Peroxisome Proliferator–Activated Receptor-α Gene Level Differently Affects Lipid Metabolism and Inflammation in Apolipoprotein E2 Knock-In Mice

Fanny Lalloyer, Kristiaan Wouters, Morgane Baron, Sandrine Caron, Emmanuelle Vallez, Jonathan Vanhoutte, Eric Baugé, Ronit Shiri-Sverdlov, Marten Hofker, Bart Staels, Anne Tailleux

Objective—Peroxisome proliferator–activated receptor-α (PPARα) is a ligand-activated transcription factor that controls lipid metabolism and inflammation. PPARα is activated by fibrates, hypolipidemic drugs used in the treatment of dyslipidemia. Previous studies assessing the influence of PPARα agonists on atherosclerosis in mice yielded conflicting results, and the implication of PPARα therein has not been assessed. The human apolipoprotein E2 knock-in (apoE2-KI) mouse is a model of mixed dyslipidemia, atherosclerosis, and nonalcoholic steatohepatitis (NASH). The aim of this study was to analyze, using homo- and heterozygous PPARα-deficient mice, the consequences of quantitative variations of PPARα gene levels and their response to the synthetic PPARα agonist fenofibrate on NASH and atherosclerosis in apoE2-KI mice.

Methods and Results—Wild-type (+/+), heterozygous (+/−), and homozygous (−/−) PPARα-deficient mice in the apoE2-KI background were generated and subjected to a Western diet supplemented with fenofibrate or not supplemented. Western diet–fed PPARα−/− apoE2-KI mice displayed an aggravation of liver steatosis and inflammation compared with PPARα+/+ and PPARα+/− apoE2-KI mice, indicating a role of PPARα in liver protection. Moreover, PPARα expression was required for the fenofibrate-induced protection against NASH. Interestingly, fenofibrate treatment induced a similar response on hepatic lipid metabolism in PPARα+/+ and PPARα+/− apoE2-KI mice, whereas, for a maximal antiinflammatory response, both alleles of the PPARα gene were required. Surprisingly, atherosclerosis development was not significantly different among PPARα+/+ , PPARα+/− , and PPARα−/− apoE2-KI mice. However, PPARα gene level determined both the antiatherosclerotic and vascular antiinflammatory responses to fenofibrate in a dose-dependent manner.

Conclusion—These results demonstrate a necessary but quantitatively different role of PPARα in the modulation of liver metabolism, inflammation, and atherogenesis. (Arterioscler Thromb Vasc Biol. 2011;31:1573-1579.)

Key Words: Atherosclerosis ■ PPARα ■ fatty liver disease ■ inflammation ■ murine model

Fibrates are lipid-lowering drugs widely used in clinical practice to treat dyslipidemia.1 Studies performed in peroxisome proliferator–activated receptor-α (PPARα)–deficient mice have demonstrated that the hypolipidemic effects of fibrates are due to activation of PPARα, a ligand-activated transcription factor that modulates lipid metabolism.2 PPARα is expressed in many tissues, particularly in tissues with high fatty acid oxidation rates, such as liver, kidney, heart, and muscle. After activation by fibrates, PPARα binds as a heterodimer with the retinoid X receptor to PPAR response elements in the promoters of genes implicated in lipid and lipoprotein metabolism. In addition to its effects on lipid metabolism, PPARα also inhibits proinflammatory pathways by negatively interfering with other signaling pathways, such as nuclear factor-κB, signal transducer and activator of transcription, or activator protein-1. Consequently, through its effects on lipid metabolism and inflammation, PPARα may modulate pathophysiological pathways implicated in fatty liver disease and atherosclerosis. Data concerning the implication of PPARα in liver steatosis and inflammation in humans is scarce. However, it has been shown that PPARα agonist treatment decreases nonalcoholic steatohepatitis (NASH) development in wild-type mice fed a methionine choline-deficient diet3,4 and apolipoprotein E2 knock-in (apoE2-KI) mice or foz/foz mice fed a high-fat diet.5,6 Moreover, PPARα is expressed in many cell types found in...
the atherosclerotic lesion, such as macrophages, endothelial cells, and smooth muscle cells. In vitro and in vivo studies have suggested that fibrates could exert antiatherogenic actions by improving lipid abnormalities or by modulating several steps of atherogenesis, such as decreasing inflammation and thrombosis, directly in the vascular wall. In humans, fibrates decrease cardiovascular disease, especially in patients with high triglyceride and low-high density lipoprotein-cholesterol levels. Moreover, we have previously shown that fenofibrate treatment reduces macrophage-laden atherosclerotic lesions in apoE2-KI mice, a mouse model of NASH, atherosclerosis, and mixed dyslipidemia. However, the role of PPARα deficiency protects against atherosclerosis progression in apoE-deficient mice and Tsukuba hypertensive mice, whereas macrophage-specific PPARα expression protects low-density lipoprotein receptor (LDLR)-deficient mice from atherosclerosis, and PPARα agonist treatment increases or decreases atherosclerosis development in different murine models.

