Reduction of Atherosclerosis by the Peroxisome Proliferator-activated Receptor α Agonist Fenofibrate in Mice*

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Several clinical and angiographic intervention trials have shown that fibrate treatment leads to a reduction of the coronary events associated with atherosclerosis. Fibrates are ligands for peroxisome proliferator-activated receptor α (PPARα) that modulate risk factors related to atherosclerosis by acting at both systemic and vascular levels. Here, we investigated the effect of treatment with the PPARα agonist fenofibrate (FF) on the development of atherosclerotic lesions in apolipoprotein (apo) E-deficient mice and human apoA-I transgenic apoE-deficient (hapoA-I Tg × apoE-deficient) mice fed a Western diet. In apoE-deficient mice, plasma lipid levels were increased by FF treatment with no alteration in the cholesterol distribution profile. FF treatment did not reduce atherosclerotic lesion surface area in the aortic sinus of 5-month-old apoE-deficient mice. By contrast, FF treatment decreased total cholesterol and esterified cholesterol contents in descending aortas of these mice, an effect that was more pronounced in older mice exhibiting more advanced lesions. Furthermore, FF treatment reduced MCP-1 mRNA levels in the descending aortas of apoE-deficient mice, whereas ABCA-1 expression levels were maintained despite a significant reduction of aortic cholesterol content. In apoE-deficient mice expressing a human apoA-I transgene, FF increased human apoA-I plasma and hepatic mRNA levels without affecting plasma lipid levels. This increase in human apoA-I expression was accompanied by a significant reduction in the lesion surface area in the aortic sinus. These data indicate that the PPARα agonist fenofibrate reduces atherosclerosis in these animal models of atherosclerosis.

Atherosclerosis is a complex vascular disease initiated by abnormal accumulation of plasma lipoproteins in the sub-endothelial space of the blood vessels (1). Macrophages within the vascular wall take up cholesterol, resulting in their transformation into foam cells. Concomitant activation of the different vascular cell types leads to a chronic inflammatory response (2). Numerous epidemiological studies have highlighted the importance of metabolic risk factors, such as dyslipidemia, in these pathological changes. Indeed, the relative abundance of the different lipoprotein fractions appears critical as increased plasma levels of atherogenic particles (such as low density lipoprotein (LDL)) or decreased levels of the anti-atherogenic lipoprotein fraction (high density lipoprotein (HDL)) are well known risk factors for the development of atherosclerosis (3, 4).

Fibrates are hypolipidemic drugs that are currently used to treat hypertriglyceridemia and mixed dyslipidemia (5). They are efficient in lowering elevated plasma triglycerides and LDL-cholesterol, and increasing HDL-cholesterol concentrations. Several clinical and angiographic intervention trials have shown that fibrate treatment leads to a reduction of coronary events (6, 7) and delays progression of coronary atherosclerosis (8–10).

Fibrates are ligands for peroxisome proliferator-activated receptor α (PPARα), a transcription factor belonging to the nuclear receptor superfamily, which mediates their lipid-normalizing effects (11). Studies performed using PPARα-deficient mice have demonstrated the obligatory role of PPARαs in mediating the effects of fibrates on lipid and lipoprotein metabolism (12). Although fibrates decrease plasma triglycerides both in man and rodents, plasma concentrations of HDL-cholesterol and apoA-I are regulated in an opposite manner in rodents (decrease) and humans (increase) (13). As in humans, fibrate-activated PPARαs induce the expression of human apoA-I in transgenic mice overexpressing the human apoA-I (hapoA-I) gene (hapoA-I Tg), thereby increasing HDL production (14). Furthermore, PPARαs are expressed in the major cell types found in the atherosclerotic lesion (macrophages, endothelial, and smooth muscle cells) (15–17), and increasing evidence indicates that PPARα agonists exert a potent anti-inflammatory action directly at the level of the vascular wall (18, 19). Therefore, both lipid-related and direct vascular activities may account for the overall beneficial action of fibrates on atherosclerosis.

