Original Article

Cholesterol Reduction and Atherosclerosis Inhibition by Bezafibrate in Low-Density Lipoprotein Receptor Knockout Mice

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Fibrates, peroxisome proliferator–activated receptor α agonists, are widely used as lipid-lowering agents with anti-atherogenic activity. However, conflicting results have been reported with regard to their pharmacological effects on plasma lipoprotein profiles as well as on atherosclerosis in animal models. Furthermore, the anti-atherogenic effects of bezafibrate, one of the most commonly used fibrates, in animal models have not been reported. In the present study, we investigated the effects of bezafibrate on lipoprotein profiles as well as on atherosclerosis in low-density lipoprotein receptor knockout (LDLR–/–) mice fed an atherogenic diet for 8 weeks. Bezafibrate decreased plasma levels of both cholesterol and triglycerides (TG), while increasing plasma levels of high-density lipoprotein-cholesterol (HDL-C). Since hepatic TG production was significantly reduced in the bezafibrate-treated mice lacking LDLR, the plasma lipid–lowering effects of bezafibrate might be primarily mediated by the suppression of hepatic production of apolipoprotein-B–containing lipoproteins. In parallel with the reduced ratio of non-HDL-C to HDL-C, bezafibrate suppressed fatty streak lesions in the aortic sinus by 51%. To determine whether or not bezafibrate directly alters the expression of genes relevant to atherosclerosis, we measured mRNA expression levels of three genes in the aorta by real-time PCR: ATP-binding cassette transporter A1, lipoprotein lipase, and monocyte chemoattractant protein-1. The results showed that there were no differences in the expression of these genes between mice treated with bezafibrate and those not. In conclusion, bezafibrate inhibits atherosclerosis in LDLR–/– mice primarily by decreasing the ratio of non-HDL-C to HDL-C. (*Hypertens Res* 2008; 31: 999–1005)

***Key Words*: fibrates, cholesterol, atherosclerosis, lipoproteins, receptor**

Introduction

Fibrates are widely used as lipid-lowering drugs for patients with hypertriglyceridemia or mixed hyperlipidemia (*1*). The lipid-lowering effects of fibrates occur mainly through the activation of peroxisome proliferator–activated receptor α (PPAR-α), a member of the nuclear hormone–receptor superfamily, which governs the transcriptional activity of genes involved in lipid and lipoprotein metabolism (*2*). Fibrates lower plasma triglyceride (TG) levels by stimulating the plasma clearance of TG-rich lipoproteins as well as by inhib-

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iting hepatic fatty acid synthesis (*3*, *4*). The stimulation of the catabolism of TG-rich lipoproteins is caused not only by upregulation of hepatic lipoprotein lipase (LPL) activity (*5*–*7*) but also by decreased synthesis of apolipoprotein (apo) CIII (*8*), which has an inhibitory effect on LPL activity (*9*). Furthermore, fibrates increase plasma levels of high-density lipoprotein-cholesterol (HDL-C) in humans (*10*), conceivably by stimulating the expression of apolipoprotein (apo)A-I and A-II genes in the liver (*11*, *12*).

In addition to organs involved in plasma lipoprotein metabolism, PPAR-α is highly expressed in vascular cells, such as macrophages, endothelial cells, and smooth muscle cells (*13*–*15*). Some fibrates may exert anti-atherogenic and anti-inflammatory effects by directly activating PPAR-α in these vascular wall cells (*16*). Therefore, fibrates may act both systemically and locally to influence lesion development.

However, fibrates do not consistently show either lipid-lowering or anti-atherogenic effects in murine models. For example, fenofibrate inhibited atherosclerosis in apoE knockout (apoE−/−) mice only when these animals harbored a fenofibrate-inducible transgene for human apoA-I (*17*). Paradoxically, ciprofibrate aggravated atherosclerosis in either low-density lipoprotein receptor knockout (LDLR−/−) mice or apoE−/− mice (*18*, *19*). In accord with this, PPAR-α knockout mice developed less severe atherosclerosis, although they had a more atherogenic lipoprotein profile and presumably have lost any potential beneficial vascular effects of PPAR-α (*20*). Therefore, the clinical efficacy of fibrates has not been recapitulated, at least in murine models of atherosclerosis. It is also of note that no experiments using bezafibrate in mice have been reported, despite ample evidence of its clinical usefulness (*21*, *22*).

