table 2, Figure 4A), but it significantly reduced the Rmax to phenylephrine in the isoproterenol-treated group (Table 2, Figure 4B). SOD did not modulate the phenylephrine-induced contraction in control rats treated with fenofibrate (Table 2, Figure 4C). However, the increased Rmax to phenylephrine in control animals treated with pioglitazone was completely reversed by SOD (Table 2, Figure 4E). This indicates a pronounced superoxide-dependent effect in the phenylephrine-induced contraction of aortas from pioglitazone-treated animals. In addition, Rmax to phenylephrine in the presence of SOD was similar among ISO, ISOα, and ISOγ, suggesting that neither fenofibrate nor pioglitazone was able to modulate the generation of superoxide anions induced by isoproterenol-treatment of isolated rat aorta (Table 2, Figure 4).

TABLE 1. Values of Phenylephrine (PHE)–induced Plateaus and the Maximal Relaxation to Acetylcholine (ACh) in Segments of Thoracic Aorta from Wistar Rats That Received Vehicle (CT) or Isoproterenol (ISO) and Were Co-treated With Fenofibrate (α) or Pioglitazone (γ) for 7 Days

	CT	CTα	CTγ	ISO	ISOα	ISOγ
PHE-induced plateaus (g)	1.82 ± 0.06	2.11 ± 0.17	2.20 ± 0.10	2.04 ± 0.09	2.25 ± 0.29	1.98 ± 0.09
ACh (%)	90 ± 1.1%	92 ± 1.6%	93 ± 1.1%	93 ± 1.2%	93 ± 2.7%	90 ± 1.3%
N	16	9	12	18	8	12

$P > 0.05$; 1-way ANOVA. N is the number of animals used in each group.

TABLE 2. Effects of Endothelium Denudation (E–) or Intact Endothelium (E+) and L-NAME or Superoxide Dismutase (SOD) Incubation on Maximal Response (Rmax) and Sensitivity (pD2) to Phenylephrine in Segments of Thoracic Aorta from Wistar Rats Which Received Vehicle (CT) or Isoproterenol (ISO) and That Were Co-treated With Fenofibrate (α) or Pioglitazone (γ) for 7 Days

	CT		CTα		CTγ	
	Rmax	pD_2	Rmax	pD_2	Rmax	pD_2
E+	59 ± 4	6.65 ± 0.08	50 ± 5	6.43 ± 0.20	72 ± 5*	6.76 ± 0.09
E−	122 ± 4‡	7.36 ± 0.10‡	128 ± 7‡	7.32 ± 0.17‡	118 ± 4‡	7.41 ± 0.12‡
E+/L-NAME	132 ± 7‡	7.11 ± 0.08‡	148 ± 8‡	7.06 ± 0.07‡	139 ± 5‡	7.20 ± 0.11‡
E+/SOD	50 ± 7	6.52 ± 0.08	46 ± 8	6.43 ± 0.15	53 ± 6‡	6.36 ± 0.12

DISCUSSION

We observed that fenofibrate and pioglitazone, PPAR-α and -γ agonists, respectively, could not reverse the enhanced vasoconstrictor response to the α-adrenoceptor agonist phenylephrine or the NO-superoxide anion imbalance present in aortas from 7-day isoproterenol-treated rats. In addition, pioglitazone treatment per se increased the vascular reactivity to phenylephrine and induced local oxidative stress in control aortas.

In certain cardiovascular diseases, such as essential hypertension and atherosclerosis, endothelial dysfunction is characterized by an increased response to vasoconstrictor stimulus and/or by impaired endothelium-dependent relaxation. This is also associated with an increased production of cytokines and other inflammatory mediators.[14,15] As reported by Davel et al[18,19] and in the present study, chronic β-adrenergic stimulation with isoproterenol for 7 days induces hyperreactivity to phenylephrine in thoracic aortic rings from rats without changes in the endothelium-dependent relaxation induced by ACh. In the absence of endothelium, contraction to the α-adrenergic agonist was similar between control and isoproterenol-treated groups, indicating that aortic rings from isoproterenol-treated animals exhibit reduced endothelial modulation in the response to phenylephrine. In addition, we have previously demonstrated that the enhanced contraction to phenylephrine and the endothelial dysfunction in isoproterenol-treated animals are associated with an incremented gene and protein expression of IL-1β and IL-6 via activation of the nuclear factor (NF)-κB in rat aortas.[19]

Previous studies suggest that PPAR-α and PPAR-γ ligands are able to inhibit synthesis of proinflammatory mediators.[25–27] This effect of PPAR activation in vascular cells seems to be due, at least partially, to inhibition of NF-κB.[28,29] In addition, some studies have shown that PPAR agonists can also prevent endothelial dysfunction.[20,30,31]

