The peroxisome-proliferator-activated receptor α agonist ciprofibrate severely aggravates hypercholesterolaemia and accelerates the development of atherosclerosis in mice lacking apolipoprotein E

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Mice lacking apolipoprotein E (apoE) are characterized by severe hypercholesterolaemia, caused by an abnormal accumulation of apolipoprotein B-48 (apoB-48)-carrying remnants of chylomicrons and very-low-density lipoproteins (VLDL) in the plasma, and by the spontaneous development of atherosclerotic lesions. Ciprofibrate is a hypolipidaemic compound that acts primarily by enhancing the oxidation of fatty acids in the liver and, consequently, decreasing the production of hepatic VLDL. In the present study, homozygous apoE-deficient mice were fed with a normal chow diet, supplemented with ciprofibrate. We report that, as anticipated, ciprofibrate treatment (a) stimulated hepatic fatty acid oxidation, as indicated by an increase in the mRNA levels of peroxisomal fatty acyl-CoA oxidase (AOX) and peroxisomal bifunctional enzyme, and (b) decreased the hepatic secretion of VLDL into the plasma, as determined by treating the animals with Triton WR-1339. Paradoxically, the apoE-deficient mice developed a 3–4-fold increase in their plasma cholesterol

levels. A similar effect was observed in apoE-deficient mice treated with other peroxisome-proliferator-activated receptor α agonists (fenofibrate, bezafibrate and WY14,643). By FPLC of the plasma and Western-blot analysis, we determined that the enhanced hypercholesterolaemia was due to an increased accumulation of apoB-48-carrying lipoprotein remnants in the plasma. Consistent with this finding, atherosclerotic lesions in animals treated with ciprofibrate for 90 days were considerably more advanced than in untreated animals. These results indicate that the ciprofibrate-induced accumulation of apoB-48-carrying remnants in apoE-deficient mice is caused by the inhibition of an as yet uncharacterized apoE-independent mechanism of removal of remnant from the circulation by the liver.

Key words: cholesterol, fibrate, lipoprotein remnant, scavenger receptor BI, triacylglycerol.

INTRODUCTION

Fibrates are a class of compounds widely used in the treatment of patients with hyperlipidaemia [1]. Studies with rodents have demonstrated that these compounds exert their hypolipidaemic effects by binding to and activating peroxisome proliferator-activated receptor α (PPAR α), a transcription factor belonging to a subfamily of nuclear hormone receptors. After activation by fibrates, PPAR α binds to specific response elements in the regulatory regions of target genes, resulting in the modulation of their expression [2]. In humans and rodents, fibrates are most efficient in decreasing plasma triacylglycerol levels. It is hypothesized that they do so, in part, by modulating the expression of genes that mediate a decrease in hepatic triacylglycerol production. This involves both a decrease in hepatic fatty acid synthesis and the stimulation of the hepatic fatty acid β -oxidation pathway [3]. In combination, these effects result in a decrease in the availability of fatty acids for the synthesis of triacylglycerols that are normally incorporated into hepatic very-low-density lipoproteins (VLDL). Thus as a result of fibrate treatment, less number of VLDL particles are secreted into the circulation, and/or the VLDL particles have a decreased triacylglycerol content.

The hypotriglyceridaemic effect of fibrates is also associated with a decreased synthesis of apolipoprotein C-III (apoC-III) [1–3]. This apolipoprotein is synthesized primarily in the liver and is transported to the plasma associated mainly with chylomicrons and VLDL. Although its exact function has not yet been fully

elucidated, there is considerable evidence that the rate of chylomicron and VLDL triacylglycerol hydrolysis by lipoprotein lipase (LPL) is greatly accelerated when these lipoproteins are depleted of apoC-III [4]. Recent evidence with apoC-III-deficient mice has also indicated that, in the absence of this apoprotein, the clearance of VLDL cholesteryl ester from the plasma is selectively enhanced. This phenomenon was particularly evident in mice deficient in both apoC-III and apolipoprotein E (apoE) [5].