These considerations prompted us to examine the effects of bezafibrate on lipoprotein profiles as well as on atherosclerosis in LDLR−/− mice. We also measured the expression of genes relevant to atherosclerosis in the aorta to determine whether or not bezafibrate has direct vascular effects.

Methods

Mice and Diets

Mice were maintained in a temperature-controlled (25°C) facility with a 12 h light/dark cycle and given free access to food and water. LDLR−/− mice (*23*) had been backcrossed to C57BL/6 for 6 generations. Two diets were used: the Paigen diet (*24*), which was normal chow (MF Diet from Oriental Yeast, Tokyo, Japan) supplemented with 15% (w/w) fat, 1.25% (w/w) cholesterol, and 0.5% (w/w) cholic acid with or without 0.1% (w/w) bezafibrate (Kissei Pharmaceutical, Matsumoto, Japan). At 4–6 months of age, male LDLR−/− mice were fed the Paigen diet with or without 0.1% (w/w) bezafibrate for 8 weeks. Mice were anesthetized with pentobarbital before sacrifice. These experiments were performed in accord with the institutional guidelines for animal experiments at Jichi Medical University School of Medicine.

Table 1. Plasma Levels of Lipids, Glucose, Insulin and Adiponectin

	Bezafibrate	
	− (n=14)	+ (n=11)
Total cholesterol (mg/dL)	2,628±381	2,009±424*
TG (mg/dL)	111±50	35±25*
Glucose (mg/dL)	56±12	64±12
Insulin (μg/dL)	581±522	919±576
Adiponectin (μg/mL)	15.5±3.2	15.8±3.1

After a 16-h fast, blood was collected. Values are expressed as means±SD. *$p<0.01$ *vs*. mice without bazafibrate treatment. TG, triglyceride.

Quantification of Atherosclerotic Lesions

At 4–6 months of age, LDLR−/− mice were fed the Paigen diet with or without bezafibrate for 8 weeks. Mice were sacrificed and the aortic cross-sectional lesion area was evaluated according to a modification of the method of Paigen *et al*. (*24*, *25*). In brief, the hearts were perfused with saline containing 4% (w/v) formalin and fixed for more than 48 h in the same solution. The basal half of each heart was embedded in Tissue-Tek OCT compound (Miles, Torrance, USA), and the serial sections were cut using a cryostat microtome (6-μm thick) as previously described. Four sections, each separated by 60 μm, were used to evaluate the lesions: two at the end of the aortic sinus and two at the junction of the sinus and ascending aorta. The sections were stained with Oil Red O and counterstained with hematoxylin. Lesion area measurements were analyzed using the Scion Image program (Scion, Frederick, USA).

Plasma Lipids and Lipoproteins

After a 16 h fast, blood was collected from the retro-orbital venous plexus into tubes containing EDTA. Plasma total cholesterol and TG concentrations were measured enzymatically (*26*). Cholesterol and TG profiles in plasma lipoproteins were analyzed by a dual-detection HPLC system with two tandem-connected TSKgel LipopropakXL columns (300 × 7.8 mm; Tosoh, Tokyo, Japan) according to the method of Usui *et al*. (*27*), Skylight Biotech (Akita, Japan).

Plasma Glucose, Insulin, and Adiponectin

Plasma glucose, insulin, and adiponectin levels were determined using the Free Style Kissei kit (Kissei Pharmaceutical), the Mouse Insulin ELISA Ultra-Sensitive kit (Shibayagi, Shibukawa, Japan) and the mouse/rat adiponectin ELISA kit (Otsuka, Tokyo, Japan), respectively.

