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Peroxisome proliferator-activated receptor-alpha gene level differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice

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Abstract

Objective

Peroxisome Proliferator-Activated Receptor α (PPAR α) is a ligand-activated transcription factor which controls lipid metabolism and inflammation. PPAR α is activated by fibrates, hypolipidemic drugs used in the treatment of dyslipidemia. Previous studies assessing the influence of PPAR α agonists on atherosclerosis in mice yielded conflicting results and the implication of PPAR α therein has not been assessed. The human apoE2 knock-in (apoE2-KI) mouse is a model of mixed dyslipidemia, atherosclerosis and non-alcoholic steatohepatitis (NASH). The aim of this study was, using homo- and heterozygous PPAR α -deficient mice, to analyze the consequences of quantitative variations of PPAR α gene levels and its response to the synthetic PPAR α agonist fenofibrate, on NASH and atherosclerosis in apoE2-KI mice.

Methods and results

Wildtype (+/+), heterozygous (+/-) and homozygous (-/-) PPAR α -deficient mice in the apoE2-KI background were generated and submitted to a western diet supplemented or not with fenofibrate. Western diet-fed PPAR α -/- apoE2-KI mice displayed an aggravation of liver steatosis and inflammation compared to PPAR α +/+ and PPAR α +/- apoE2-KI mice, indicating a role of PPAR α in liver protection. Moreover, PPAR α expression was required for the fenofibrate-induced protection against NASH. Interestingly, fenofibrate treatment induced a similar response on hepatic lipid metabolism in PPAR α +/+ and PPAR α +/- apoE2-KI mice, whereas, for a maximal anti-inflammatory response, both alleles of the PPAR α gene were required.

Surprisingly, atherosclerosis development was not significantly different between PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice. However, PPAR α gene level determined both the anti-atherosclerotic and vascular anti-inflammatory responses to fenofibrate in a dose-dependent manner.

Conclusions

These results demonstrate a necessary, but quantitatively different role of PPAR α in the modulation of liver metabolism, inflammation and atherogenesis.

MESH Keywords Analysis of Variance ; Animals ; Anti-Inflammatory Agents ; pharmacology ; Aorta ; drug effects ; metabolism ; pathology ; Apolipoprotein E2 ; genetics ; metabolism ; Atherosclerosis ; drug therapy ; genetics ; metabolism ; pathology ; Disease Models, Animal ; Fatty Liver ; drug therapy ; genetics ; metabolism ; pathology ; Female ; Fenofibrate ; pharmacology ; Gene Expression Regulation ; Gene Knock-In Techniques ; Heterozygote ; Homozygote ; Humans ; Hypolipidemic Agents ; pharmacology ; Inflammation ; drug therapy ; genetics ; metabolism ; pathology ; Lipid Metabolism ; drug effects ; genetics ; Lipids ; blood ; Liver ; drug effects ; metabolism ; pathology ; Mice ; Mice, Inbred C57BL ; Mice, Knockout ; Mice, Transgenic ; PPAR alpha ; agonists ; genetics ; metabolism

Author Keywords PPARalpha ; fatty liver disease ; atherosclerosis ; inflammation ; lipid metabolism ; murine model

Introduction

Fibrates are lipid-lowering drugs widely used in clinical practice to treat dyslipidemia [1]. Studies performed in Peroxisome Proliferator-Activated Receptor α (PPAR α)-deficient mice have demonstrated that the hypolipidemic effects of fibrates are due to activation of PPAR α , a ligand-activated transcription factor which modulates lipid metabolism [2]. PPAR α 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, PPAR α binds as a heterodimer with the Retinoid X Receptor (RXR) to PPAR response elements (PPRE) in the promoters of genes implicated in lipid and lipoprotein metabolism. In addition to its effects on lipid metabolism, PPAR α also inhibits pro-inflammatory pathways by negatively interfering with other signalling pathways such as NF- κ B, STATs (Signal Transducer and Activator of Transcription) or AP-1 (Activator