In the present study, we aimed to analyze the consequences of PPARα deficiency on lipid metabolism and inflammation in the vascular wall and the liver using apoE2-KI mice, a model of mixed dyslipidemia, atherosclerosis, and NASH, and to further explore the implication of PPARα in the response to fenofibrate treatment. Therefore, wild-type (+/+), heterozygous (+/−), and homozygous PPARα-deficient (−/−) apoE2-KI mice were fed a Western diet with or without fenofibrate during 9 weeks. Surprisingly, homozygous PPARα deficiency did not modify plasma lipid concentrations; however, it aggravated liver steatosis and inflammation. Interestingly, PPARα gene levels differently affected the response to fenofibrate on hepatic lipid metabolism and inflammation. In addition, whereas PPARα deficiency did not influence atherosclerosis development, the PPARα gene level dose-dependently controlled the response to fenofibrate on vascular inflammation and atherogenesis.

Materials and Methods

Animals
Homozygous PPARα-deficient mice on the C57BL/6 background were crossed with homozygous human apoE2-KI mice, which express human apoE2 instead of mouse apoE under control of the endogenous promoter, to generate PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice. Three-month-old female mice of the 3 genotypes (n=11 per group) were fed a Western diet containing 0.2% cholesterol and 21% fat (wt/wt) (UAR) without (control group) or with fenofibrate for 9 weeks. Based on food consumption, the dosage of fenofibrate corresponded to ≈100 mg/kg of body weight. Mice were maintained under a 12-hour light/dark cycle and had free access to water. All animal experiments were conducted with the approval of the Pasteur Institute review board, Lille, France.

Plasma Lipid and Lipoprotein Analyses
Mice were fasted for 4 hours before retro-orbital puncture under isoflurane-induced anesthesia. Plasma concentrations of total cholesterol (TC) and triglycerides (TG) were measured using commercially available kits (Biomerieux).

Hepatic Lipid Analysis
Frozen liver tissue (50 mg) was homogenized in SET buffer (1 mL; 250 mmol/L sucrose, 2 mmol/L EDTA, and 10 mmol/L Tris), followed by 2 freeze-thaw cycles, and 3 passages through a 27-gauge syringe needle, and a final freeze-thaw cycle to ensure complete cell lysis. Protein content was determined with the BCA method, and TG and cholesterol was measured as described above.

Liver Immunohistochemistry
Seven-micrometer frozen-cut liver sections were fixed in acetone and stained with Mac1 (M1/70) antibodies, as described.

Isolation of Primary Hepatocytes
Primary hepatocytes were isolated from the livers of fed mice, as previously described.

RNA Extraction and Quantitative Polymerase Chain Reaction Analysis
RNA, isolated from livers using the acid guanidinium thiocyanate/phenol/chloroform method, was reverse transcribed using random hexamer primers and Moloney murine leukemia virus–reverse transcriptase (Invitrogen). RNA levels were determined by real-time quantitative polymerase chain reaction on a MX-4000 apparatus (Stratagene) using the Brilliant SYBR Green QPCR master mix (Stratagene) and specific primers. Results are expressed normalized to cyclophilin.