Table 2. HPLC Lipoprotein Analyses

Bezafibrate	*n*	Cholesterol (mg/dL)				Triglyceride (mg/dL)			
		CM	VLDL	LDL	HDL	CM	VLDL	LDL	HDL
–	14	6±5	1,817±283	798±119	34±22	7±5	77±34	22±15	6±4
+	11	3±3	1,360±294*	585±129*	61±17*	3±4**	22±16*	6±4*	4±2

Ten microliters of plasma from each mouse were subjected to HPLC lipoprotein analyses, and cholesterol and triglyceride contents in effluents were measured enzymatically. Values are expressed as the means±SD. *$p<0.01$, **$p<0.05$ *vs*. mice without bezafibrate treatment. CM, chylomicrons; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

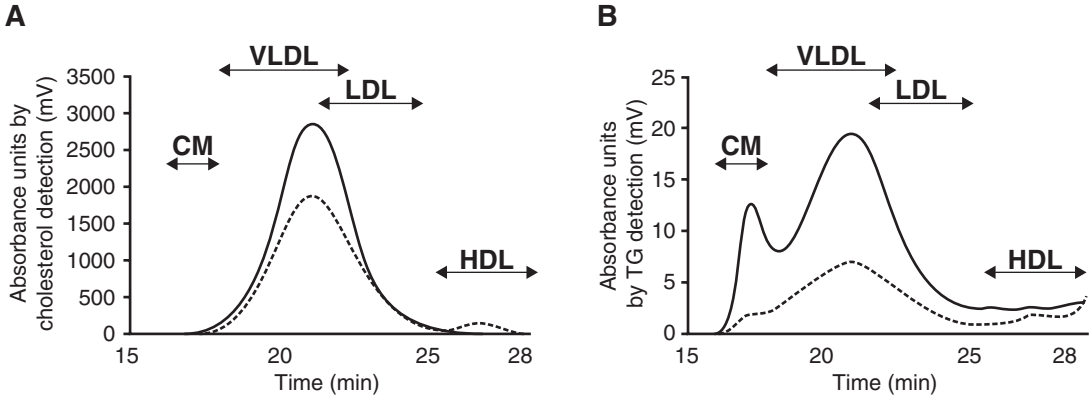

Fig. 1. *Lipoprotein profiles. Distributions of cholesterol (A) and TG (B) in plasma lipoproteins. Male LDLR−/− mice were maintained on the Paigen diet without (solid line) or with 0.1% (w/w) bezafibrate (dashed line). Ten microliters of plasma was subjected to HPLC analysis, and cholesterol and TG were measured enzymatically.*

Intraperitoneal Glucose Tolerance Test

Intraperitoneal glucose tolerance test (IPGTT) was performed after a 16 h fast. Blood was collected from mice to measure the baseline plasma glucose levels, and a bolus dose of glucose (2 g/kg body weight) was injected into the peritoneal cavity. Blood was obtained before and at 30, 60, 90, and 120 min after the injection. Plasma glucose was determined as described.

Very Low-Density Lipoprotein-TG Production

Very low-density lipoprotein (VLDL)-TG production rates were determined as described previously (*28*). In brief, a solution containing 15% (v/v) Triton WR-1339 (0.5 mg/g body weight; Sigma, St. Louis, USA) in 0.9% (w/v) NaCl was injected into the jugular veins of mice after a 16 h fast. Blood (50 μL) was obtained before and at 60 and 180 min after the injection for measurement of plasma TG levels.

LPL Activity

Post-hepalin plasma LPL activity was analyzed as described previously (*29*). After the 16 h fast, we injected male LDLR−/− mice intravenously with 10 units of heparin and collected blood 10 min later. Plasma samples were assayed in triplicate for LPL activity.

Real-Time PCR Analyses

Total RNA was isolated from the aorta using TRIzol reagent (GIBCO-BRL, Gaithersburg, USA). Two micrograms of total RNA was reverse-transcribed (TaqMan Reverse Transcription Reagents; Applied Biosystems, Foster City, USA), and the synthesized cDNA was quantified by TaqMan quantitative PCR analysis of each gene with the ABI Prism 7700 sequence detection system (Applied Biosystems) according to the manufacturer's protocol. The specific primer pairs for ATP-binding cassette transporter A1 (ABCA-1) (GenBank accession No. NM_031168), monocyte chemoattractant protein-1 (MCP-1) (NM_011333), LPL (NM_008509), and β-actin (NM_007393) were purchased from Applied Biosystems. PCR data were normalized by the β-actin level in each sample and calculated by the $2^{-\Delta\Delta C_T}$ method (*30*).