Protein-1). Consequently, through its effects on lipid metabolism and inflammation, PPAR α may modulate pathophysiological pathways implicated in fatty liver disease and atherosclerosis. Data concerning the implication of PPAR α in liver steatosis and inflammation in humans is scarce. However, it has been shown that PPAR α agonist treatment decreases non-alcoholic steatohepatitis (NASH) development in wild-type mice fed a methionine choline-deficient (MCD) diet [3, 4] and apoE2 knock-in (apoE2-KI) mice or foz/foz mice fed a high-fat diet [5, 6]. Moreover, PPAR α is expressed in many cell types found in the atherosclerotic lesion such as macrophages, endothelial cells and smooth muscle cells (SMC) [7]. *In vitro* and *in vivo* studies have suggested that fibrates could exert anti-atherogenic actions by improving lipid abnormalities and/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 (TG) and low high density lipoprotein-cholesterol (HDL-C) levels [1]. 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 [8]. However, the role of PPAR α in atherosclerosis is controversial, since PPAR α -deficiency protects against atherosclerosis progression in apoE-deficient mice [9] and Tsukuba hypertensive mice [10], whereas macrophage-specific PPAR α expression protects Low Density Lipoprotein-Receptor (LDL-R)-deficient mice from atherosclerosis [11], and PPAR α agonist treatment increases [12] or decreases [8, 13, 14] atherosclerosis development in different murine models.

In the present study, we aimed to analyze the consequences of PPAR α -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 PPAR α in the response to fenofibrate treatment. Therefore, wildtype (+/+), heterozygous (+/-) and homozygous PPAR α -deficient (-/-) apoE2-KI mice were fed a western diet with or without fenofibrate during 9 weeks. Surprisingly, homozygous PPAR α -deficiency did not modify plasma lipid concentrations, however it aggravated liver steatosis and inflammation. Interestingly, PPAR α gene levels differently affected the response to fenofibrate on hepatic lipid metabolism and inflammation. In addition, whereas PPAR α -deficiency did not influence atherosclerosis development, the PPAR α gene level dose-dependently controlled the response to fenofibrate on vascular inflammation and atherogenesis.

Materials and Methods

Animals

Homozygous PPAR α -deficient mice on the C57BL/6 background [15] were crossed with homozygous human apoE2-KI mice [16], which express human apoE2 instead of mouse *apoe* under control of the endogenous promoter, to generate PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice. Three month-old female mice of the three genotypes (n=11 per group) were fed a Western diet containing 0.2 % cholesterol and 21% fat (wt/wt) (UAR, France) without (control group, CON) or with fenofibrate (FF) for 9 weeks. Based on food consumption, the dose 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, France).

Hepatic lipid analysis

Frozen liver tissue (50 mg) was homogenized in SET buffer (1 mL; sucrose 250 mM, EDTA 2 mM and Tris 10 mM), followed by two freeze-thaw cycles and three times passing 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

7 μ m frozen-cut liver sections were fixed in acetone and stained with Mac1 (M1/70) antibodies, as described [5].

Isolation of primary hepatocytes

Primary hepatocytes were isolated from the livers of fed mice, as previously described [17].

RNA extraction and quantitative PCR analysis

RNA, isolated from livers using the acid guanidinium thiocyanate/phenol/chloroform method [18], was reverse transcribed using random hexamer primers and Moloney murine leukemia virus-reverse transcriptase (Invitrogen, France). RNA levels were determined by real-time quantitative PCR 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, the hearts were perfused with cold Krebs-Ringer buffer and fixed in a solution containing 4 % phosphate-buffered paraformaldehyde. Serial 10 μ m-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 Mikroskopische und System GmbH, Germany). Cryosections from aortic lesions were stained with anti-mouse MOMA-2 (Santa Cruz Biotechnology) or anti-mouse MCP-1 (Santa Cruz Biotechnology). MCP-1 protein levels were semi-quantitatively scored for staining on lesions of 4 mice per group.