In the present study, we investigated whether treatment with PPAR-α and -γ agonists could ameliorate the impaired endothelial modulation of phenylephrine-induced contraction in aortas of isoproterenol-treated rats. We observed that co-treatment of isoproterenol with fenofibrate or pioglitazone did not reverse the altered vascular reactivity and impaired endothelial modulation observed in animals treated only with isoproterenol. In contrast, some studies have demonstrated a beneficial role for PPAR agonists in experimental models of cardiovascular diseases. Diep et al[30] showed that treatment with the PPARα agonist docosahexaenoic acid for 7 days could prevent blood pressure elevation and improve vascular dysfunction in angiotensin II–infused rats. This effect was associated with a reduction of ROS generation and inflammation of the vascular wall. It was also reported by Diep et al[32] that treatment with pioglitazone (10 $mg \cdot kg^{-1} \cdot day^{-1}$) or rosiglitazone (5 $mg \cdot kg^{-1} \cdot day^{-1}$) for 7 days attenuated the development of hypertension, corrected vascular structural abnormalities, and improved endothelial dysfunction induced by angiotensin II infusion. In the present study, rats were co-treated with isoproterenol and fenofibrate (100 $mg \cdot kg^{-1} \cdot day^{-1}$) or with pioglitazone (2.5 $mg \cdot kg^{-1} \cdot day^{-1}$) for 7 days, which is comparable to doses used in previous studies. However, we did not find any beneficial vascular effects of PPAR agonists in this experimental model.

Our results are in contrast to other results obtained for PPAR agonists in the cardiovascular system. This could be explained by differences in the experimental designs of each study, regardless of the presence of metabolic disorders or hypertension. It is possible that different kinds of PPAR

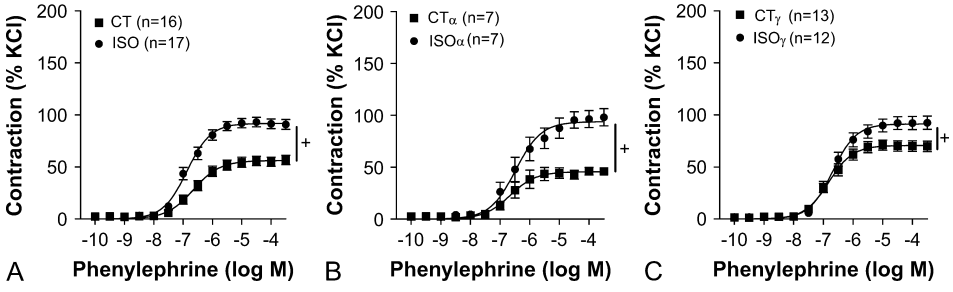

FIGURE 1. Concentration-response curves to phenylephrine in thoracic aortic rings from Wistar rats that (A) received vehicle (CT) or isoproterenol subcutaneously for 7 days (ISO) and were co-treated with (B) the PPAR-α agonist fenofibrate (α) or with (C) the PPAR-γ agonist pioglitazone (γ). Results (means ± SEM) are expressed as a percentage of the response to 75 mM KCl in each case. Number of animals is indicated in parentheses for each group. $^{+}P < 0.05$ versus respective CT, two-way ANOVA.

TABLE 2. (*Continued*) Effects of Endothelium Denudation (E–) or Intact Endothelium (E+) and L-NAME or Superoxide Dismutase (SOD) Incubation on Maximal Response (Rmax) and Sensitivity (pD2) to Phenylephrine in Segments of Thoracic Aorta from Wistar Rats Which Received Vehicle (CT) or Isoproterenol (ISO) and That Were Co-treated With Fenofibrate (α) or Pioglitazone (γ) for 7 Days

	ISO		ISOα		ISOγ	
	Rmax	pD_2	Rmax	pD_2	Rmax	pD_2
E+	94 ± 5*	6.81 ± 0.08	98 ± 8†	6.38 ± 0.21	94 ± 6 &	6.63 ± 0.10
E−	121 ± 3‡	7.33 ± 0.09‡	117 ± 3‡	7.24 ± 0.11‡	117 ± 2‡	7.35 ± 0.07‡
E+/L-NAME	145 ± 6‡	7.14 ± 0.09‡	143 ± 4‡	7.09 ± 0.23‡	134 ± 6‡	7.08 ± 0.08‡
E+/SOD	65 ± 5‡	6.50 ± 0.11	74 ± 6‡	6.34 ± 0.13	74 ± 5‡	6.39 ± 0.07

Values represent mean ± SEM. One-way ANOVA: *$P < 0.05$ versus CT; †$P < 0.05$ versus CTα; and $P < 0.05$ versus CTγ; ‡$P < 0.05$ versus E+. N = 7 to 12.