Atherosclerotic Lesion Analysis
At the end of the diet, mice were euthanized, and the hearts were perfused with cold Krebs-Ringer buffer and fixed in a solution containing 4% phosphate-buffered paraformaldehyde. Serial 10-μm-thick cryosections were cut between the valves and the aortic arch, and atherosclerotic lesions were quantified by Oil Red O staining. Images were captured using a JVC 3-CCD video camera and analyzed using the computer-assisted Quips Image analysis system (Leica Mikroskopie und System GmbH). Cryosections from aortic lesions were stained with anti-mouse monocyte/macrophage marker-2 (MOMA-2) (Santa Cruz Biotechnology) or anti-mouse monocyte chemoattractant protein-1 (MCP-1) (Santa Cruz Biotechnology). MCP-1 protein levels were semiquantitatively scored for staining on lesions of 4 mice per group.

Statistical Analysis
Results are expressed as the means±SE. Data were compared by ANOVA. Significant differences were subjected to post hoc analysis by using the Scheffe test. A value of P<0.05 was considered statistically significant.

Results
Homozygous PPARα Deficiency Does Not Modify Plasma Lipid Concentrations but Aggravates Liver Steatosis and Inflammation in apoE2-KI Mice Fed a Western Diet
Female PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice were generated, and PPARα mRNA levels were found to be intermediate and undetectable, respectively, in the livers of PPARα+/− and PPARα−/− mice versus PPARα+/+ apoE2-KI mice (Supplemental Figure IA, available online at http://atvb.ahajournals.org). These mice were fed a Western diet that was previously shown to induce liver steatosis and inflammation. After 9 weeks of Western diet feeding, plasma TG and TC concentrations were similar in the 3 groups of mice (Figure 1A and 1B). Relative liver weight was not different among the 3 genotypes (Supplemental Figure IB), and liver cholesterol content was slightly but not significantly increased in PPARα−/− apoE2-KI compared with PPARα+/+ and PPARα+/− apoE2-KI mice (Supplemental Figure IC). PPARα−/− apoE2-KI mice displayed more severe steatohepatitis, as illustrated by higher
levels of liver TG (Figure 1C) and increased numbers of Mac1-positive cells (Figure 1D), compared with PPARα+/+ mice. Interestingly, heterozygous PPARα+/− apoE2-KI mice displayed a phenotype similar to that of PPARα+/+ mice (Figure 1C and 1D). These results demonstrate that PPARα deficiency does not modify plasma lipid concentrations, and only homozygous PPARα deficiency aggravates liver steatosis and macrophage content in Western diet–fed apoE2-KI mice.

PPARα Activation Improves Plasma and Hepatic Lipid Homeostasis in PPARα+/+ and PPARα+/− but not PPARα−/− apoE2-KI Mice Fed a Western Diet

To determine the role of the PPARα gene level on the hepatic and plasma response to its agonist fenofibrate, female PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice were fed a Western diet supplemented with fenofibrate for 9 weeks. The response to the PPARα agonist was compared with the respective placebo-treated PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice, whose values were set at 100%.

As expected, fenofibrate treatment decreased plasma TC and TG concentrations (Figure 2A and 2B) and liver TG content (Figure 2C) in PPARα+/+ apoE2-KI mice. In marked contrast, fenofibrate-treated PPARα−/− apoE2-KI mice did not exhibit any significant decrease in plasma TC and TG concentrations and liver TG levels compared with placebo-treated mice, showing that fenofibrate improves dyslipidemia and hepatic steatosis in a PPARα-dependent manner in apoE2-KI mice (Figure 2A to 2C). Interestingly, fenofibrate reduced plasma TC and TG concentrations and liver TG levels to the same extent in PPARα+/+ apoE2-KI mice as in PPARα+/+ apoE2-KI mice. Because PPARα is a transcription factor which regulates the expression of genes involved in fatty acid uptake and oxidation in parenchymal cells of the liver, mRNA levels for fatty acid uptake and oxidation genes in PPARα+/+ mice were measured (Figure 3A to 3D).