Statistical analysis

Results are expressed as the means $\pm$ 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 as statistically significant.

Results

Homozygous PPAR α -deficiency does not modify plasma lipid concentrations, but aggravates liver steatosis and inflammation in apoE2-KI mice fed a western diet

Female PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice were generated and PPAR α mRNA levels were found to be respectively intermediate and undetectable in the livers of PPAR α +/- and PPAR α -/- vs PPAR α +/+ apoE2-KI mice (supplemental figure 1A). These mice were fed a western diet which was previously shown to induce liver steatosis and inflammation [5]. After 9 weeks of western diet feeding, plasma TG and TC concentrations were similar in the three groups of mice (figure 1, A–B). Relative liver weight was not different between the three genotypes (supplemental figure 1B) and liver cholesterol content was slightly, but not significantly increased in PPAR α -/- apoE2-KI compared to PPAR α +/+ and PPAR α +/- apoE2-KI mice (supplemental figure 1C). PPAR α -/- 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 to PPAR α +/+ mice. Interestingly, heterozygous PPAR α +/- apoE2-KI mice displayed a similar phenotype as PPAR α +/+ mice (figure 1, C–D). These results demonstrate that PPAR α -deficiency does not modify plasma lipid concentrations, and only homozygous PPAR α -deficiency aggravates liver steatosis and macrophage content in western diet-fed apoE2-KI mice.

PPAR α activation improves plasma and hepatic lipid homeostasis in PPAR α +/+ and PPAR α +/-, but not PPAR α -/- apoE2-KI mice fed a western diet

To determine the role of the PPAR α gene level on the hepatic and plasma response to its agonist fenofibrate, female PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice were fed a western diet supplemented with fenofibrate for 9 weeks. The response to the PPAR α agonist was compared to the respective placebo-treated PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice whose values were set at 100%. As expected [8], fenofibrate treatment decreased plasma TC and TG concentrations (figure 2, A–B) and liver TG content (figure 2C) in PPAR α +/+ apoE2-KI mice. In marked contrast, fenofibrate-treated PPAR α -/- apoE2-KI mice did not exhibit any significant decrease in plasma TC and TG concentrations and liver TG levels compared to placebo-treated mice, showing that fenofibrate improves dyslipidemia and hepatic steatosis in a PPAR α -dependent manner in apoE2-KI mice (figure 2, A–C). Interestingly, fenofibrate reduced plasma TC and TG concentrations and liver TG levels to the same extent in PPAR α +/- apoE2-KI as in PPAR α +/+ apoE2-KI mice. Since PPAR α 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 translocase (FAT), very long chain acyl-CoA dehydrogenase (VLCAD), long chain acyl-CoA dehydrogenase (LCAD) and medium chain acyl-CoA dehydrogenase (MCAD) were measured (figure 3, A–D). Fenofibrate increased the hepatic expression levels of all these genes in PPAR α +/+ but not in PPAR α -/- apoE2-KI mice. However, in PPAR α +/- apoE2-KI mice, the expression of these genes increased to a similar extent as in PPAR α +/+ apoE2-KI mice upon fenofibrate treatment. A comparable induction of genes implicated in hepatic lipid metabolism was also observed in primary hepatocytes isolated from PPAR α +/+ and PPAR α +/- apoE2-KI mice and treated with the specific PPAR α agonist, GW647, whereas this induction was not observed in PPAR α -/- apoE2-KI hepatocytes (supplemental figure 2, A–B). Thus, only one allele of the PPAR α gene is required for an optimal response to fenofibrate on dyslipidemia and liver steatosis.