The aim of this study was to investigate the influence of fibrate treatment on atherosclerosis in vivo. ApoE-deficient mice were treated with the PPARα agonist fenofibrate (FF), and the lesion surface area was determined in the aortic sinus and aortic cholesterol content in the descending aorta. Furthermore, to assess the influence of hapoA-I induction by FF on atherosclerosis, human apoA-I transgenic apoE-deficient

1 The abbreviations used are: LDL, low density lipoprotein; HDL, high density lipoprotein; PPARα, peroxisome proliferator-activated receptor α; hapoA-I, human apoA-I; apo, apolipoprotein; FF, fenofibrate; VLDL, very low density lipoprotein; IDL, intermediery density lipoprotein; PBS, phosphate-buffered saline; ABCA-1, ATP-binding cassette A-1; MCP-1, monocyte chemotactrant protein-1.
Together these data indicate that fibrate treatment can modulate lesion surface area in apoA-I Tg mice. Decrease was associated with lowered aortic monocyte chemotactant protein-1 (MCP-1) and unchanged ATP-binding cassette A-1 (ABCA-1) mRNA levels in the descending aortas of the fenofibrate-treated mice. Furthermore, induction of human apoA-I by FF treatment resulted in a decreased aortic sinus lesion surface area in apoA-I Tg × apoE-deficient mice. Altogether these data indicate that fibrate treatment can modulate atherogenesis in vivo in a manner independent of changes in absolute plasma lipid levels.

**EXPERIMENTAL PROCEDURES**

**Animals**—Homozygous human apoA-I transgenic (20) and apoE-deficient C57Bl/6 mouse strains were obtained from Charles River-Iffa Credo (L’Arbresle, France). Human apoA-I transgenic mice were crossed with apoE-deficient mice to generate heterozygous human apoA-I transgenic mice in a heterozygous apoE-deficient background. These mice were further mated with homozygous apoE-deficient mice to generate human apoA-I transgenic mice in a homozygous apoE-deficient background (apoA-I Tg × apoE-deficient mice). Human apoA-I transgene expression was analyzed in plasma by enzyme-linked immunosorbent assay using specific antibodies (14). Homozygous apoE-deficient mice were identified after determination of total cholesterol levels. After weaning, apoE-deficient and hapaA-I Tg × apoE-deficient male mice were maintained on a standard pellet rodent chow (Usine d’Alimentation Rationelle, Villelmoisson, France).

In a first experiment, 10-week-old apoE-deficient male mice were given a Western diet containing 20% (g/100 g) fat and 0.2% cholesterol for 2 weeks before the treatment period. Then each genotype of mice was divided into two groups (12 mice/group); one was treated with fenofibrate (FF) added to the Western diet (0.05% w/w corresponding to 100 mg/kg/day), and the other group (control) was maintained on the Western diet. The treatment period lasted 8 weeks. Animals were housed in a facility with a light/dark cycle (12 h/12 h) and had free access to food and water. At the end of the study, animals were anesthetized by ether inhalation, and blood was collected after a 4-h fasting period by retro-orbital puncture. Animals were euthanized by cervical dislocation, and tissues were collected for further analysis.

In a second experiment, apoE-deficient male mice on a C57Bl/6 background were purchased from Jackson Laboratories (Bar Harbor, ME) and bred at Merck Research Laboratories. At weaning they were fed and maintained on Western diet. Starting at 20 weeks of age, groups of 10 mice were treated by gavage with either vehicle or FF (100 mg/kg/day suspended in 0.5% methylcellulose solution). After 6 weeks of treatment, mice were euthanized, and their aortic cholesterol and cholesteryl ester contents were determined (see below).

Ten-week-old hapaA-I Tg × apoE-deficient male mice (12 mice/group) were given a Western diet for 2 weeks before treatment with either control or FF (0.05% w/w corresponding to 100 mg/kg/day) for another 8 weeks. At the end of the study, blood and tissue samples were collected for further analysis. All animal experiments were approved by the Institutional Animal Care and Use Committees from both Institut Pasteur de Lille and Merck Research Laboratories.

**Lipids, Apolipoprotein, and Lipoprotein Measurements**—Plasma cholesterol and triglyceride concentrations were determined by an enzymatic assay adapted to microtiter plates using commercially available reagents (Roche Molecular Biochemicals, Germany). Plasma human apoA-I levels were measured by an immunonephelometric assay using specific monoclonal antibodies (Roche Molecular Biochemicals). Plasma lipoprotein profiles were obtained by fast-protein liquid chromatography. This system allows the separation of the three major lipoprotein classes, very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and LDL according to their size. 200 µl of pooled plasma samples was injected onto a Superose 6HR 10/30 column (Amersham Biosciences, Sweden), and lipoproteins were separated at a flow rate of 0.28 ml/min with a 0.5 M potassium phosphate buffer (pH 7.4) containing 0.01% EDTA and 0.01% NaN3. The effluent was monitored at 280 nm and collected in 0.27-ml fractions. Cholesterol concentration was measured in each fraction.