**Figure 1.** Homozygous PPARα deficiency does not modify plasma lipid concentrations but aggravates liver steatosis and inflammation in apoE2-KI mice. Blood samples were collected after a 4-hour fast for measurements of plasma TC (A) and TG (B) levels in female PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice fed a Western diet for 9 weeks. Liver TG content (C) and MAC-1 staining (D) were quantified in the liver. n = 11 mice/group. Results are expressed as means ± SE. NS indicates nonsignificant. #P < 0.05 vs PPARα+/+ apoE2-KI mice.

**Figure 2.** Fenofibrate improves plasma and hepatic lipid homeostasis in PPARα+/+ and PPARα+/− but not PPARα−/− apoE2-KI mice. Shown are plasma TC (A) and TG (B) levels and liver TG content (C) in female PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice fed a Western diet supplemented (FF, ■) or not (CON, □) with fenofibrate for 9 weeks. n = 11 mice/group. Results are expressed as means ± SE. **P < 0.001 vs untreated mice; ###P < 0.001 vs fenofibrate-treated PPARα+/+ apoE2-KI mice.

**Figure 3.** Fenofibrate increases the expression of fatty acid uptake and oxidation genes in PPARα+/+ and PPARα+/− but not PPARα−/− apoE2-KI mice. Shown are hepatic mRNA levels of fatty acid translocase (FAT) (A), very-long-chain acyl-CoA dehydrogenase (VLCAD) (B), long-chain acyl-CoA dehydrogenase (LCAD) (C), and medium-chain acyl-CoA dehydrogenase (MCAD) (D) in female PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice fed a Western diet supplemented (FF, ■) or not (CON, □) with fenofibrate for 9 weeks. n = 11 mice/group. Results are expressed as means ± SE. **P < 0.01 vs untreated mice; ###P < 0.001 vs fenofibrate-treated PPARα+/+ apoE2-KI mice.
increased the hepatic expression levels of all these genes in PPARα+/+ but not in PPARα−/− apoE2-KI mice. However, in PPARα+/− apoE2-KI mice, the expression of these genes increased to an extent similar to that in PPARα+/+ apoE2-KI mice on fenofibrate treatment. A comparable induction of genes implicated in hepatic lipid metabolism was also observed in primary hepatocytes isolated from PPARα+/+ and PPARα+/− apoE2-KI mice and treated with the specific PPARα agonist GW647, whereas this induction was not observed in PPARα−/− apoE2-KI hepatocytes (Supplemental Figure IIA and IIB). Thus, only 1 allele of the PPARα gene is required for an optimal response to fenofibrate on dyslipidemia and liver steatosis.

PPARα Activation Decreases Hepatic Inflammation and Macrophage Content in PPARα+/+ but not PPARα+/− or PPARα−/− apoE2-KI Mice Fed a Western Diet

Hepatic inflammation was analyzed in the fenofibrate-treated mice. Fenofibrate treatment decreased the number of Mac-1 positive cells, indicative of the number of macrophages, in livers of PPARα+/+ but not PPARα+/− or PPARα−/− apoE2-KI mice (Figure 4A and 4B). The expression of monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), which are implicated in monocyte/macrophage recruitment in the liver (Figure 4C to 4E), were decreased in fenofibrate-treated PPARα+/+ apoE2-KI mice. By contrast, fenofibrate did not influence the expression of these inflammatory markers in livers of PPARα−/− apoE2-KI mice. Interestingly, fenofibrate-treated PPARα+/− apoE2-KI mice exhibited an intermediary gene expression level of MCP-1, VCAM-1, and ICAM-1. Of note, repression of lipopolysaccharide-induced MCP-1 and VCAM-1 expression by the specific PPARα agonist GW647 was most pronounced in isolated primary hepatocytes from PPARα+/+ apoE2-KI mice, whereas an intermediary response was seen in PPARα+/− apoE2-KI cells (Supplemental Figure IIC and IID). Thus, both PPARα alleles are necessary for an optimal inhibition of the hepatic inflammatory response by fenofibrate in apoE2-KI mice fed a Western diet.