PPAR α activation decreases hepatic inflammation and macrophage content in PPAR α +/+, but not PPAR α +/- and PPAR α -/- apoE2-KI mice fed a western diet

Hepatic inflammation was analysed in the fenofibrate-treated mice. Fenofibrate treatment decreased the number of Mac-1 positive cells, indicative of the number of macrophages, in livers of PPAR α +/+ but not PPAR α +/- or PPAR α -/- apoE2-KI mice (figure 4, A–B). 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 4, C–E), were decreased in fenofibrate-treated PPAR α +/+ apoE2-KI mice. By contrast, fenofibrate did not influence the expression of these inflammatory markers in livers of PPAR α -/- apoE2-KI mice. Interestingly, fenofibrate-treated PPAR α +/- apoE2-KI mice exhibited an intermediary gene expression level of MCP-1, VCAM-1 and ICAM-1. Of note, repression of LPS-induced MCP-1 and VCAM-1 expression by the specific PPAR α agonist GW647 was most pronounced in isolated primary hepatocytes from PPAR α +/+ apoE2-KI mice, whereas an intermediary

response was seen in PPAR α +/- apoE2-KI cells (supplemental figure 2, C–D). Thus, both PPAR α alleles are necessary for an optimal inhibition of the hepatic inflammatory response by fenofibrate in apoE2-KI mice fed a western diet.

PPAR α gene levels do not influence atherogenesis, but determine the atheroprotective response to fenofibrate in apoE2-KI mice fed a western diet

Since non-alcoholic fatty liver disease is now considered a risk factor for cardiovascular disease [19], atherogenesis and vascular inflammation were assessed in PPAR α +/+, PPAR α +/- and PPAR α -/- 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 between PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice (figure 5, A–B). As expected [8], mean lesion area was significantly reduced by about 80 % in fenofibrate-treated PPAR α +/+ mice compared to controls (median: 0.030 mm² in treated mice vs 0.166 mm² in control mice, $p < 0.001$) (figure 5, A–B). By contrast, no effect was observed in fenofibrate-treated PPAR α -/- apoE2-KI mice (median: 0.137 mm² in treated mice vs 0.123 mm² in control mice, NS). Interestingly, treatment of PPAR α +/- apoE2-KI mice with fenofibrate resulted in a significant, intermediary decrease in atherosclerotic lesion area (median: 0.138 mm² in treated mice vs 0.185 in control mice, $p < 0.05$), indicating a dose-response effect of PPAR α 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 5, D–E) co-localized with oil-red-O staining, were intense and did not differ between PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice, in accordance with the comparable lesion areas between the three genotypes (figure 5A). Treatment with fenofibrate strongly decreased MOMA-2 and MCP-1 staining in the lesions of PPAR α +/+ but not PPAR α -/- apoE2-KI mice, indicating PPAR α -dependency. Interestingly, fenofibrate treatment of PPAR α +/- apoE2-KI mice resulted in an intermediary phenotype with a slight decrease of MOMA-2 and MCP-1 staining in the lesions. Thus, while PPAR α 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 mouse model of mixed dyslipidemia and NASH [16], we show that homozygous PPAR α -deficiency aggravates western diet-induced steatosis and inflammation in the liver. This result is consistent with the reported increased hepatic steatosis observed in PPAR α -deficient mice in response to the physiological stimulus fasting [20]–[22], further illustrating the role of PPAR α in hepatic lipid metabolism. In apoE2-KI mice, aggravation of NASH induced by PPAR α -deficiency was not accompanied by changes of plasma TC and TG concentrations nor atherosclerosis lesion development, suggesting a dissociation between both pathological states. We also analyzed the effects of PPAR α gene level on the response to treatment with the PPAR α agonist fenofibrate on NASH. Fenofibrate treatment protected against NASH in western diet-fed PPAR α +/+ but not PPAR α -/- apoE2-KI mice, showing that the previously reported effects of fenofibrate on NASH in apoE2-KI mice [5] occur via PPAR α . Interestingly, fenofibrate treatment of PPAR α +/- apoE2-KI mice resulted in decreased hepatic steatosis to an extent similar as PPAR α +/+ 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 PPAR α +/- and PPAR α +/+ apoE2-KI mice. However, in contrast with the regulation of lipid metabolism, the anti-inflammatory response to fenofibrate in the liver depends on both PPAR α alleles. Indeed, fenofibrate treatment strongly reduced inflammation and macrophage content in livers of PPAR α +/+ apoE2-KI mice (associated with decreased expression levels of MCP-1, ICAM-1, VCAM-1), whereas little or no response to fenofibrate was observed in livers of PPAR α +/- apoE2-KI mice. Similar observations were made in isolated heterozygous primary hepatocytes treated with the specific PPAR α agonist GW647. Thus, PPAR α gene level differently influences fenofibrate's effects on hepatic steatosis and inflammation. A single PPAR α allele is sufficient for an optimal response of lipid metabolism to fenofibrate, whereas both alleles are required to obtain maximal anti-inflammatory effects in the liver.