**RNA Analysis**—Total RNA was isolated from each liver or descending aorta by the acid guanidinium thiocyanate/phenol/chloroform method (21). Dot blot analysis of 1 µg of total RNA was performed using the human apoA-I cDNA as probe. Rat 36B4 cDNA was used as a control probe. mRNA were quantified using a GS525 phosphorimaging device (Bio-Rad Laboratories, Inc., Hercules, CA). Human apoA-I mRNA levels were expressed as fold induction, and an arbitrary value of 1 was assigned to the average of the control. ABCA-1 and MCP-1 mRNA levels were determined by reverse transcription followed by real-time PCR using a LightCycler (Roche Diagnostics, Meylan, France) and normalized to a β-actin internal control. The following specific primer pairs were used: ABCA-1 sense, 5’-GGT CTA AGA CGC TCA GAG GC-3’; ABCA-1 antisense, 5’-CAC GAG CTC GAC AGT AG-3’; MCP-1 sense, 5’-TGT CAT GCT TCT GCC CCT GC-3’; MCP-1 antisense, 5’-GGG TCA GCA CAG CCT TTC TG-3’. Reactions were carried out using the LightCycler-FastStart DNA Master SYBRGreen mix (3 mM MgCl2) as recommended by the manufacturer.

**Analysis of Atherosclerotic Lesions in the Aortic Sinus**—Hearts and ascending aortas of animals were fixed in formaldehyde, and serial 10-µm-thick cryosections were cut from the aortic arch to the ventricles for quantitative analysis of atherosclerosis. The distance from each section was measured. The sections were stained with Oil Red O and counterstained with hematoxylin. The atherosclerotic lesion and Oil Red O-stained areas of each section were quantified using a computer-assisted video imaging system (Leica Mikroskop System, Wetzlar, Germany). To avoid morphological heterogeneity between mice, the expression of average atherosclerotic lesion areas in the region of the aortic sinus, from the appearance to the disappearance of the aortic valves, was normalized at 10 equal sections.

**Aortic Cholesterol Measurement in the Descending Aorta**—After collection of the blood, the vasculature was gently perfused through the left ventricle with cold PBS and 5 mM EDTA. For collection of aorta for biochemical analysis, all branches and adipose tissue connected to the aorta were removed, and each aorta was carefully excised from the aortic root to the right renal artery. The aortas were kept briefly on ice in PBS and then blotted dry, weighed, minced, and extracted with chloroform/methanol (2:1), according to the method of Folch et al. (22).

The lipid extracts were dried down, resuspended quantitatively in chloroform/methanol (2:1), and stored at −20°C until the time of assay. Total and free cholesterol levels in the aortic extracts were determined with an enzymatic fluorometric assay as previously described (23). Briefly, the solvent was evaporated and the lipid residue was dissolved in 100 µl of reagent grade ethanol. Aliquots of cholesterol (Aldrich) and cholesterol oleate (Aldrich) standard solutions prepared in chloroform/methanol (1:1) were treated similarly. To determine free cholesterol, samples and standards were incubated for 1 h at 37°C. A total volume of 1.0 ml of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.03% Triton X-100 and 0.9 mM sodium cholate. Cholesterol oxide (Roche Molecular Biochemicals), peroxidase (Roche Molecular Biochemicals), and p-hydroxyphenylacetic acid (Aldrich) were added for an additional 1-h incubation at 37°C. The fluorescent product was measured (excitation 325 nm, emission 415 nm). For total cholesterol determinations, cholesterol esterase (Calbiochem) was included in the first incubation step, and cholesteryl oleate was used as a standard. The esterified cholesterol content in each sample was calculated by subtracting the value of free cholesterol from that for total cholesterol. Samples of individual aortas were run in duplicate at two different concentrations. All values are expressed as nmol/g aorta.

**Statistical Analyses**—A nonparametric Mann-Whitney test was used to analyze for significant differences between the experimental groups. Analysis of variance (ANOVA) and Tukey post-hoc tests were used for analysis of the lesion area data.