PPARα Gene Levels Do Not Influence Atherogenesis but Determine the Atheroprotective Response to Fenofibrate in apoE2-KI Mice Fed a Western Diet

Because nonalcoholic fatty liver disease is now considered a risk factor for cardiovascular disease,19 atherogenesis and vascular inflammation were assessed in PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice fed a Western diet supplemented or not with fenofibrate. Interestingly, the mean aortic lesion area, as measured by lipid staining with Oil Red O, did not differ significantly among PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice (Figure 5A and 5B). As expected,8 mean lesion area was significantly reduced by ~80% in fenofibrate-treated PPARα+/+ mice compared with controls (median: 0.030 mm² in treated mice versus 0.166 mm² in control mice; P<0.001) (Figure 5A and 5B). By contrast, no effect was observed in fenofibrate-treated PPARα−/− apoE2-KI mice (median: 0.137 mm² in treated mice versus 0.123 mm² in control mice; not significant). Interestingly, treatment of PPARα+/− apoE2-KI mice with fenofibrate resulted in a significant intermediary decrease in atherosclerotic lesion area (median: 0.138 mm² in treated mice versus 0.185 in control mice; P<0.05), indicating a dose-response effect of PPARα gene expression on arterial wall lipid accumulation. To determine whether the modifications in atherosclerotic lipid accumulation were associated with altered inflammation in the arterial wall, immunostaining for MOMA-2 (specific of macrophages) and MCP-1 was performed in the lesions. Both MOMA-2 (Figure 5C) and MCP-1 (Figure 5D and 5E) colocalized with Oil Red O staining, were intense, and did not differ among PPARα+/+, PPARα+/−, and PPARα−/− apoE2-KI mice, in accordance with the comparable lesion areas among the 3 genotypes (Figure 5A). Treatment with fenofibrate strongly decreased MOMA-2 and MCP-1 staining in the lesions of PPARα+/+ but not PPARα−/− apoE2-KI mice, indicating
PPARα dependency. Interestingly, fenofibrate treatment of PPARα+/− apoE2-KI mice resulted in an intermediary phenotype with a slight decrease of MOMA-2 and MCP-1 staining in the lesions. Thus, although PPARα gene levels do not influence atherosclerosis development, they determine the response to fenofibrate on both lipid deposition and inflammation in the arterial wall of apoE2-KI mice.

Discussion

Using apoE2-KI mice, a humanized model of mixed dyslipidemia and NASH, we show that homozygous PPARα deficiency aggravates Western diet-induced steatosis and inflammation in the liver. This result is consistent with the reported increased hepatic steatosis observed in PPARα-deficient mice in response to the physiological stimulus fasting, further illustrating the role of PPARα in hepatic lipid metabolism. In apoE2-KI mice, aggravation of NASH in response to treatment with the PPARα agonist fenofibrate on NASH. Fenofibrate treatment protected against NASH in Western diet-fed PPARα+/+ but not PPARα−− apoE2-KI mice, showing that the previously reported effects of fenofibrate on NASH in apoE2-KI mice occur via PPARα. Interestingly, fenofibrate treatment of PPARα+/− apoE2-KI mice resulted in decreased hepatic steatosis to an extent similar to that of PPARα+/+ apoE2-KI mice. In parallel, the hepatic expression of genes implicated in fatty acid uptake and oxidation increased to a similar extent in fenofibrate-treated PPARα+/+ and PPARα+/− apoE2-KI mice. However, in contrast with the regulation of lipid metabolism, the antiinflammatory response to fenofibrate in the liver depends on both PPARα alleles. Indeed, fenofibrate treatment strongly reduced inflammation and macrophage content in livers of PPARα+/+ apoE2-KI mice (associated with decreased expression levels of MCP-1, ICAM-1, and VCAM-1), whereas little or no response to fenofibrate was observed in livers of PPARα+/− apoE2-KI mice. Similar observations were made in isolated heterozygous primary hepatocytes treated with the specific PPARα agonist GW647. Thus, PPARα gene level differently influences fenofibrate’s effects on hepatic steatosis and inflammation. A single PPARα allele is sufficient for an optimal response of lipid metabolism to fenofibrate, whereas both alleles are required to obtain maximal antiinflammatory effects in the liver.