Until now, no null mutations of PPAR α have been identified in humans, but the hepatic PPAR α expression levels vary largely among individuals [23] and several PPAR α mutations have been reported [24 , 25]. Presently, the implication of PPAR α in fatty liver disease and a possible modulation by PPAR α agonists is still unclear. Magnetic Resonance Imaging (MRI) analysis of liver fat in fifteen type 2 diabetic patients has failed to show any changes in response to fenofibrate [26]. A pilot study in NAFLD 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]. Further studies are needed to evaluate the impact of PPAR α agonists on NAFLD, and in particular on the inflammatory component of NASH.

We show that PPAR α is required for the fenofibrate-induced improvement of dyslipidemia in apoE2-KI mice and a single PPAR α allele is sufficient to mediate this effect, showing that PPAR α gene level does not determine the plasma lipid-response of fenofibrate. In the Lower Extremity Arterial Disease Event Reduction (LEADER) trial, PPAR α gene variation did not influence the magnitude of plasma

TG lowering in response to bezafibrate, whereas genetic variation in the PPAR α gene affected the changes in plasma fibrinogen, an inflammatory response marker, to fibrate treatment [29]. Together with our data, variation in PPAR α gene activity or expression level appears of lesser impact on the lipid response to fibrate, but of higher impact on the inflammatory response.

Since growing evidence links fatty liver disease to cardiovascular disease [19] and since apoE2-KI mice develop dyslipidemia and NASH as well as atherosclerosis, we also assessed the role of PPAR α on vascular inflammation and atherosclerosis. Interestingly, despite the more severe NASH progression, PPAR α +/+, PPAR α +/- and PPAR α -/- apoE2-KI mice developed quantitatively similar atherosclerotic lesion areas (as assessed by lipid, macrophage and MCP-1 content of the atherosclerotic plaques), indicating that PPAR α does not modulate atherogenesis and vascular inflammation in apoE2-KI mice. This result is surprising since a study performed in LDLR-deficient mice demonstrated that macrophage PPAR α confers anti-atherogenic effects via modulation of macrophage cholesterol trafficking and inflammatory activity [11]. Similar as in apoE2-KI mice, plasma lipid concentrations were not modified by PPAR α -deficiency in these LDLR-deficient mice, indicating that differences in plasma lipids do not explain the observed discrepancies of lesion formation between both models. The lesions in apoE2-KI mice mainly consist 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 PPAR α gene may not modulate the first stages of lesion formation, but could influence the progression of atherosclerosis to more complex stages. Surprisingly, PPAR α -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 atherosclerotic lesion formation upon western diet feeding, notwithstanding a pro-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 PPAR α agonists as humans, displaying no change [13] or an increase [12] in plasma lipids upon fibrate treatment, and may therefore not be a suitable model to study the impact of PPAR α agonists and possibly other lipid-lowering drugs on atherosclerosis [30 , 32].