ligands at different doses and used for different treatment periods could induce varying effects. Another feature that could explain the differing results is the route of PPAR agonist administration. Here, pioglitazone and fenofibrate were administrated by gavage, as in the study reported by Frantz et al.[9] Others investigators[7,32] have used a dietary supplement allowing different peak and steady-state concentrations.[33]

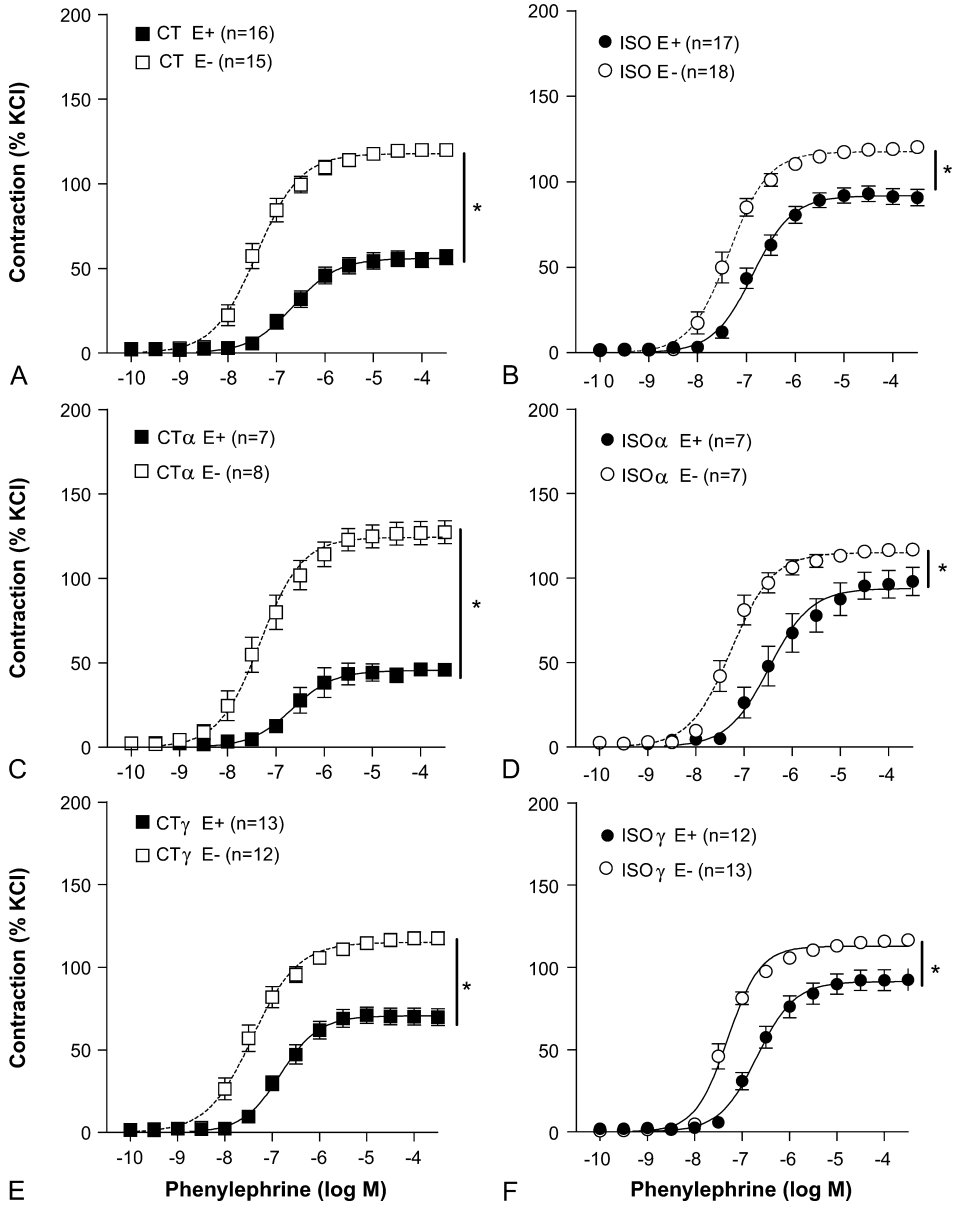

FIGURE 2. Concentration-response curves to phenylephrine in intact (E+) and endothelium-denuded (E–) thoracic aortic rings from Wistar rats that received (A) vehicle (CT) or (B) isoproterenol subcutaneously for 7 days (ISO) and that were co-treated with (C and D) the PPAR-α agonist fenofibrate (α) or with (E and F) the PPAR-γ agonist pioglitazone (γ). Results (mean ± SEM) are expressed as a percentage of the response to 75 mM KCl in each case. Number of animals is indicated in parentheses for each group. *$P < 0.05$ versus respective E+, two-way ANOVA.