**Figure 5.** PPARα gene levels do not influence atherogenesis but determine the atheroprotective response to fenofibrate in apoE2-KI mice. Shown is the atherosclerotic lesion area (A) in the aorta of female PPARα+/+, PPARα+/−, and PPARα−− apoE2-KI mice fed a Western diet supplemented (filled symbols) or not (open symbols) with fenofibrate for 9 weeks. The graph represents the mean area of lesions of the analyzed sections, and each symbol represents 1 mouse. The horizontal bar corresponds to the median of the values. *P<0.05, **P<0.001 vs untreated mice; ###P<0.001 vs fenofibrate-treated PPARα+/+ apoE2-KI mice. B and C, Representative microphotographs showing Oil Red O staining (B) and MOMA-2-stained macrophages (C) in the atherosclerotic lesions. D and E, Representative photomicrographs showing MCP-1 staining of atherosclerotic lesions (D) and score of MCP-1 staining (E) graded as follows: −, not detected; +, slight; ++, marked; ++++, pronounced expression in lesions. n=4 mice/group. Scale bars=500 μm (B), 100 μm (C and D).
Until now, no null mutations of PPARα have been identified in humans, but the hepatic PPARα expression levels vary largely among individuals,23 and several PPARα mutations have been reported.24-25 Presently, the implication of PPARα in fatty liver disease and a possible modulation by PPARα agonists is still unclear. Magnetic resonance imaging analysis of liver fat in 15 type 2 diabetic patients has failed to show any changes in response to fenofibrate.26 A pilot study in non-alcoholic fatty liver disease patients has shown that treatment with fenofibrate improves metabolic syndrome parameters, including the lipid profile, and has beneficial effects on certain liver function parameters, but its impact on liver histology was small.27 Another study has suggested a beneficial effect of fenofibrate treatment, particularly in combination with statins, in reducing fatty liver disease.28 Additional studies are needed to evaluate the impact of PPARα agonists on non-alcoholic fatty liver disease, and in particular on the inflammatory component of NASH.

We showed that PPARα is required for the fenofibrate-induced improvement of dyslipidemia in apoE2-KI mice and a single PPARα allele is sufficient to mediate this effect, showing that PPARα gene level does not determine the plasma lipid-response of fenofibrate. In the Lower Extremity Arterial Disease Event Reduction trial, PPARα gene variation did not influence the magnitude of plasma TG lowering in response to bezafibrate, whereas genetic variation in the PPARα gene affected the changes in plasma fibrinogen, an inflammatory response marker, to fibrate treatment.29 Together with our data, variation in PPARα gene activity or expression level appears of lesser impact on the lipid response to fibrate but of higher impact on the inflammatory response.

Because growing evidence links fatty liver disease to cardiovascular disease19 and because apoE2-KI mice develop dyslipidemia and NASH, as well as atherosclerosis, we also assessed the role of PPARα on vascular inflammation and atherosclerosis. Interestingly, despite the more severe NASH progression, PPARα+/+, PPARα−/−, and PPARα−/− apoE2-KI mice developed quantitatively similar atherosclerotic lesion areas (as assessed by lipid, macrophage, and MCP-1 content of the atherosclerotic plaques), indicating that PPARα does not modulate atherogenesis or vascular inflammation in apoE2-KI mice. This result is surprising because a study performed in LDLR-deficient mice demonstrated that macrophage PPARα confers antiatherogenic effects via modulation of macrophage cholesterol trafficking and inflammatory activity.11 Similar to the case in apoE2-KI mice, plasma lipid concentrations were not modified by PPARα deficiency in these LDLR-deficient mice, indicating that differences in plasma lipids do not explain the observed discrepancies of lesion formation between the two models. The lesions in apoE2-KI mice consist mainly of foam cells, as occurs in the initial stages of atherogenesis in humans, whereas LDLR-deficient mice develop more advanced atherosclerotic plaques.30 Thus, the PPARα gene may not modulate the first stages of lesion formation but could influence the progression of atherosclerosis to more complex stages. Surprisingly, PPARα deficiency in apoE-deficient mice, another mouse model of atherosclerosis characterized by a wide spectrum of lesions going from fatty streaks to fibro-proliferative lesions,31 resulted in a protection against atherogenic lipid profile characterized by higher levels of TG and TC.9 However, in contrast to apoE2-KI mice, apoE-deficient mice do not respond to PPARα agonists as humans do, displaying no change17 or an increase12 in plasma lipids on fibrate treatment, and therefore may not be a suitable model to study the impact of PPARα agonists and possibly other lipid-lowering drugs on atherosclerosis.30,32