PPAR α activation with fenofibrate protected western diet-fed PPAR α +/+ but not PPAR α -/- apoE2-KI mice against atherosclerosis progression, showing PPAR α -dependency of the response to fenofibrate. Treatment of PPAR α +/- apoE2-KI mice with fenofibrate resulted in an intermediary level of atheroprotection and vascular anti-inflammatory response (assessed by MOMA-2 and MCP-1 staining). Thus, the PPAR α gene level dose-dependently controls the response to its agonist on vascular inflammation and atherogenesis, despite a similar plasma lipid response in PPAR α +/+ and PPAR α +/- apoE2-KI mice. These observations suggest that atheroprotection upon PPAR α 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 PPAR α in atherosclerosis has been investigated using two different and complementary strategies: genetic deficiency and a pharmacological intervention. PPAR α -deficiency did not result in the opposite phenotype of fenofibrate-induced activation of PPAR α 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 and/or the absence of active repression of target genes by the unliganded nuclear receptor via co-repressor recruitment. However, the effects of fenofibrate did require PPAR α expression, since it was absent in PPAR α -/- apoE2-KI mice.

In the Lipid Coronary Angiography Trial (LOCAT) study, the PPAR α V162 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 V162 variant displays higher PPARE-dependent transcriptional activity [24 , 33], whereas the intron 7 C allele was hypothesized to be associated with lower expression levels of PPAR α [29]. Collectively, these results suggest that variations in PPAR α gene level or activity are associated with differential progression of atherosclerosis in humans. Both PPAR α variants did not influence plasma lipid concentrations in either study, suggesting that, in line with our results, PPAR α gene variation may influence atherosclerosis via mechanisms complementary to the PPAR α regulation of plasma lipid concentrations. Finally, since PPAR α agonists may selectively modulate the different activities of PPAR α (SPPARM effect) [34], the effects of PPAR α variants on the response to its agonists could be different according to the PPAR α agonist used. Thus, it will be of interest to study the impact of PPAR α 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 PPAR α polymorphisms.

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Footnotes:

Disclosure None

References:

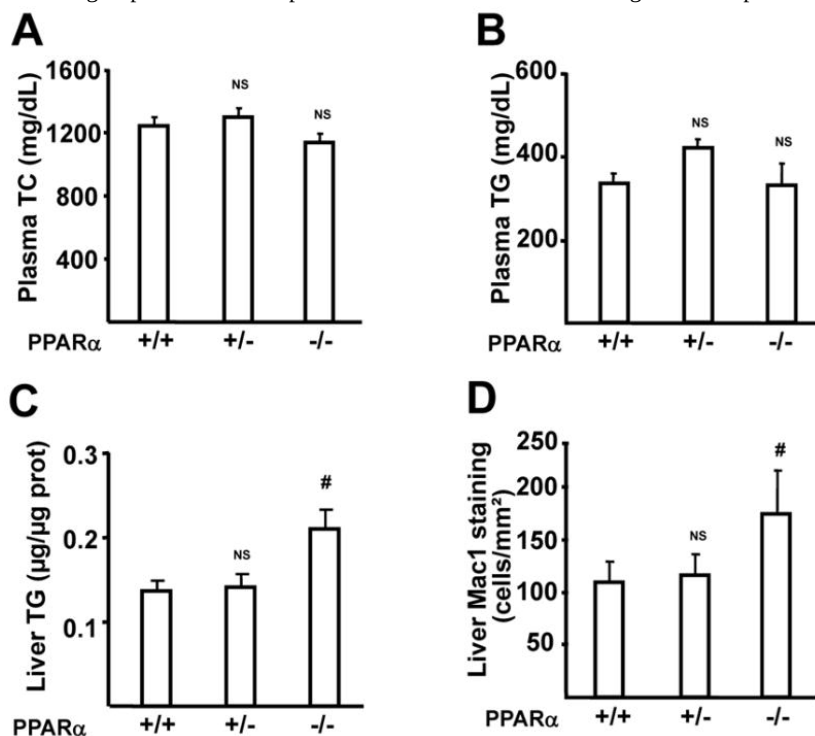
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Figure 1

Homozygous PPAR α -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 PPAR α +/+, PPAR α +/- and PPAR α -/- 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 $\pm$ SE. NS=Non Significant. # p<0.05 versus PPAR α +/+ apoE2-KI mice.