Statistics

Data are presented as means±SD. Student's *t*-test was used to compare the mean values between two sets of mice.

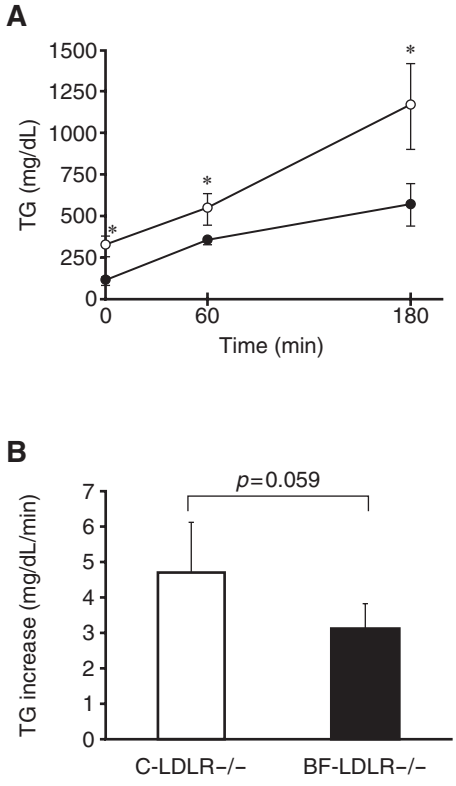

Fig. 2. *Hepatic VLDL-TG production. A: After a 16 h fast, mice were injected with Triton WR-1339 (0.5 mg/g body weight). Blood was obtained before, as well as at 60 and 180 min after, injection for measurement of plasma TG levels. Male LDLR−/− mice were maintained on the Paigen diet without (n=11; open circles) or with 0.1% (w/w) bezafibrate (n=10; closed circles). B: Increment in the plasma TG level from the baseline to 180 min was calculated. Values are expressed as means±SD. *p<0.01.*

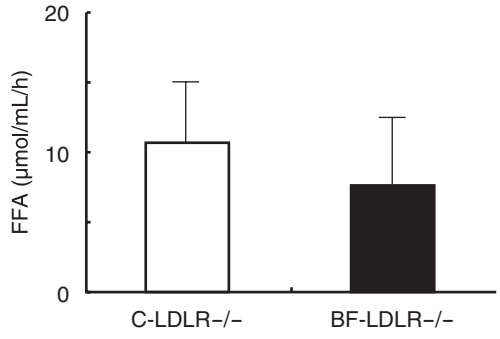

Fig. 3. *LPL activity. Post-heparin plasma LPL activity did not differ between LDLR−/− mice without bezafibrate treatment (open bars, n=9) and those with the treatment (closed bars, n=11). Values are expressed as means±SD.*

Results

Plasma Lipids and Lipoproteins

Bezafibrate lowered the plasma levels of cholesterol and TG by 24% and 69%, respectively (Table 1). Lipoprotein analyses revealed that the reduction in cholesterol was largely attributable to the reduction of cholesterol in non-HDL lipoprotein fractions (Table 2 and Fig. 1A). TG contents in chylomicrons (CM), VLDL, and LDL were reduced by 57, 71, and 73%, respectively (Table 2 and Fig. 1B). On the other hand, bezafibrate increased the plasma level of HDL-C by 56% (Table 2 and Fig. 1A).

Hepatic VLDL-TG Production

To determine whether or not the cholesterol-lowering effects of bezafibrate result from the suppression of VLDL production in the liver, mice were injected with Triton WR-1339 after a 16 h fast, and then their plasma TG levels were measured at various times. As shown in Fig. 2, treatment with bezafibrate suppressed the hepatic VLDL-TG production rate 26% in LDLR−/− mice ($p<0.01$).

LPL Activity

Post-heparin plasma of LDLR−/− mice with and without bezafibrate treatment was assayed for LPL activity in triplicate. LPL activity did not differ between the treated and untreated mice ($p=0.46$) (Fig. 3).

Plasma Glucose Tolerance

To determine whether or not bezafibrate affected plasma glucose metabolism, we measured the plasma glucose and insulin levels after a 16 h fast and found no significant differences between treated and untreated mice (Table 1). Glucose response curves after the intraperitoneal glucose load were indistinguishable between treated and untreated mice (Fig. 4). In agreement with the lack of improvement in glucose tolerance, bezafibrate did not change plasma adiponectin levels (Table 1).