**RESULTS**

**FF Treatment Does Not Decrease Plasma Lipid Levels or Affect Cholesterol Distribution Profiles in ApoE-deficient Mice**—Ten-week-old apoE-deficient mice were fed a Western diet 2 weeks before treatment with either a control or FF (0.05% w/w corresponding to 100 mg/kg/day)-supplemented Western diet for another 8 weeks. First, the effects of FF treatment on plasma lipid levels were examined. Plasma cholesterol...
levels were not decreased by FF treatment and even slightly increased as compared with control mice (1657 ± 477 versus 2186 ± 415 mg/dl, p < 0.05) (Fig. 1A). Similarly, FF treatment did not decrease, but rather increased triglyceride levels (127 ± 42 versus 275 ± 64 mg/dl, p < 0.001) (Fig. 1A).

To assess whether FF treatment may lead to changes in lipoprotein profiles, the cholesterol lipoprotein distribution profile was then analyzed by subjecting a representative pooled plasma sample from each group to gel filtration chromatography. As previously shown, in apoE-deficient mice almost all the cholesterol is contained in the low density fractions (VLDL, IDL + LDL). The cholesterol distribution profile after FF treatment was slightly skewed toward smaller VLDL or IDL remnant particles, but the changes were very small (Fig. 1B).

**FF Treatment Does Not Decrease Lesion Surface Area in the Aortic Sinus but Leads to Reduced Cholesterol Content in Descending Aorta of 5-Month-old ApoE-deficient Mice.**—Next, the extent of atherosclerotic lesions was measured in serial sections between the valves and the aortic cross (aortic sinus). Fig. 2 shows the distribution of average surface of total atherosclerotic lesions (panel A) and Oil Red O-stained areas from the ventricle to the aortic arch (panel B), and mean lesion areas are reported in panel C. FF treatment did not affect atherosclerotic lesion size nor distribution within the aortic sinus of apoE-deficient mice. Moreover, FF treatment did not alter the macrophage content of the atherosclerotic lesion as revealed by macrophage-specific staining of the sections (data not shown). Recent studies indicated that regional differences in atherosclerosis susceptibility can exist (24). For instance, deletion of NADPH oxidase in apoE-deficient mice resulted in less atherosclerotic lesion formation in the descending aorta but not in the aortic arch (24). Therefore, the influence of FF treatment on aortic cholesterol content was determined also in the descending aorta. Interestingly, FF treatment tended to decrease aortic total and esterified cholesterol content in the descending aortas of these mice (Fig. 2D).

**FF Treatment Decreases Cholesterol Content in the Descending Aorta of Older ApoE-deficient Mice with More Advanced Lesions.**—Because the relative modest effect of FF treatment on aortic cholesterol content in these apoE-deficient mice may be related to the limited lesion development in the descending aorta at this age (25), it was determined whether FF treatment would be more efficacious to reduce aortic cholesterol content in older mice with more advanced lesions. Thus, apoE-deficient mice were weaned on a Western diet, and, at the age of 20 weeks, treated orally with either FF, at a similar dose as administered in the previous study, or vehicle for another 6 weeks (Fig. 3). As expected, feeding a Western diet for 16 weeks led to a large increase in both total and esterified cholesterol content of the descending aorta compared with baseline (Fig. 3). Most interestingly, FF treatment strongly decreased aortic cholesterol content in Western diet-fed apoE-deficient mice. Total cholesterol was decreased by 39% (533.3 ± 59.4 versus 324.5 ± 31.7 mmol/aorta, p = 0.009) and esterified cholesterol by 48% (216.6 ± 22.9 versus 112.4 ± 12.9 mmol/aorta, p = 0.002) (Fig. 3). This reduction of aortic cholesterol content by FF treatment occurred in the absence of any decrease in plasma total cholesterol or triglyceride levels (data not shown).

**FF Treatment Decreases Lesion Surface Area in the Aortic Sinus of hApoA-I Tg × ApoE-deficient Mice.**—Because systemic effects of fibrate treatment leading to an increase in the antiatherogenic HDL fraction and its major protein component apoA-I may also influence atherogenesis, we next aimed to determine whether induction of apoA-I by the PPARα agonist FF would be efficient to decrease atherosclerotic lesion development in the aortic sinus of apoE-deficient mice. Thus, human apoA-I transgenic mice were crossed with apoE-deficient mice and subsequently fed a control or FF (0.05% w/w corresponding to 100 mg/kg/day) supplemented Western diet for 8 weeks and atherosclerotic lesion surface areas in the aortic sinus were then determined. hApoA-I Tg × apoE-deficient FF-treated mice showed significantly decreased atherosclerotic lesion surface areas (~31%) when compared with control mice (160,802 ± 19,259 versus 110,236 ± 12,856 μm², p < 0.05) (Fig. 4).