Mice that lack apoE are characterized by severe hypercholesterolaemia caused by the accumulation of cholesterol-rich apolipoprotein B-48 (apoB-48)-carrying lipoproteins in the plasma. [6,7]. These lipoproteins are LPL-generated remnants of chylomicrons and hepatic VLDL, which, because they lack apoE, are not taken up by the liver and remain in the circulation. ApoE-deficient mice are also characterized by the spontaneous development of atherosclerotic lesions – a process that can be greatly accelerated by increasing the accumulation of remnants in the circulation, e.g. by feeding the animals with high-fat, high-cholesterol diets [8].

In the present study, we set out to examine how treatment of apoE-deficient mice with fibrates might affect their plasma cholesterol levels. We hypothesized that fibrate treatment would cause a decrease in the secretion of VLDL particles by the liver and, consequently, fewer cholesterol-rich VLDL remnants would accumulate in the plasma. We also anticipated that, as a result of the fibrate-induced decrease in apoC-III synthesis, the VLDL cholesteryl esters might be cleared from the circulation

Abbreviations used: AOX, fatty acyl-CoA oxidase; apoB-100, apolipoprotein B-100; apoB-48, apolipoprotein B-48; apoC-III, apolipoprotein C-III; apoE, apolipoprotein E; HL, hepatic lipase; LDL, low-density lipoprotein; LDLR, LDL receptor; L-PBE, peroxisomal bifunctional enzyme; LPL, lipoprotein lipase; PPAR α , peroxisome-proliferator-activated receptor α ; SR-BI, scavenger receptor BI; VLDL, very-LDL.

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at a higher rate. Together, these two processes could lead to a significant decrease in plasma cholesterol levels and, probably, retard the development of atherosclerotic lesions. The results of the present study show that treatment of apoE-deficient mice with ciprofibrate as well as other PPAR α agonists resulted in a paradoxical and significant increase, instead of a decrease, in plasma cholesterol levels. We provide evidence that this enhanced hypercholesterolaemia is due to a further accumulation of apoB-48-carrying lipoprotein remnants in the plasma caused by the inhibition of their clearance from the circulation. We also show that ciprofibrate treatment accelerated the development of atherosclerotic lesions in apoE-deficient mice.

MATERIALS AND METHODS

Animals

Homozygous male and female apoE^{-/-} mice on the C57Bl/6J background and normal male C57Bl/6J mice (6–8-week-old), as well as low-density lipoprotein (LDL) receptor (LDLR^{-/-}) mice, were purchased from Jackson Laboratories (Bar Harbor, ME, U.S.A.). They were housed (5 animals/cage) under 12 h cycles of light (06:00–18:00 h) and darkness, and were fed with a normal rodent chow diet (Teklad diet TD 7022; Harlan Teklad, Madison, WI, U.S.A.) with or without the addition of 0.05 % (w/w) ciprofibrate. Some animals were also fed with chow, supplemented with 0.05 % (w/w) fenofibrate or bezafibrate purchased from Sigma (St. Louis, MO, U.S.A.) or 0.02 % (w/w) WY14,643 (Wyeth Laboratories, Madison, NJ, U.S.A.). At indicated time intervals, blood samples (40–100 μ l) were obtained from the tail of conscious animals in heparinized capillary tubes, always between 07:00 and 09:00 h. When indicated, mice were deprived of food for 4 h before being sampled for blood from the tail. They were then injected through a tail vein with 0.2 ml of a 10 % (v/v) solution of Triton WR-1339 in saline, and blood samples were collected 90 and 180 min later. During the entire period, the animals had free access to water.

Lipid and lipoprotein analysis

Plasma triacylglycerols and cholesterol were measured by a microplate assay technique using enzymic assay reagent kits obtained from Sigma. Lipoproteins were fractionated using equal volumes of pooled plasma from 5 animals/group on tandem Superose 6 FPLC columns in 200 mmol/l sodium phosphate (pH 7.4), 50 mmol/l NaCl, 0.03 % EDTA and 0.02 % sodium azide. Fractions (400 μ l) were collected and the amount of cholesterol was determined in the even-numbered fractions.