Atherosclerosis

Atherosclerotic lesion size was evaluated at the aortic root after feeding with the Paigen diet with or without bezafibrate for 2 months (Fig. 5). Treatment with bezafibrate robustly reduced lesion size by 51% (440,000±160,000 *vs.* 900,000±180,000 μm^2, $p<0.001$).

Gene Expression in the Aorta

To determine whether or not bezafibrate influences athero-

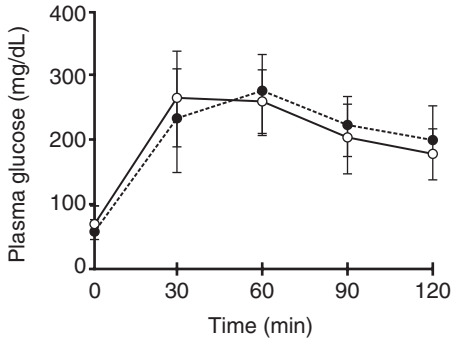

Fig. 4. *Glucose tolerance. After a 16 h fast, mice were injected with a bolus dose of glucose (2 g/kg body weight) into the peritoneal cavity. Blood was obtained before, as well as at 30, 60, 90, and 120 min after the injection. Plasma glucose levels were determined. Male LDLR−/− mice were maintained on the Paigen diet without (n=14; open circles) or with 0.1% (w/w) bezafibrate (n=11; closed circles). Values are expressed as means±SD.*

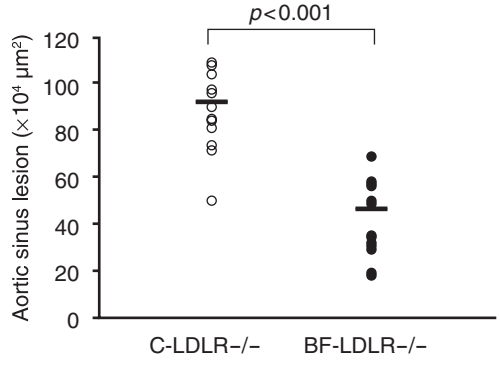

Fig. 5. *Atherosclerotic lesion area. Cross-sectional lesion area in the aortic sinus from LDLR−/− mice without bezafibrate treatment (open circles, n=14) and those with the treatment (closed circles, n=11). Fatty streak lesion areas were determined as described in Methods. Mean values are indicated by horizontal lines.*

sclerosis by directly altering the expression of genes relevant to atherosclerosis, we measured the mRNA levels of ABCA-1, MCP-1, and LPL by real-time PCR. There were no changes in the mRNA expression levels of ABCA-1, MCP-1, or LPL between mice with and without bezafibrate treatment (Table 3).

Table 3. mRNA Expression in the Aorta

	Bezafibrate	
	− (*n*=14)	+ (*n*=11)
MCP-1	1.0±0.52	0.71±0.43
LPL	1.0±0.97	0.65±0.40
ABCA-1	1.0±0.86	0.84±0.71

mRNA expression levels for monocyte chemoattractant protein-1 (MCP-1), lipoprotein lipase (LPL) and ATP-binding casette transporter A1 (ABCA-1) in the aorta were measured by real-time PCR. Data were normalized for β-actin levels in each sample and calculated by the $2^{-\Delta\Delta C_T}$ method. Data are shown as the ratio between the values of the treated and non-treated mice. Values are expressed as means±SD.

Discussion

In the present study, we showed that bezafibrate markedly inhibited atherosclerosis in LDLR−/− mice. The anti-atherogenic effects were associated with decreases in plasma cholesterol and TG levels as well as an increase in the plasma HDL-C level, but not with changes in the expression of genes relevant to atherosclerosis in the aorta. These results suggest that bezafibrate inhibits atherosclerosis primarily by affecting the plasma lipoprotein profile.