PPARα activation with fenofibrate protected Western diet-fed PPARα+/+ but not PPARα−/− apoE2-KI mice against atherosclerosis progression, showing PPARα dependency of the response to fenofibrate. Treatment of PPARα+/− apoE2-KI mice with fenofibrate resulted in an intermediary level of atheroprotection and vascular antiinflammatory response (assessed by MOMA-2 and MCP-1 staining). Thus, the PPARα gene level dose-dependently controls the response to its agonist on vascular inflammation and atherogenesis, despite a similar plasma lipid response in PPARα+/+ and PPARα−/− apoE2-KI mice. These observations suggest that atheroprotection on PPARα activation, in addition to being determined by the plasma lipid concentrations, also depends on other parameters, such as inflammation in the artery wall and the liver.

The pathophysiological role of PPARα in atherosclerosis has been investigated using 2 different and complementary strategies: genetic deficiency and a pharmacological intervention. PPARα deficiency did not result in the opposite phenotype of fenofibrate-induced activation of PPARα on atherosclerotic lesion areas. It is well established that nuclear-receptor deficiency does not always mirror ligand activation, which may be due to possible compensation mechanisms or the absence of active repression of target genes by the unliganded nuclear receptor via corepressor recruitment. However, the effects of fenofibrate did require PPARα expression, because it was absent in PPARα−/− apoE2-KI mice.

In the Lopid Coronary Angiography Trial study, the PPARα Val162 allele was associated with reduced progression of atherosclerosis, whereas the intron C allele was associated with greater progression of atherosclerosis.29 In vitro, it has been shown that the Val162 variant displays higher PPAR response element–dependent transcriptional activity,24,33 whereas the intron 7 C allele was hypothesized to be associated with lower expression levels of PPARα.29 Collectively, these results suggest that variations in PPARα gene level or activity are associated with differential progression of atherosclerosis in humans. Neither PPARα variant influenced plasma lipid concentrations in either study, suggesting that, in line with our results, PPARα gene variation may influence atherosclerosis via mechanisms complementary to the PPARα regulation of plasma lipid concentrations. Finally, because PPARα agonists may selectively modulate the different activities of PPARα (selective PPAR modulator effect),34 the effects of PPARα variants on the response to its agonists could be different according to the PPARα agonist used. Thus, it will be of interest to study the impact of PPARα variants on the responses to different agonists on fatty liver disease and to assess the association of modifications of lipid
metabolism and inflammatory parameters with PPARα polymorphisms.

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Disclosures
None.

References

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Peroxisome Proliferator–Activated Receptor-α Gene Level Differently Affects Lipid Metabolism and Inflammation in Apolipoprotein E2 Knock-In Mice

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Liver PPARα mRNA levels (A), liver weight (B) and liver TC (C) were measured in PPARα+/+, PPARα+/- and PPARα-/- apoE2-KI mice. N=11 mice/group. Results are expressed as means ± SE. NS=Non Significant. ##p<0.01, ### p<0.001 versus PPARα+/+ apoE2-KI mice.
Primary hepatocytes were isolated from PPARα+/+, PPARα+/− and PPARα−/− apoE2-KI mice. A-B, Hepatocytes were in vitro treated with GW647 (600 nM) or vehicle (CON, 0.1% Me2SO) for 24h. C-D, Hepatocytes were in vitro treated with GW647 (600 nM) or vehicle (0.1% Me2SO) for 24h and stimulated with LPS (100 ng/mL) for an additional 6-h period.

RNAs were extracted and subjected to quantitative RT-PCR analysis to measure VLCAD (A), LCAD (B), MCP-1 (C) and VCAM-1 (D) mRNA levels.

* p<0.05, ** p<0.001 versus CON; *p<0.05, **p<0.01, ***p<0.001 versus GW647-treated PPARα+/+ apoE2-KI cells.