Northern-blot analysis

Total RNA was extracted from frozen livers using TRIzol[®] reagent (Life Technologies, Carlsbad, CA, U.S.A.). RNA was glyoxylated, electrophoresed on 0.8 % agarose gel, transferred on to a nylon membrane and hybridized at 42 °C in 50 % (v/v) formamide using random-primed ³²P-labelled cDNA probes of peroxisomal bifunctional enzyme (L-PBE) and fatty acyl-CoA oxidase (AOX), obtained as described previously [9,10]. To ensure equal loading of RNA, the blots were probed using 18 S rRNA probe.

Western-blot analysis

Plasma pooled from 5 mice was diluted (1:8, v/v) with sample buffer [3 % (w/v) SDS/12 % (v/v) glycerol/70 mM Tris

Table 1 Body weights (g) of mice before and after treatment with ciprofibrate

Ten mice (6–8 weeks old) were weighed, separated into two groups (5 animals/group) and fed with a chow diet with or without 0.05 % ciprofibrate. Results are expressed as means $\pm$ S.D.

	Before treatment	After 90 days	
		– Ciprofibrate	+ Ciprofibrate
Wild-type	19.2 $\pm$ 1.7	28.2 $\pm$ 1.4	26.2 $\pm$ 1.8
Male apoE ^{-/-}	16.7 $\pm$ 1.3	27.9 $\pm$ 1.3	25.6 $\pm$ 1.6
Female apoE ^{-/-}	16.2 $\pm$ 1	23.4 $\pm$ 1.2	20.7 $\pm$ 0.6

(pH 6.8)/0.0015 % Bromophenol Blue/5 % 2-mercaptoethanol]. The samples were boiled for 5 min, and equal volumes were loaded and run on 4–15 % Tris/HCl gel (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Separated proteins were blocked for 3 h in TBST buffer [10 mM Tris/HCl (pH 7.4)/150 mM NaCl/0.1 % Tween 20] containing 5 % (w/v) non-fat dried milk and then probed overnight with rabbit polyclonal antibodies (Bioscience International, Saco, ME, U.S.A.) against mouse apoB-48/apoB-100 (apolipoprotein B-100) (1:300 dilution in TBST). Reactions were detected with goat anti-rabbit secondary antibody (1:2000 dilution in TBST) conjugated with horseradish peroxidase using enhanced chemiluminescence (ECL[®]; Amersham Biosciences).

Assessment of atherosclerotic lesions

Mice were anaesthetized with Nembutal intraperitoneally (0.65 mg/10 g body weight) and their vasculature was perfused via the left ventricle of the heart with PBS containing 7 international units/ml heparin for 2 min and then perfused with 10 % (v/v) buffered formaldehyde for 5 min. The entire aorta, with the heart attached, was removed and fixed overnight in buffered formaldehyde. The heart, adventitia and branched vessels were then removed, the aorta was cut open and stained with Sudan IV. The stained aortas were then mounted on glass slides covered with tissue paper (Kimwipes; Kimberly-Clark Corp., Dallas, TX, U.S.A.) and their luminal surface-scanned using a Canon Color Image Scanner (CanoScan N670U). The *en face* surface areas occupied by the Sudan IV-stained lesions in the arch and thoracic aorta were then quantified using MetaMorph software (Universal Imaging Corp., Downingtown, PA, U.S.A.).

RESULTS

Male and female normal and apoE^{-/-} mice (all 6–8 weeks old) were fed with a regular chow diet with or without 0.05 % ciprofibrate. Their body weights after 90 days of treatment are given in Table 1. All animals on the ciprofibrate-supplemented diet gained less weight than their respective untreated controls. We also observed that, at the time of killing, animals treated with ciprofibrate had less adipose tissue (results not shown) and that they had also developed the characteristic fibrate-induced hepatomegally associated with the proliferation of peroxisomes in the hepatocytes [11].