**FF Treatment Does Not Decrease Lipid Levels in hApoA-I Tg × ApoE-deficient Mice.**—To determine whether the reduction in atherosclerotic lesion area in hApoA-I Tg × apoE-deficient mice is due to changes in plasma lipids, plasma cholesterol and triglyceride levels were determined. Analysis of plasma lipid levels demonstrated that total cholesterol levels were not decreased by FF treatment as compared with their respective controls (2991 ± 394 versus 2306 ± 271 mg/dl, p = 0.181) (Fig. 5A). For unknown reasons, triglyceride levels were higher in hApoA-I Tg × apoE-deficient than in apoE-deficient mice (Figs. 1 and 5). However, triglyceride levels were not affected by FF treatment (968 ± 447 versus 916 ± 155 mg/dl, p = 0.291) (Fig. 5A).

Cholesterol distribution profiles were also analyzed. Although the overall profile was not altered by FF treatment, FF
Fig. 2. FF treatment does not reduce atherosclerotic lesion size in the aortic sinus but tends to decrease cholesterol contents in the descending aorta of 5-month-old apoE-deficient mice. A and B, 10-week-old apoE-deficient mice were fed a Western diet for 2 weeks, followed by either a control (CON) or fenofibrate (FF) (0.05% w/w corresponding to 100 mg/kg/day) (filled symbols)-supplemented Western diet for another 8 weeks. Distribution of atherosclerotic lesion (A) and Oil Red O-stained areas (B) in the aortic sinus. The portion of aorta ranging from the ventricle to the distal part of the aortic sinus was divided into 10 sections to avoid morphological differences between mice. Results (n = 7/group) are expressed as the mean lesion area in each section as a function of the distance to the aortic valves. Mean atherosclerotic lesion sizes (± S.E.) are plotted in panel C. D, bar graph showing the aortic content of total cholesterol and esterified cholesterol in the descending aortas (n = 12/group). The results are expressed as mean ± S.E.

Fig. 3. FF treatment decreases cholesterol contents in the descending aorta of apoE-deficient mice with more advanced atherosclerotic lesions. ApoE-deficient mice (n = 10/group) were weaned and maintained on a Western diet for 16 weeks. Starting at 20 weeks of age (Baseline), they were given either vehicle (CON) or fenofibrate (FF) (100 mg/kg/day) by gavage for 6 weeks. Bar graph showing the aortic content of total cholesterol and esterified cholesterol in the descending aortas of apoE-deficient mice. The results are expressed as mean ± S.E.; *, p = 0.009; †, p = 0.002.

induced a slight increase in the HDL fraction as well as in the HDL fraction as compared with control (Fig. 5B).

Fig. 4. FF treatment decreases atherosclerotic lesion surface area in hapoA-I Tg × apoE-deficient mice. HapoA-I Tg × apoE-deficient mice were fed a control (CON; n = 9) (open circles) or fenofibrate (FF; n = 10) (0.05% w/w corresponding to 100 mg/kg/day) (filled circles)-supplemented Western diet for 8 weeks. Oil Red O staining of atherosclerotic lesions in serial sections between the valves and aortic crosses was measured. The graph shows atherosclerotic lesion size area quantification. Statistically significant differences between groups are indicated by asterisks (Tukey test; *, p < 0.05).

levels of ABCA-1, which mediates cholesterol efflux from foam cells and whose expression is positively controlled by macrophage cholesterol content (26), and MCP-1, a pro-atherogenic chemokine stimulating monocyte recruitment to the atherosclerotic lesion (27), were determined in the descending aortas from apoE-deficient and hapoA-I Tg × apoE-deficient mice (Fig. 7). Interestingly, but not unexpectedly, considering the atheroprotective activity of hapoA-I (compare Figs. 2C and 4), aortic ABCA-1 and MCP-1 mRNA levels were significantly lower in hapoA-I Tg × apoE-deficient compared with apoE-deficient mice (Fig. 7). Although FF treatment led to a reduction of atherosclerotic lesion cholesterol content in apoE-deficient mice (Figs. 2D and 3), ABCA-1 mRNA levels were not reduced compared with control treated apoE-deficient mice (Fig. 7A). By contrast, FF treatment reduced aortic MCP-1 mRNA levels (Fig. 7B). FF treatment did not influence ABCA-1 nor MCP-1 mRNA levels in hapoA-I Tg × apoE-deficient mice (Fig. 7), which correlates with the absence of significant lesions in the descending aorta of the hapoA-I Tg mice.
apoE-deficient mice (n indicated by control mice. Statistically significant differences between groups are from FF-treated mice are expressed as -fold induction compared with expressed as mean plasma and hepatic mRNA levels. Plasma human apoA-I levels are complemented Western diet for 8 weeks.