Many clinical trials have demonstrated the clinical efficacy and safety of fibrates (*22*, *31–33*). Animal experiments should be useful to determine how fibrates reduce the risk of cardiovascular events. However, somehow conflicting results have been reported with regard to their effects even on lipoprotein profiles. Cipofibrate did not influence the plasma HDL-C levels in apoE−/− or LDLR−/− mice (*18*, *19*). Fenofibrate increased plasma HDL-C levels in LDLR−/− mice only when human apoA-I was overexpressed in those animals (*17*). GW7647, a PPAR-α agonist, did not change the lipoprotein profile in LDLR−/− mice fed an atherogenic diet (*34*). In rodents, fibrates paradoxically lowered plasma HDL-C levels by decreasing the transcription of the apoA-I gene (*35*). The lack of induction of apoA-I expression by fibrates has been ascribed to the three nucleotide differences in the rodent apoA-I promoter, which eliminates the binding of PPAR-α to the promoter (*11*). Therefore, bezafibrate's cholesterol-lowering and HDL-C–raising effects are rather unique compared to the effects of other fibrates. Unlike ciprofibrate and fenofibrate, whose active metabolites are dual agonists for PPAR-α and PPAR-γ, with 10-fold selectivity for PPAR-α, bezafibrate is a panagonist activating all of PPAR-α, PPAR-γ, and PPAR-δ (*36*). This receptor selectivity may account for the significant influence on the lipoprotein profile in the present study.

How did bezafibrate lower the plasma cholesterol level in LDLR−/− mice? Since VLDL production was significantly reduced in the mice treated with bezafibrate, we speculate that decreased lipoprotein production plays a central role in the reduction in cholesterol. Previous studies have shown that fibrates stimulate lipolysis by activating LPL (*6*, *7*) and decreasing the plasma concentration of apoC-III (*8*), an apolipoprotein with an antagonistic property against lipolysis (*9*). In the present study, we failed to detect an increase in LPL

activity in post-heparin plasma (Fig. 3). However, it is still possible that a decrease in plasma levels of apoC-III stimulates lipolysis, thereby contributing to the lowering of the TG content in TG-rich lipoproteins (Fig. 1) as well as plasma cholesterol levels (*37*). With regard to the increased level of HDL-C, increased lipolysis, though not directly demonstrated in the present study, may be a central player, because we failed to detect an increase in the expression of the ABCA-1 gene (Table 3), a key molecule mediating reverse cholesterol transport. Fibrates paradoxically decrease the expression of apoA-I in rodents (*12*).

Were other systemic factors involved in the anti-atherogenic effects of bezafibrate? Impaired glucose tolerance, insulin resistance, and/or changes in some humoral factors are risk factors for atherosclerosis. We have examined glucose tolerance, as well as fasting plasma levels of both insulin and adiponectin, an emerging cytokine linking obesity/diabetes to atherosclerosis (*38*, *39*). Bezafibrate did not affect these potential factors modulating atherogenesis. This was rather unexpected, because amelioration of insulin sensitivity by bezafibrate has been shown in previous animal studies (*40*). Clinically, it was also proved that bezafibrate reduces insulin resistance (*41*) and delays the onset of type-2 diabetes in patients with impaired fasting glucose (*42*).

In addition to the systemic actions mentioned above, fibrates can directly act on vascular wall cells. PPAR-α agonists are known to exert anti-inflammatory effects at the vascular level by reducing the cytokine-induced activation of vascular cell adhesion molecule-1 in vascular endothelial cells (*13*). Moreover, Chinetti *et al*. reported that PPAR-α agonists stimulate cholesterol efflux from foam cell macrophages *in vitro* by inducing the expression of ABCA-1 through the stimulation of nuclear-receptor liver-X receptor expression (*43*). In the present study, however, bezafibrate did not reduce the mRNA expression level of MCP-1, which plays a crucial role in the transendothelial migration of monocytes into the vascular subendothelium, resulting in predisposition to atherosclerotic lesions (*44*). Bezafibrate did not affect the expression of LPL or ABCA-1 either. A similar failure of ABCA-1 induction was reported by other investigators (*17*, *33*). In mice with severe hypercholesterolemia, such as LDLR−/− mice fed an atherogenic diet or apoE−/− mice, ABCA-1 expression in the lesions may be maximally stimulated by the liver-X receptor pathway induced by massive cholesterol accumulation. PPAR-α agonists may not have additional effects on the maximal induction.