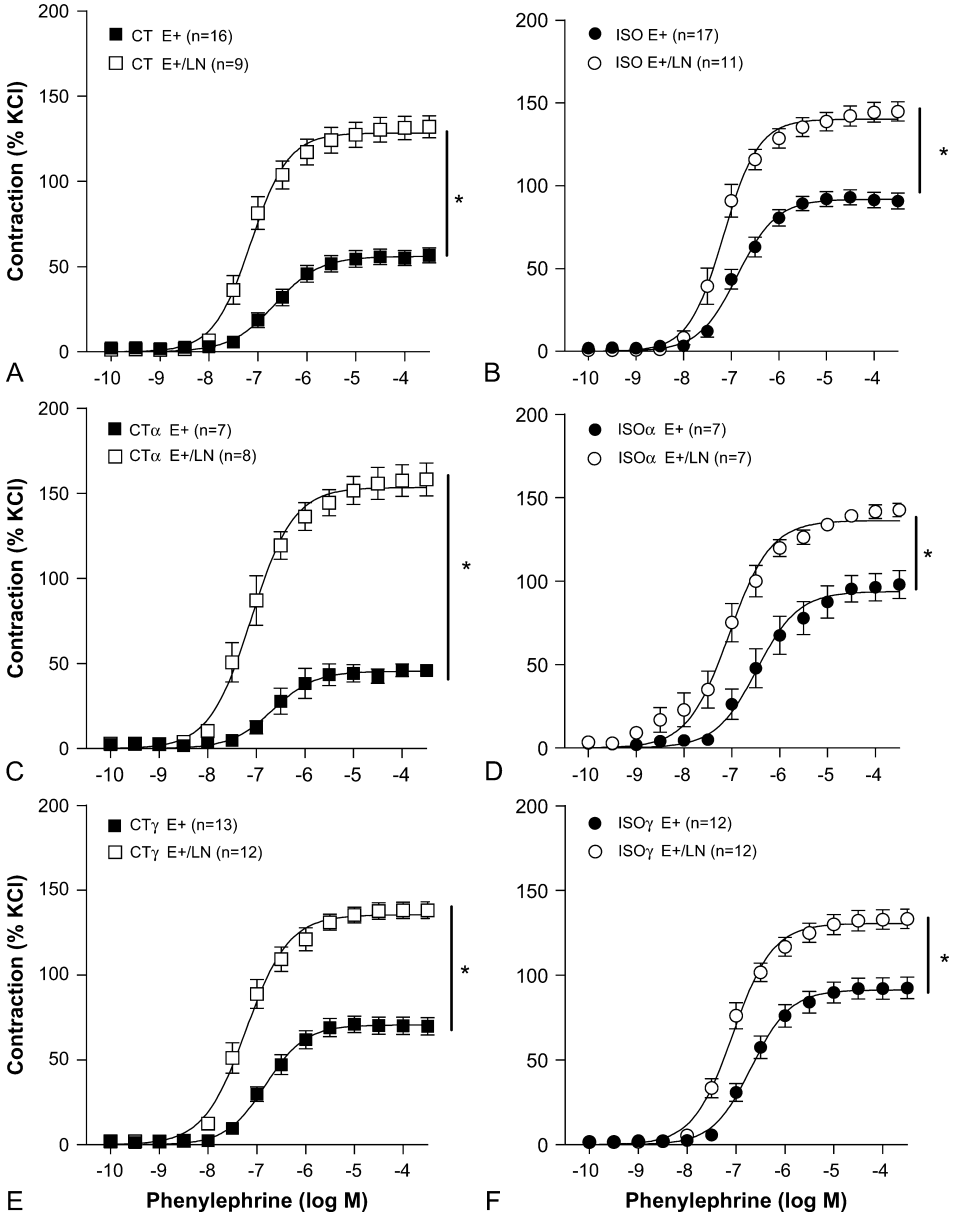

FIGURE 3. Effect of L-NAME incubation (LN, 100 μM) on concentration-response curve to phenylephrine in thoracic aorta rings from Wistar rats that received (A) vehicle (CT) or (B) isoproterenol subcutaneously for 7 days (ISO) and that were co-treated with (C and D) the PPAR-α agonist fenofibrate (α) or with (E and F) the PPAR-γ agonist pioglitazone (γ). Results (mean ± SEM) are expressed as a percentage of the response to 75 mM KCl in each case. Number of animals is indicated in parentheses for each group. $^{*}P < 0.05$ versus respective E+, two-way ANOVA.