**Fenofibrate**

Human apoA-I Tg mRNA levels in hapoA-I Tg/H11547 tors related to atherosclerosis (28). In addition, PPAR metabolism, thereby positively affecting plasma lipid risk fac-

**DISCUSSION**

PPARα is an important regulator of lipid and lipoprotein metabolism, thereby positively affecting plasma lipid risk factors related to atherosclerosis (28). In addition, PPARα is known to exert anti-inflammatory and anti-thrombotic effects at both systemic and vascular levels (29). Furthermore, it positively regulates macrophage lipid homeostasis (26). Therefore, we hypothesized that both local and systemic effects could account for the clinically observed benefit of fibrate PPARα agonists on cardiovascular disease.

In the first part of this study, apoE-deficient mice were used to assess the effect of FF on atherosclerosis. ApoE-deficient mice represent a well-established murine model of hypercholesterolemia (30, 31). These animals develop severe lesions similar to those found in humans, with characteristic evolution from foam cell accumulation over lipid-laden fatty streaks to fibro-proliferative lesions (25, 32, 33). As previously reported (33), apoE-deficient mice fed a Western-type diet were highly susceptible to develop atherosclerotic lesions (Fig. 2). However, FF treatment did not reduce atherosclerotic lesion surface area in the aortic sinus. Because recent studies using NADPH oxidase-deficient mice have demonstrated the existence of regional differences in atherosclerotic lesion susceptibility (24), the effect of FF treatment in the descending aortas of these mice was also analyzed. Our results show that FF treatment tended to reduce cholesterol content in the descending aortas of these younger mice. These observations suggested a potential beneficial effect of FF treatment on lesion formation. ApoE-deficient mice develop lesions throughout the arterial tree, with lesions appearing first in the aortic arch in young mice and progressing in the thoracic and abdominal aorta in older mice (25). Furthermore, as the lesions progress, they increase in size and complexity to advanced lesions in older mice (32).

Because the first experiment was performed in relatively young mice with little lesions in the abdominal aorta, a second experiment performed in older mice fed a Western diet for 16 weeks before the treatment period with FF was started. In these mice with more advanced lesions, FF treatment significantly reduced aortic cholesterol content in the descending aortas. FF was given orally in both experiments, mixed in the food in the first and by gavage in the second, at comparable doses of 100 mg/kg/d. Furthermore, the increase in liver weights, which is a parameter for hepatic PPARα activation (34), was comparable in both experiments (data not shown). Moreover, in both experiments cholesterol content in the descending aorta decreased, although the effect was more pronounced in the older mice. Therefore, it appears unlikely that galenic differences in drug administration would cause the slight differences in the effects of FF treatment observed in both experiments. Altogether, it appears that the protective effect of FF is more pronounced when analyzed in mice developing more advanced lesions.

In the second part of this study, we aimed to determine whether induction of apoA-I expression and the HDL lipoprotein fraction by FF treatment would further influence lesion formation in apoE-deficient mice. Because PPARα agonists stimulate production of HDL and its major protein component apoA-I in human apoA-I transgenic mice (14), hapoA-I Tg mice were crossed into the apoE-deficient background and subsequently treated with either FF or control supplemented Western diet. As previously reported (35, 36), human apoA-I expression resulted in a reduction of atherosclerotic lesions in the apoE-deficient mice (Figs. 2 and 4). In this model, FF treatment
led to an increase in human apoA-I plasma and hepatic mRNA levels as previously shown in human apoA-I transgenic mice on a normal background (14). Strikingly, FF treatment resulted in a significant reduction in atherosclerotic lesion area in the aortic sinus of these hapaO-I Tg × apoE-deficient mice. Furthermore, atherosclerotic lesion surface areas were inversely correlated with plasma human apoA-I levels (r = 0.74, data not shown). These data suggest that FF treatment leading to increased human apoA-I production efficiently contributes to a reduction of atherosclerotic lesion size. Interestingly, in these apoE-deficient mice, pharmacological induction of human apoA-I expression by FF resulted in only a slight increase in the HDL fraction. However, because this mouse model has very low levels of HDL-cholesterol, subtle changes in this lipoprotein fraction may have robust effects on atherosclerosis development, due to a stimulation of reverse cholesterol transport and apoA-I-mediated cholesterol efflux. Alternatively, apoA-I may exert lipid-independent anti-atherosclerotic activities, which may contribute to the observed protection by apoA-I induction (37). For instance, it has been shown in apoE-deficient mice that macrophage human apoA-I expression within the vessel wall can reduce atherosclerotic lesion area (38). Furthermore, it has been demonstrated that liver-directed apoA-I gene transfer in LDL receptor-deficient mice led to a significant regression of atherosclerotic lesion despite the fact that HDL-cholesterol was only modestly increased (39). Finally, it has also been shown that apoA-I exerts anti-inflammatory effects (40).