In conclusion, we have demonstrated for the first time the anti-atherogenic effects of bezafibrate in mice, corroborating the findings of clinical trials. Based on our results, we presume that the favorable changes in the lipoprotein profile, with a decreased ratio of non-HDL-C to HDL-C, primarily account for the anti-atherogenic effects of bezafibrate, and that the roles of other systemic and direct vascular effects are less important.

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References

1. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC: Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 1998; **98**: 2088–2093.
2. Willson TM, Brown PJ, Sternbach DD, Henke BR: The PPARs: from orphan receptors to drug discovery. *J Med Chem* 2000; **43**: 527–550.
3. Shepherd J: Mechanism of action of fibrates. *Postgrad Med J* 1993; **69** (Suppl 1): S34–S41.
4. Caslake MJ, Packard CJ, Gaw A, *et al*: Fenofibrate and LDL metabolic heterogeneity in hypercholesterolemia. *Arterioscler Thromb* 1993; **13**: 702–711.
5. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, *et al*: PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response *via* a PPRE in the lipoprotein lipase gene. *EMBO J* 1996; **15**: 5336–5348.
6. Heller F, Harvengt C: Effects of clofibrate, bezafibrate, fenofibrate and probucol on plasma lipolytic enzymes in normolipaemic subjects. *Eur J Clin Pharmacol* 1983; **25**: 57–63.
7. Staels B, Auwerx J: Perturbation of developmental gene expression in rat liver by fibric acid derivatives: lipoprotein lipase and alpha-fetoprotein as models. *Development* 1992; **115**: 1035–1043.
8. Staels B, Vu-Dac N, Kosykh VA, *et al*: Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. *J Clin Invest* 1995; **95**: 705–712.
9. Wang CS, McConathy WJ, Kloer HU, Alaupovic P: Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J Clin Invest* 1985; **75**: 384–390.
10. Torra IP, Chinetti G, Duval C, Fruchart JC, Staels B: Peroxisome proliferator–activated receptors: from transcriptional control to clinical practice. *Curr Opin Lipidol* 2001; **12**: 245–254.
11. Staels B, Auwerx J: Regulation of apo A-I gene expression by fibrates. *Atherosclerosis* 1998; **137** (Suppl): S19–S23.
12. Berthou L, Saladin R, Yaqoob P, *et al*: Regulation of rat liver apolipoprotein A-I, apolipoprotein A-II and acyl-coenzyme A oxidase gene expression by fibrates and dietary fatty acids. *Eur J Biochem* 1995; **232**: 179–187.
13. Marx N, Sukhova GK, Collins T, Libby P, Plutzky J: PPARalpha activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. *Circulation* 1999; **99**: 3125–3131.
14. Shu H, Wong B, Zhou G, *et al*: Activation of PPARalpha or gamma reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. *Biochem Biophys Res Commun* 2000; **267**: 345–349.
15. Neve BP, Corseaux D, Chinetti G, *et al*: PPARalpha ago-