In line with our results, Frantz et al[9] showed that pioglitazone treatment (20 $mg \cdot kg^{-1} \cdot day^{-1}$) was not able to decrease the vasoconstrictor response induced by phenylephrine or alter the impaired relaxation to ACh and sodium nitroprusside in isolated aortas from mice after coronary artery ligation. The authors also demonstrated that pioglitazone treatment did not change mortality, ventricular dilatation, and collagen deposition in infarcted animals. Similar to the myocardial infarction model induced by coronary artery ligation, chronic isoproterenol-treatment is accompanied by pronounced β-adrenergic stimulation without development of hypertension, suggesting similarities in the neurohumoral pathways involved in the mechanisms that induce vascular dysfunction in these models.

The increased vasoconstrictor response of aortic rings from isoproterenol-treated animals compared to controls was associated with reduced NO bioavailability, as incubation with L-NAME abolished the difference between control and isoproterenol-treated rats. Neither fenofibrate nor pioglitazone was able to change the effect of L-NAME in the isoproterenol-treated animals. Together, these data suggest that neither PPAR agonist was able to improve the reduced NO bioavailability induced by chronic isoproterenol treatment.

In contrast to the present results, it has been reported that PPAR-α and -γ agonists can increase NO bioavailability in cultured cells.[34–36] In vivo, PPAR-α and -γ agonists have been shown to reduce superoxide generation, restore endothelial dysfunction, and improve vasorelaxation to ACh in aorta of diabetic rats.[8,37] However, Blanco-Rivero et al[10] describe how, despite increasing NO production, fenofibrate can induce endothelial dysfunction in a time-dependent manner. The authors observed that after 6 weeks of fenofibrate treatment