**Figure 2**

Fenofibrate improves plasma and hepatic lipid homeostasis in PPAR α +/+ and PPAR α +/-, but not PPAR α -/- apoE2-KI mice

Plasma TC (A) and TG (B) levels as well as liver TG content (C) in female PPAR α +/+, PPAR α +/- and PPAR α -/- 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 $\pm$ SE. * p<0.05, ***p<0.001 vs untreated mice; # p<0.05, ###p<0.001 versus fenofibrate-treated PPAR α +/+ apoE2-KI mice.

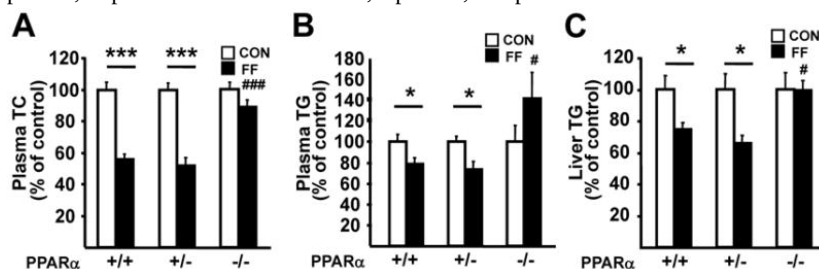
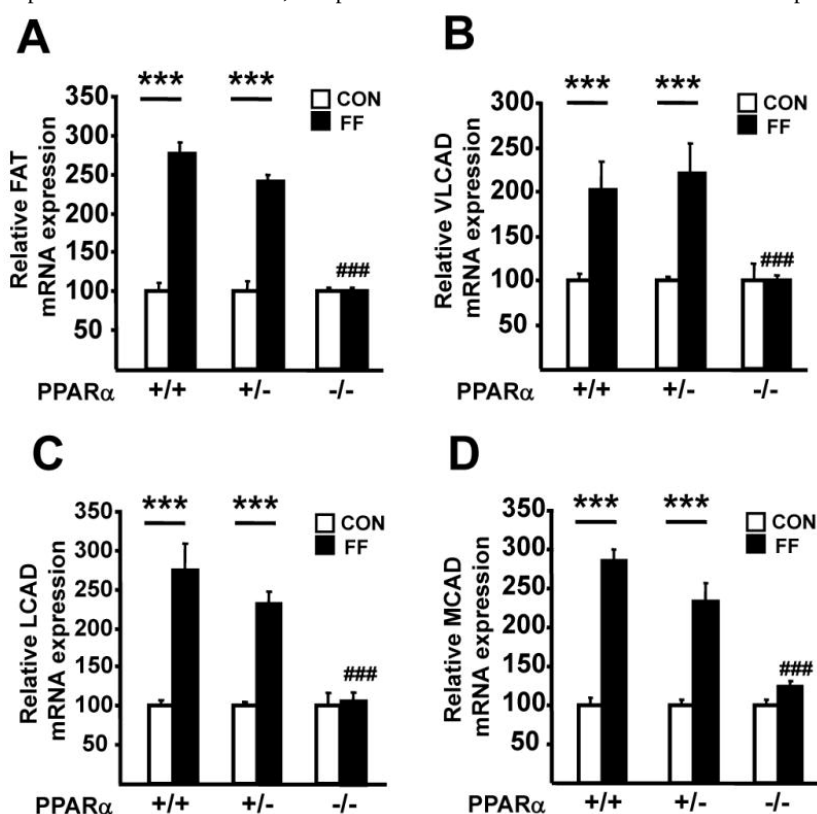