Figure 1 shows the plasma cholesterol levels, measured at various time intervals for up to 90 days, in normal mice and in male and female apoE^{-/-} mice fed with chow with or without ciprofibrate. As described previously [6,7], the plasma cholesterol of the untreated apoE^{-/-} mice was significantly higher than that of untreated normal animals. The initial measurements revealed levels of 94 $\pm$ 7, 436 $\pm$ 31 and 520 $\pm$ 34 mg/dl (means $\pm$ S.D.,

Figure 1 Plasma cholesterol levels in normal mice and male and female apoE^{-/-} mice

Normal mice (A) and male (B) and female (C) apoE^{-/-} mice were maintained on a chow diet with or without 0.05 % ciprofibrate for the indicated time periods. Plasma was separated from blood samples obtained from the tail of animals fed *ad libitum*. Results are expressed as means $\pm$ S.D. ($n = 5$). Statistically significant differences between groups are indicated by asterisks (* $P < 0.001$). □, - ciprofibrate; ■, + ciprofibrate.

Figure 2 Plasma triacylglycerol levels in normal mice and male and female apoE^{-/-} mice

Details are as for Figure 1.

$n = 5$) for the untreated normal mice, male apoE^{-/-} mice and female apoE^{-/-} mice respectively. These levels remained relatively unchanged throughout the duration of the study. Treatment with ciprofibrate caused no significant changes in the plasma cholesterol level of normal animals. In contrast, ciprofibrate treatment caused a remarkable increase in plasma cholesterol of both male and female apoE^{-/-} mice. In male mice, a significant increase ($P < 0.001$) was already apparent after 6 days of treatment. These levels remained increased and, after 90 days, the levels in the treated mice were approx. 4-fold higher than those of the untreated mice (1366 ± 97 versus 351 ± 64 mg/dl; means $\pm$ S.D., $n = 5$). In female apoE^{-/-} mice, a significant increase was observed on day 10 of treatment and, after 90 days,

the levels in the treated mice were approx. 3-fold higher than those of the untreated mice (1297 ± 128 versus 473 ± 41 mg/dl; means $\pm$ S.D., $n = 5$).

The plasma triacylglycerol levels are shown in Figure 2. There was considerable intragroup variability at all time points, which may be explained by the fact that measurements were made on blood samples obtained from animals fed *ad libitum*. In normal mice, as anticipated, ciprofibrate caused a marked hypotriglyceridaemic effect. After 6 days, the plasma triacylglycerol levels in the treated animals were about half that of the untreated group (40 ± 7 versus 113 ± 20 ; means $\pm$ S.D., $n = 5$). This significant difference ($P < 0.001$) persisted for the duration of the present study. A significant hypotriglyceridaemic effect was also observed

Table 2 Plasma lipids of apoE^{-/-} mice treated with PPAR α agonists

Groups of 6–8-week-old mice were fed with a chow diet supplemented with 0.05 % fenofibrate, 0.05 % bezafibrate or 0.02 % WY14,643 for 20 days. Cholesterol and triacylglycerol levels are expressed in mg/dl (means $\pm$ S.D.). Number of animals/group is given in parentheses.

	No treatment (14)	Fenofibrate (4)	Bezafibrate (5)	WY14,643 (5)
Cholesterol	492 $\pm$ 79	1038 $\pm$ 132*	1026 $\pm$ 163*	1131 $\pm$ 97*
Triacylglycerol	187 $\pm$ 42	230 $\pm$ 76	300 $\pm$ 128	217 $\pm$ 41

* $P < 0.005$.

Figure 3 FPLC gel filtration of mouse plasma

Equal volumes of plasma ($n = 5$) from male apoE^{-/-} mice fed with chow with or without 0.05 % ciprofibrate for 30 days were pooled, and 200 μ l was subjected to gel-filtration chromatography using a Superose column. Fractions (0.4 ml) were collected, and cholesterol concentration was measured in the even-numbered fractions. ●, – ciprofibrate; ○, + ciprofibrate.

in the male and female apoE^{-/-} mice, but only until 6 days of treatment with ciprofibrate. After 10, 15 and 20 days of treatment, the hypotriglyceridaemic effect was no longer observed. After 90 days, the plasma triacylglycerol levels in both male and female apoE^{-/-} treated mice were lower than those in the untreated controls, although a statistically significant lower level ($P < 0.001$) was observed only in the male apoE^{-/-} mice.