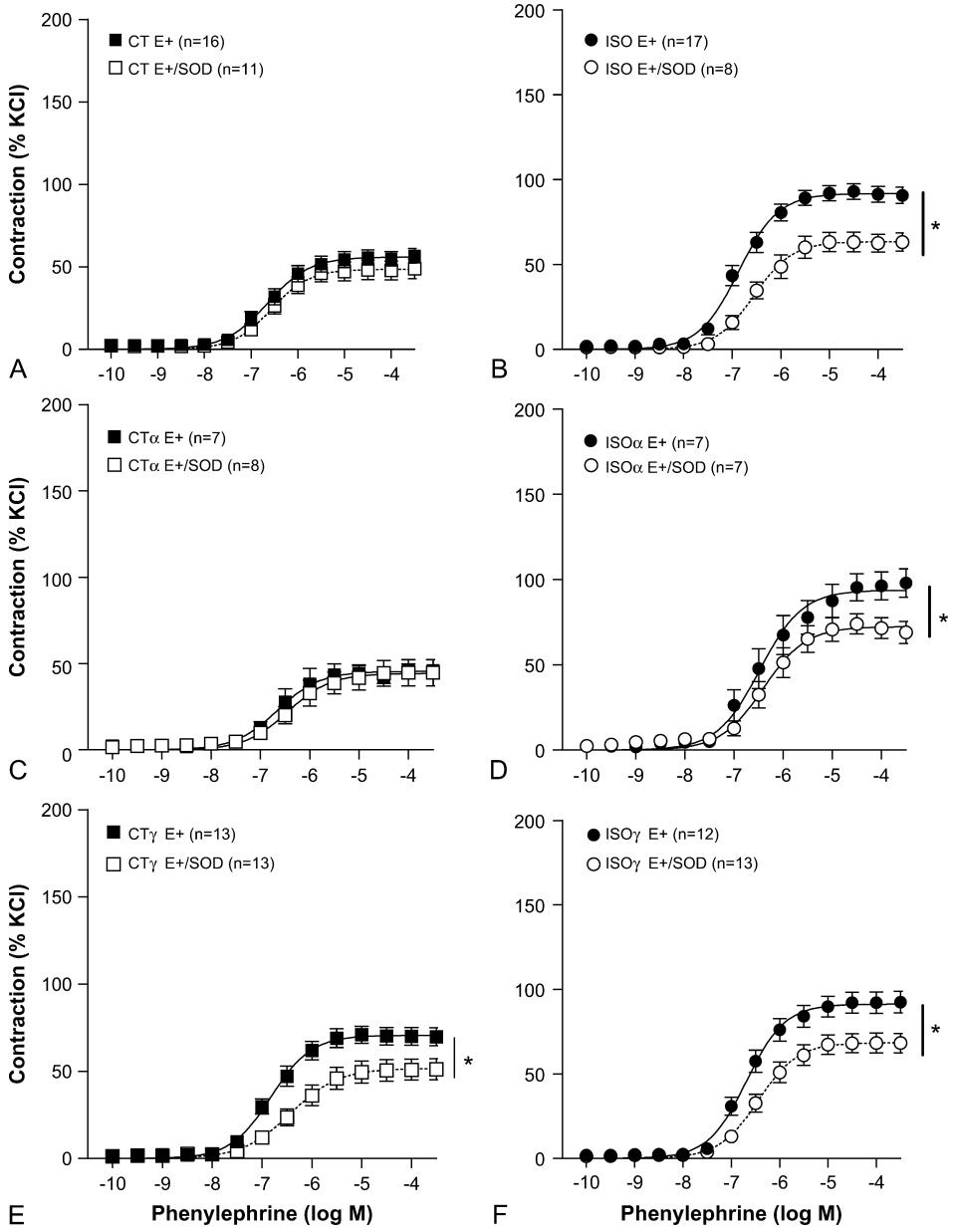

FIGURE 4. Effect of superoxide dismutase incubation (SOD, 150 U/mL) on concentration-response curve to phenylephrine in segments of thoracic aorta from Wistar rats that received (A) vehicle (CT) or (B) isoproterenol subcutaneously for 7 days (ISO) and that were co-treated with (C and D) the PPAR-α agonist fenofibrate (α) or with (E and F) the PPAR-γ agonist pioglitazone (γ). Results (mean ± SEM) are expressed as a percentage of the response to 75 mM KCl in each case. Number of animals is indicated in parentheses for each group. $^{*}P < 0.05$ versus respective E+, two-way ANOVA.

(100 $mg \cdot kg^{-1} \cdot day^{-1}$), the endothelium-dependent relaxation to ACh were reduced in isolated rat aortic rings. The effects of chronic treatment with PPAR ligands in the cardiovascular system are therefore still controversial.

Incubation of aorta with SOD did not alter contraction to phenylephrine in the control group, but this contraction was found to be reduced in isoproterenol-treated rats. In addition, Davel et al[18] demonstrated that these isoproterenol-treated animals show elevated levels of superoxide generation in the aorta. The increased superoxide generation could explain the reduced NO bioavailability because the O_2^- radical may have several effects either directly or indirectly through the generation of other radicals, such as $ONOO^-$. This can lead to rapid inactivation of NO, leading to endothelial dysfunction.[38,39]

In the present study, neither fenofibrate nor pioglitazone were able to reverse the superoxide-dependent contraction induced by isoproterenol treatment. In contrast, some studies describe an increase in gene and protein expression for Cu/Zn-SOD and a suppression of NADPH oxidase by PPAR agonists in endothelial cells.[40,41] Likewise, rosiglitazone and fenofibrate were able to prevent the vascular increase in superoxide anion production in DOCA-salt animals.[7] However, there are some studies demonstrating oxidative and apoptotic effects of glitazones.[42,43] In the present study, the results also suggest an oxidative effect of pioglitazone per se in aortas from isoproterenol-treated rats that seems to increase the vasoconstrictor response to phenylephrine. On the other hand, fenofibrate-treatment for 7 days did not significantly modify

the vascular reactivity to phenylephrine in aorta from control animals, in line with recent results from Blanco-Rivero et al.[10]

The PROspective pioglitAzone Clinical Trial In macro-Vascular Events (PROACTIVE) recently showed that treatment with the PPAR-γ agonist results in a reduction in coronary and stroke events in diabetic and hypertensive patients. However, this benefit was counterbalanced by an increase in congestive heart failure as well as symptomatic edema.[44] It is possible that, the beneficial vascular role of PPARs is more prominent in vascular diseases associated with lipid metabolism and energy balance disorders, such as atherosclerosis,[45] diabetes mellitus,[8,37] and obesity.[46,47] In addition, FIELDS study showed nonsignificant alterations in thrombotic events and coronary and sudden deaths in patients treated with fenofibrate.[44] A link between rosiglitazone and a significantly increased risk of myocardial infarction has also been demonstrated.[48]

It is known that many patients have baseline cardiovascular alterations and take fenofibrate and/or pioglitazone as anti-lipidemic and anti-diabetic drugs, respectively. Our findings reinforce the idea that PPAR protective effects cannot be uniformly observed and warn that extrapolation of the protective properties of those compounds to any clinical employment must be done with caution, and further study is required to clarify the effects of PPAR agonists in the cardiovascular system.

CONCLUSION

In summary, neither fenofibrate nor pioglitazone ameliorates the increased vasoconstrictor response to phenylephrine and the NO/O_2^- imbalance present in aorta from 7-day isoproterenol-treated rats. Moreover, pioglitazone treatment per se increased the contraction to phenylephrine in aortas, probably related to increased O_2^- levels associated with reduced NO bioavailability.

ACKNOWLEDGMENT

We gratefully acknowledge Natasha Hausman for helpful suggestions in the preparation of the article.