nists inhibit tissue factor expression in human monocytes and macrophages. *Circulation* 2001; **103**: 207–212.
16. Chinetti G, Griglio S, Antonucci M, *et al*: Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. *J Biol Chem* 1998; **273**: 25573–25580.
17. Duez H, Chao YS, Hernandez M, *et al*: Reduction of atherosclerosis by the peroxisome proliferator–activated receptor alpha agonist fenofibrate in mice. *J Biol Chem* 2002; **277**: 48051–48057.
18. Fu T, Kashireddy P, Borensztajn J: The peroxisome-proliferator–activated receptor alpha agonist ciprofibrate severely aggravates hypercholesterolaemia and accelerates the development of atherosclerosis in mice lacking apolipoprotein E. *Biochem J* 2003; **373**: 941–947.
19. Fu T, Mukhopadhyay D, Davidson NO, Borensztajn J: The peroxisome proliferator–activated receptor alpha (PPAR-alpha) agonist ciprofibrate inhibits apolipoprotein B mRNA editing in low density lipoprotein receptor–deficient mice: effects on plasma lipoproteins and the development of atherosclerotic lesions. *J Biol Chem* 2004; **279**: 28662–28669.
20. Tordjman K, Bernal-Mizrachi C, Zemany L, *et al*: PPAR-alpha deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. *J Clin Invest* 2001; **107**: 1025–1034.
21. Ericsson CG, Hamsten A, Nilsson J, Grip L, Svane B, de Faire U: Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet* 1996; **347**: 849–853.
22. Group TBS: Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the Bezafibrate Infarction Prevention (BIP) study. *Circulation* 2000; **102**: 21–27.
23. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J: Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest* 1993; **92**: 883–893.
24. Paigen B, Morrow A, Brandon C, Mitchell D, Holmes P: Variation in susceptibility to atherosclerosis among inbred strains of mice. *Atherosclerosis* 1985; **57**: 65–73.
25. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA: Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis* 1987; **68**: 231–240.
26. Yagyu H, Kitamine T, Osuga J, *et al*: Absence of ACAT-1 attenuates atherosclerosis but causes dry eye and cutaneous xanthomatosis in mice with congenital hyperlipidemia. *J Biol Chem* 2000; **275**: 21324–21330.
27. Usui S, Hara Y, Hosaki S, Okazaki M: A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by HPLC. *J Lipid Res* 2002; **43**: 805–814.
28. Kako Y, Huang LS, Yang J, Katopodis T, Ramakrishnan R, Goldberg IJ: Streptozotocin-induced diabetes in human apolipoprotein B transgenic mice. Effects on lipoproteins and atherosclerosis. *J Lipid Res* 1999; **40**: 2185–2194.
29. van Vlijmen BJ, Rohlmann A, Page ST, *et al:* An extrahepatic receptor–associated protein–sensitive mechanism is involved in the metabolism of triglyceride-rich lipoproteins. *J Biol Chem* 1999; **274**: 35219–35226.
30. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods* 2001; **25**: 402–408.
31. Frick MH, Elo O, Haapa K, *et al*: Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med* 1987; **317**: 1237–1245.
32. Rubins HB, Robins SJ, Collins D, *et al*: Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 1999; **341**: 410–418.
33. Keech A, Simes RJ, Barter P, *et al*: Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; **366**: 1849–1861.
34. Li L, Beauchamp MC, Renier G: Peroxisome proliferator–activated receptor alpha and gamma agonists upregulate human macrophage lipoprotein lipase expression. *Atherosclerosis* 2002; **165**: 101–110.
35. Peters JM, Hennuyer N, Staels B, *et al*: Alterations in lipoprotein metabolism in peroxisome proliferator–activated receptor alpha-deficient mice. *J Biol Chem* 1997; **272**: 27307–27312.
36. Berger J, Moller DE: The mechanisms of action of PPARs. *Annu Rev Med* 2002; **53**: 409–435.
37. Shimada M, Ishibashi S, Inaba T, *et al*: Suppression of diet-induced atherosclerosis in low density lipoprotein receptor knockout mice overexpressing lipoprotein lipase. *Proc Natl Acad Sci U S A* 1996; **93**: 7242–7246.
38. Yamauchi T, Kamon J, Waki H, *et al*: Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem* 2003; **278**: 2461–2468.
39. Okamoto Y, Kihara S, Ouchi N, *et al*: Adiponectin reduces atherosclerosis in apolipoprotein E–deficient mice. *Circulation* 2002; **106**: 2767–2770.
40. Guerre-Millo M, Gervois P, Raspe E, *et al*: Peroxisome proliferator–activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* 2000; **275**: 16638–16642.
41. Kim JI, Tsujino T, Fujioka Y, Saito K, Yokoyama M: Bezafibrate improves hypertension and insulin sensitivity in humans. *Hypertens Res* 2003; **26**: 307–313.
42. Tenenbaum A, Motro M, Fisman EZ, *et al*: Peroxisome proliferator–activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease. *Circulation* 2004; **109**: 2197–2202.
43. Chinetti G, Lestavel S, Bocher V, *et al*: PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* 2001; **7**: 53–58.
44. Chinetti G, Fruchart JC, Staels B: Peroxisome proliferator–activated receptors: new targets for the pharmacological modulation of macrophage gene expression and function. *Curr Opin Lipidol* 2003; **14**: 459–468.