Interestingly, plasma lipid levels were increased by FF treatment and a slight change in VLDL size distribution occurred. Although the influence of these slight size changes on atherogenicity of the VLDL particles is unknown, an increase in plasma lipid levels usually results in more pronounced lesions. Therefore, it appears plausible that FF exerts anti-atherosclerotic effects beyond its plasma lipid-lowering activity. PPARα agonists have anti-inflammatory and anti-thrombotic activities through a direct action on the vascular cells (41), which may provide a potential explanation for these anti-atherosclerotic effects independent of systemic lipid changes. PPARα agonists have been shown to inhibit interleukin-6 production by smooth muscle cells (19) and decrease expression of cytokine-induced genes such as vascular cell adhesion molecule 1, matrix metalloproteinase-9 (MMP-9), and tissue factor in endothelial cells and macrophages (15–17). In line with these reports, a decrease of aortic MCP-1 mRNA was observed in the descending aorta of the FF-treated apoE-deficient mice. In hapaO-I Tg × apoE-deficient mice, however, MCP-1 mRNA levels in the descending aorta were very low compared with apoE-deficient mice, and FF treatment did not further decrease MCP-1 expression. These latter findings are not surprising since hapaO-I Tg mice are protected against atherosclerosis and it is expected that very few lesions are present in the descending aorta.

Furthermore, PPARα agonists improve macrophage lipid homeostasis. PPARα activators induce the expression of the HDL receptor CLA-1/SR-BI, which may play a role in macrophage cholesterol efflux in vitro and in vivo in macrophages and as such may have a beneficial effect on atherosclerotic lesion development (42). Moreover, PPARα agonists induce the expression of ABCA-1, a transporter involved in apoA-I-mediated cholesterol efflux and whose expression is positively controlled by macrophage cholesterol content (26). In agreement with the positive effect of PPARα agonists on ABCA-1 expression, aortic ABCA-1 mRNA levels were not decreased in FF-treated apoE-deficient mice compared with controls despite the fact that FF treatment resulted in a significant decrease of aortic cholesterol content. Therefore, it appears that FF treatment maintains ABCA-1 expression levels elevated under conditions where aortic cholesterol contents are decreased. This effect of FF may contribute to the atheroprotective actions of FF. Finally, PPARα agonists may modulate apoptosis susceptibility in tumor necrosis factor-α-stimulated human macrophages, which can result in a general inhibition of macrophage activation and prevent foam cell formation (18).

Hence, fibrate-mediated activation of PPARα inhibits atherogenesis in apoE-deficient mice and in hapaO-I Tg × apoE-deficient mice. This improvement is in line with previous observations from in vitro and animal studies using PPARα agonists, which were shown to improve atherosclerosis risk factors (43–45). However, apparently conflicting data were recently reported by Semenkovich and co-workers (46). These authors showed that PPARα deficiency confers protection against atherosclerosis. This discordance may be explained by the fact that PPARα deficiency does not necessarily lead to the opposite phenotype as that resulting from PPARα activation by its ligands. Importantly, the deficiency of PPARα improved insulin sensitivity and lowered blood pressure in the studies of Semenkovich et al., and these effects may have outweighed those on lipid metabolism or vascular inflammation.

In conclusion, our results indicate that fenofibrate inhibits atherosclerosis development in vivo, an effect that is most likely independent of the changes in plasma lipid levels. These results provide additional data supporting the clinical benefit of fibrates and novel PPARα agonists under development.

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