To examine whether the ciprofibrate-induced effects described above on the plasma cholesterol and triacylglycerols of apoE^{-/-} mice could be observed when these animals were treated with other PPAR α agonists, male apoE^{-/-} mice were treated with fenofibrate, bezafibrate or WY14,643. The results in Table 2 show that, by 20 days of treatment, the three PPAR α agonists tested, like ciprofibrate, caused severe hypercholesterolaemia ($P < 0.001$).

To determine the cholesterol distribution within the plasma lipoproteins, we used FPLC to fractionate the pooled plasma of male apoE^{-/-} mice maintained on chow alone or chow supplemented with ciprofibrate for 30 days (Figure 3). As demonstrated

Figure 4 Assessment of apoB levels in the plasma of apoE^{-/-} mice treated with or without ciprofibrate

Equal volumes of plasma were pooled ($n = 5$) from male apoE^{-/-} mice fed with a chow diet with or without 0.05 % ciprofibrate for the indicated time periods, and processed as described in the Materials and methods section. Pooled plasma from 3 LDLR^{-/-} mice was used as reference. Western blots were performed with rabbit polyclonal antibodies specific for mouse apoB-48 and apoB-100.

previously [6], most of the cholesterol in the plasma of the apoE^{-/-} mice on the chow diet alone was associated primarily with the VLDL (remnant) fraction. The significantly increased amount of plasma cholesterol in the ciprofibrate-treated mice was also eluted almost entirely with the remnant fraction.

Next, we investigated whether the increased cholesterol content of the remnant fraction of the ciprofibrate-treated mice (Figure 3) was due to an enrichment of the particles with cholesterol or, alternatively, to an increased number of remnant particles. For this purpose, we performed Western-blot analysis of apoB in the plasma of apoE^{-/-} mice fed with chow supplemented with ciprofibrate after their plasma cholesterol levels reached a level 3-fold higher than that of the untreated animals. Figure 4 shows the results obtained with mice treated with ciprofibrate for 10 and 20 days. It is apparent that there was considerably more accumulation of apoB-48 in the plasma of the ciprofibrate-treated animals than in the plasma of the untreated mice, clearly indicating that the enhanced hypercholesterolaemia in the treated group was due to an increased number of circulating remnant particles. With the method used, apoB-100 was not detected in either group of animals, despite the fact that the antibody employed could detect both apoB-48 and -100, as demonstrated with the use of pooled plasma from mice lacking the LDLR (Figure 4).

We next examined the possibility that the higher number of apoB-48-carrying remnants in circulation of the ciprofibrate-treated mice might have resulted from an increased influx of triacylglycerol-rich lipoproteins into the plasma. ApoE^{-/-} mice that had been fed with chow supplemented with or without ciprofibrate for 30 days were injected with Triton WR-1339 to inhibit the action of LPL [12] and the triacylglycerols accumulated in their plasma measured 90 and 180 min later. Because all animals were deprived of food for 4 h before the Triton injection and for the subsequent 3 h of the experiment, the secretion of chylomicrons was minimized and most of the accumulated triacylglycerols were presumably those associated with VLDL secreted by the liver. From the results shown in Figure 5, we determined that the rate of triacylglycerol accumulation in the plasma of the ciprofibrate-treated mice was significantly lower than that of the untreated animals (17.3 ± 3.1 versus 23.7 ± 1.6 mg $\cdot$ h⁻¹ $\cdot$ (g body weight)⁻¹; means $\pm$ S.D., $n = 4$; $P < 0.01$). In addition to the plasma triacylglycerol levels, we also measured the plasma cholesterol levels in the animals injected with Triton WR-1339. Before the injection, the cholesterol levels in the untreated and

Figure 5 Triacylglycerol influx into the plasma of apoE^{-/-} mice treated with or without ciprofibrate

Male apoE^{-/-} mice maintained on a chow diet with or without 0.05 % ciprofibrate for 30 days were deprived of food for 4 h and then injected into a tail vein with 0.2 ml of a 10 % solution of Triton WR-1339. Blood samples were obtained from the tail for the measurement of plasma triacylglycerol levels. All animals had access to water but not food for the duration of the experiment. Results are expressed as means $\pm$ S.D. ($n = 4$). ○, - ciprofibrate; ●, + ciprofibrate.

ciprofibrate-treated animals were 462 ± 44 and 1045 ± 143 mg/dl respectively and, after 180 min, 446 ± 41 and 1044 ± 103 mg/dl respectively (means $\pm$ S.D., $n = 4$). These results indicate that the livers of the ciprofibrate-treated animals, like those of the untreated mice, did not secrete VLDL particles enriched in cholesterol.