Figure 3

Fenofibrate increases the expression of fatty acid uptake and oxidation genes in PPAR α ^{+/+} and PPAR α ^{+/-}, but not PPAR α ^{-/-} apoE2-KI mice. Hepatic mRNA levels of FAT (A), VLCAD (B), LCAD (C), MCAD (D) in female PPAR α ^{+/+}, PPAR α ^{+/-} and PPAR α ^{-/-} 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 $\pm$ SE. ***p<0.001 vs untreated mice; ###p<0.001 versus fenofibrate-treated PPAR α ^{+/+} apoE2-KI mice.

**Figure 4**

Fenofibrate decreases hepatic inflammation and macrophage content in PPAR α ^{+/+}, but not PPAR α ^{+/-} and PPAR α ^{-/-} apoE2-KI mice. Liver MAC-1 staining (A) with representative microphotographs (B) and hepatic mRNA levels of MCP-1 (C), ICAM-1 (D) and VCAM-1 (E) in female PPAR α ^{+/+}, PPAR α ^{+/-} and PPAR α ^{-/-} 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 $\pm$ SE. *p<0.05, ***p<0.001 vs untreated mice; #p<0.05, ##p<0.01, ###p<0.001 versus fenofibrate-treated PPAR α ^{+/+} apoE2-KI mice.

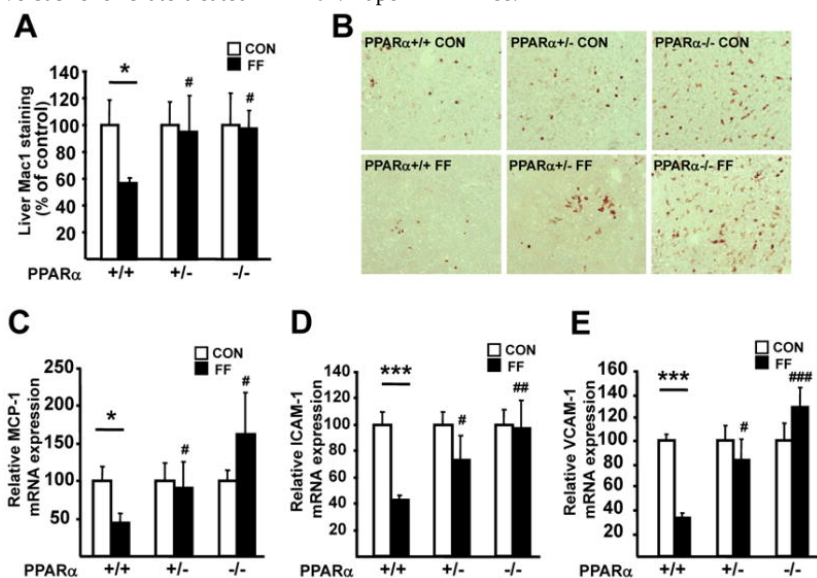


Figure 5

PPAR α gene levels do not influence atherogenesis, but determines the atheroprotective response to fenofibrate in apoE2-KI mice. Atherosclerotic lesion area (A) in the aorta of female PPAR α +/+, PPAR α +/- and PPAR α -/- 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 analysed sections and each symbol represents one mouse. The horizontal bar corresponds to the median of the values. n=11 mice/group. *p<0.05, ***p<0.001 versus untreated mice; ### p<0.001 versus fenofibrate-treated PPAR α +/+ apoE2-KI mice. Representative microphotographs showing Oil Red-O staining (B) or MOMA-2 stained macrophages (C) in the atherosclerotic lesions. Representative photomicrographs showing MCP-1 staining of atherosclerotic lesions (D) and score of MCP-1 staining (E) graded as: (-) not detected, (+) slight, (++) marked, (+++) pronounced expression in lesions. n=4 mice/group. (B, scale bar = 500 μ m; C-D, scale bar = 100 μ m).