REFERENCES

1. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*. 1990;347: 645–650.
2. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev.* 1999;20:649–688.
3. Braissant O, Foufelle F, Scotto C, et al. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology.* 1996; 137:354–366.
4. Marx N, Duez H, Fruchart JC, et al. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res*. 2004;94:1168–1178.
5. Bishop-Bailey D. Peroxisome proliferator-activated receptors in the cardiovascular system. *Br J Pharmacol*. 2000;129:823–834.
6. Schiffrin EL. Peroxisome proliferator-activated receptors and cardiovascular remodeling. *Am J Physiol Heart Circ Physiol.* 2005;288:H1037–H1043.
7. Iglarz M, Touyz RM, Amiri F, et al. Effect of peroxisome proliferator-activated receptor-alpha and -gamma activators on vascular remodeling in endothelin-dependent hypertension. *Arterioscler Thromb Vasc Biol*. 2003; 23:45–51.
8. Majithiya JB, Paramar AN, Balaraman R. Pioglitazone, a PPARgamma agonist, restores endothelial function in aorta of streptozotocin-induced diabetic rats. *Cardiovasc Res*. 2005;66:150–161.
9. Frantz S, Hu K, Widder J, et al. Peroxisome proliferator activated-receptor agonism and left ventricular remodeling in mice with chronic myocardial infarction. *Br J Pharmacol*. 2004;141:9–14.
10. Blanco-Rivero J, Marquez-Rodas I, Xavier FE, et al. Long-term fenofibrate treatment impairs endothelium-dependent dilation to acetylcholine by altering the cyclooxygenase pathway. *Cardiovasc Res*. 2007; 75:398–407.
11. von der Thusen JH, Kuiper J, van Berkel TJ, et al. Interleukins in atherosclerosis: molecular pathways and therapeutic potential. *Pharmacol Rev.* 2003;55:133–166.
12. Vila E, Salaices M. Cytokines and vascular reactivity in resistance arteries. *Am J Physiol Heart Circ Physiol*. 2005;288:H1016–H1021.
13. Jimenez-Altayo F, Briones AM, Giraldo J, et al. Increased superoxide anion production by interleukin-1beta impairs nitric oxide-mediated relaxation in resistance arteries. *J Pharmacol Exp Ther*. 2006;316:42–52.
14. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999; 340:115–126.
15. Sanz-Rosa D, Oubina MP, Cediel E, et al. Effect of AT1- receptor antagonis on vascular and circulating inflammatory mediators in SHR: role of NF-κB/ iκB system. *Am J Physiol Heart Circ Physiol*. 2005;288: H111–H115.
16. Rona G, Chappel CT, Balazs T, et al. An infarct like myocardial lesion and other true menifestation produced by isoproterenol in the rat. *Arch Path*. 1959;67:443–435.
17. Vassallo DV, Vasquez EC, Cabral AM. Contractile performance of papillary muscles of renovascular hypertensive and isoproterenol-pretreated rats. *Pharmacol Res Commun*. 1988;20:61–72.
18. Davel AP, Kawamoto EM, Scavone C, et al. Changes in vascular reativicty following administration of isoproterenol for one week: A role for endothelial modulation. *Br J Pharmacol*. 2006;148:629–639.
19. Davel AP, Fukuda LE, Sá LL, et al. Effects of isoproterenol-treatment for 7 days on inflammatory mediators in rat aorta. *Am J Physiol Heart Circ Physiol*. 2008;295:H211–H219.
20. Diep QN, Benkirane K, Amiri F, et al. PPAR-α activator fenofibrate inhibits myocardial inflammation and fibrosis in angiotensin II-infused rats. *J Mol Cell Cardiol*. 2004;36:295–304.
21. Nagisa Y, Kato K, Watanabe K, et al. Changes in glycated haemoglobin levels in diabetic rats measured with an automatic affinity HPLC. *Clin Exp Pharmacol Physiol*. 2003;30:752–758.
22. Gaillard V, Casellas D, Seguin-Devaux C, et al. Pioglitazone improves aortic wall elasticity in a rat model of elastocalcinotic arteriosclerosis. *Hypertension*. 2005;46:372–379.
23. Desouza CV, Murthy SN, Diez J, et al. Differential effects of peroxisome proliferator activator receptor-alpha and gamma ligands on intimal hyperplasia after balloon catheter-induced vascular injury in Zucker rats. *J Cardiovasc Pharmacol Ther.* 2003;8:297–305.
24. Rossoni LV, Salaices M, Miguel M, et al. Ouabain-induced hypertension is accompanied by increases in endothelial vasodilator factors. *Am J Physiol Heart Circ Physiol.* 2002;283:H2110–H2118.
25. Staels B, Koenig W, Habib A, et al. Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. *Nature*. 1998;393:790–793.
26. Goetze S, Xi XP, Kawano H, et al. PPAR gamma-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells. *J Cardiovasc Pharmacol*. 1999;33:798–806.
27. Takano H, Nagai T, Asakawa M, et al. Peroxisome proliferator-activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor-alpha expression in neonatal rat cardiac myocytes. *Circ Res*. 2000; 87:596–602.
28. Delerive P, De Bosscher K, Besnard S, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem*. 1999;274:32048–32054.
29. De Martin R, Hoeth M, Hofer-Warbinek R, et al. The transcription factor NF-kappa B and the regulation of vascular cell function. *Arterioscler Thromb Vasc Biol*. 2000;20:E83–E88.
30. Diep QN, Amiri F, Touyz RM, et al. PPARalpha activator effects on Ang II-induced vascular oxidative stress and inflammation. *Hypertension*. 2002;40:866–871