The decreased secretion of hepatic VLDL in normal mice treated with fibrates is ascribed, to a large extent, to a diversion of fatty acids from triacylglycerol synthesis to the β -oxidation pathway [1]. We therefore examined whether the decreased secretion of VLDL by the livers of the ciprofibrate-treated apoE^{-/-} mice was, likewise, associated with an enhanced oxidation of fatty acids by hepatocytes. For this purpose, we used Northern blotting to assess the mRNA levels of the peroxisomal AOX and L-PBE in the livers of normal mice and apoE^{-/-} mice treated with ciprofibrate for 35 days. Figure 6 shows that, in agreement with previous reports [13], the levels of AOX and L-PBE mRNA significantly increased in the livers of the treated normal animals. Significant increases in mRNA levels were also observed in the ciprofibrate-treated apoE-deficient mice. It is noteworthy that these changes in the expression of AOX and L-PBE, which are associated with the hypolipidaemic effects of fibrates in normal animals [3], were observed in the apoE^{-/-} mice at a time when their plasma triacylglycerol levels were not significantly decreased (Figure 2).

We also examined how the ciprofibrate-induced increased plasma accumulation of lipoprotein remnants might affect the development of atherosclerotic lesions in apoE-deficient mice. The apoE-deficient mice were killed 90 days after being placed on the chow diets supplemented with ciprofibrate. The aortas were fixed, as described in the Materials and methods section, cut longitudinally, stained with Sudan IV and the *en face* lesions

Figure 6 Northern-blot analysis of total RNA extracted from the liver of normal and apoE^{-/-} mice

A representative Northern blot showing the differences in L-PBE and AOX mRNA levels in normal and apoE^{-/-} mice maintained on a chow diet with or without 0.05 % ciprofibrate for 35 days.

quantified in the arch and thoracic aorta. The male animals maintained on the chow diet supplemented with ciprofibrate had significantly ($P < 0.005$) more atherosclerotic lesions than the animals maintained on the control diet (17.7 ± 7.4 versus 4.6 ± 3.3 % of the surface area; means $\pm$ S.D., $n = 5$). Significantly more atherosclerotic lesions ($P < 0.005$) were also observed in the female mice treated with ciprofibrate, compared with the animals on the control diet (16.8 ± 6.2 versus 3.8 ± 2.1 % of the surface area; means $\pm$ S.D., $n = 5$). Further, some of the treated animals developed raised lipid-laden lesions in the abdominal aorta, but none were observed in the untreated animals (results not shown).

DISCUSSION

Mice that lack the apoE gene are naturally hypercholesterolaemic because of their inability to clear efficiently apoB-48-carrying cholesterol-rich lipoprotein remnants from the circulation [6,7]. In the present study, we demonstrate that when such apoE-deficient mice are treated with the hypolipidaemic PPAR α agonists ciprofibrate, fenofibrate, bezafibrate or WY 14,643, they develop a paradoxical and significantly more severe hypercholesterolaemia. We further demonstrate that this enhanced hypercholesterolaemia results from a greatly increased plasma accumulation of apoB-48-carrying lipoprotein remnants. This overaccumulation of remnants cannot be explained by an increased influx of apoB-48-carrying lipoproteins into the bloodstream. The results of the experiment in which mice deprived of food were injected with Triton WR-1339 (Figure 5) indicate that ciprofibrate treatment caused a decrease in VLDL influx into the plasma. The finding that the plasma cholesterol levels before and 180 min after Triton injection were not significantly different also suggests that ciprofibrate treatment did not cause the production of cholesterol-rich VLDL. As in normal animals treated with fibrates, the diminished influx of VLDL into the circulation in the treated apoE^{-/-} mice can be attributed to the increased expression of hepatic fatty acid-oxidizing enzymes (Figure 6), which makes fewer fatty acids available for triacylglycerol production [3]. Ciprofibrate administration did not cause an increase in food consumption by either normal or apoE-deficient mice (results not shown). In fact, the treated animals gained less weight than their untreated controls (Table 1). It is unlikely, therefore, that the overaccumulation of apoB-48-carrying remnants in the

circulation of the ciprofibrate-treated apoE-deficient mice resulted from an increased secretion of chylomicrons from the intestine. Thus in the absence of an increased plasma influx of hepatic and intestinal lipoproteins, the fibrate-induced hyperlipidaemia in the apoE-deficient mice can be best explained as resulting from a defect in the removal of the apoB-48-carrying lipoprotein remnants from circulation.