31. De Ciuceis C, Amiri F, Iglarz M, et al. Synergistic vascular protective effects of combined low doses of PPARalpha and PPARgamma activators in angiotensin II-induced hypertension in rats. *Br J Pharmacol*. 2007;151: 45–53.
32. Diep QN, Mabrouk ME, Cohn JS, et al. Structure, Endothelial Function, Cell Growth, and Inflammation in Blood Vessels of Angiotensin II–Infused Rats Role of Peroxisome Proliferator–Activated Receptor-γ. *Circulation*. 2002;105:2296–2302.
33. Kapetanovic IM, Krishnaraj R, Martin-Jimenez T, et al. Effects of oral dosing paradigms (gavage versus diet) on pharmacokinetics and pharmacodynamics. *Chem Biol Interact*. 2006;164:68–75.
34. Calnek DS, Mazzella L, Roser S, et al. Peroxisome proliferator-activated receptor gamma ligands increase release of nitric oxide from endothelial cells. *Arterioscler Thromb Vasc Biol*. 2003;23:52–57.
35. Goya K, Sumitani S, Xu X, et al. Peroxisome proliferator-activated receptor alpha agonists increase nitric oxide synthase expression in vascular endothelial cells. *Arterioscler Thromb Vasc Biol*. 2004;24:658–663.
36. Wang Y, Wang Y, Yang Q, et al. Effects of bezafibrate on the expression of endothelial nitric oxide synthase gene and its mechanisms in cultured bovine endothelial cells. *Atherosclerosis*. 2006;187:265–273.
37. Kanie N, Matsumoto T, Kobayashi T, et al. Relationship between peroxisome proliferator-activated receptors (PPAR alpha and PPAR gamma) and endothelium-dependent relaxation in streptozotocin-induced diabetic rats. *Br J Pharmacol*. 2003;140:23–32.
38. Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. *Am J Physiol Regul Integr Comp Physiol*. 2004;287:R1014–R1030.
39. Landmesser U, Harrison DG, Drexler H. Oxidant stress-a major cause of reduced endothelial nitric oxide availability in cardiovascular disease. *Eur J Clin Pharmacol*. 2006;62(Suppl 13):13–19.
40. Inoue I, Goto S, Matsunaga T, et al. The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma increase Cu2+, Zn2+-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. *Metabolism*. 2001;50: 3–11.
41. Hwang J, Kleinhenz DJ, Lassegue B, et al. Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. *Am J Physiol Cell Physiol*. 2005;288:C899–C905.
42. Gouni-Berthold I, Berthold HK, Weber AA, et al. Troglitazone and rosiglitazone induce apoptosis of vascular smooth muscle cells through an extracellular signal-regulated kinase-independent pathway. *Naunyn Schmiedebergs Arch Pharmacol*. 2001;363:215–221.
43. Narayanan PK, Hart T, Elcock F, et al. Troglitazone-induced intracellular oxidative stress in rat hepatoma cells: A flow cytometric assessment. *Cytometry A*. 2003;52A:28–35.
44. Robinson JG. Update on PPAR agonists: The clinical significance of FIELD and PROACTIVE. *Curr Atheroscler Rep*. 2007;9:64–71.
45. Corti R, Osende J, Hutter R, et al. Fenofibrate induces plaque regression in hypercholesterolemic atherosclerotic rabbits: In vivo demonstration by high-resolution MRI. *Atherosclerosis*. 2007;190:106–113.
46. Walker AB, Chattington PD, Buckingham RE, et al. The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats. *Diabetes*. 1999; 48:1448–1453.
47. Naderali EK, Fatani S, Williams G. Fenofibrate lowers adiposity and corrects metabolic abnormalities, but only partially restores endothelial function in dietary obese rats. *Atherosclerosis*. 2004;177:307–312.
48. Nissen SE, Wolski K. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. *N Engl J Med*. 2007; 356:2457–2471.