In normal animals, the efficient removal of chylomicron and VLDL remnants from the circulation requires the mediation of apoE present on the surface of the lipoproteins. Apo-E, through its property of binding to proteoglycans, is believed to initiate the removal process by sequestering the circulating remnants in the space of Disse [14]. This sequestration allows the apoE on the remnants ready access and binding to the LDLR and/or the LDLR-related protein on the surface of hepatocytes, leading to the rapid endocytosis of the lipoproteins [14]. In the absence of apoE, remnants of VLDL can still be efficiently taken up by the liver, provided they carry apoB-100 – a ligand for the LDLR. However, chylomicrons, as well as a large fraction of VLDL secreted by the liver of mice, carry apoB-48 that is not a ligand for the LDLR or LDLR-related protein. Therefore in mice that lack apoE, remnants carrying apoB-48 cannot be endocytosed and they accumulate in the circulation, giving rise to their characteristic hypercholesterolaemia. It is notable, however, that in apoE-deficient mice fed with a normal chow diet this hypercholesterolaemia is maintained within narrow limits (between 400 and 600 mg/dl) [15], despite the continuous generation of apoB-48-carrying remnants from the VLDL and chylomicrons that are secreted into the circulation. Evidently, in the absence of apoE, other mechanisms come into play that can mediate the hepatic uptake of remnants, albeit not as efficiently as the apoE-mediated process [16,17]. The results of the present study suggest that the increased accumulation of remnants in the plasma of apoE^{-/-} mice is caused by a fibrate-induced inhibition of such apoE-independent mechanisms of hepatic remnant uptake.

Several apoE-independent mechanisms of hepatic remnant uptake have been proposed. Hepatic lipase (HL) has been hypothesized to function in the uptake of remnants by the liver by acting as a ligand, or through its catalytic action [14,16]. A fibrate-induced inhibition of HL could, therefore, explain the enhanced hypercholesterolaemia of the apoE^{-/-} mice described in the present study. Studies with mice doubly deficient in apoE and HL demonstrated that these animals do, in fact, display a more severe hypercholesterolaemia when compared with mice lacking apoE alone [18]. However, unlike the findings of the present study, the more severe hypercholesterolaemia in the doubly deficient mice is not due to an increased number of apoB-48-carrying remnants in the plasma [18], but rather due to the accumulation of unique cholesterol-carrying lamellar lipoproteins [19]. Also, in the present study, the development of atherosclerotic lesions in apoE-deficient mice was greatly accelerated as a result of ciprofibrate treatment, whereas in the studies by Mezdoor et al. [18] HL deficiency decreased susceptibility to atherosclerosis in apoE-deficient mice. Further, other studies have shown that the administration of fibrates to mice does not affect their HL expression [20]. It is unlikely, therefore, that inhibition of this enzyme is involved in the fibrate-induced remnant accumulation described in the present study. LPL has also been proposed to mediate the hepatic uptake of remnants by the liver by acting as a ligand [16]. A decrease in the expression of this enzyme could, in theory, contribute to a more severe hypercholesterolaemia in apoE-deficient mice. There is ample evidence, however, that fibrate administration does not inhibit, but actually enhances, the expression of LPL, especially in the liver [21]. Work from our laboratory has provided evidence that remnants generated by LPL

or HL can be readily taken up by hepatocytes through a pathway that is apoE-independent [22,23], and we hypothesized that this apoE-independent pathway may involve the scavenger receptor BI (SR-BI) [23]. Therefore it is of great interest that Mardones et al. [24] reported very recently that when normal mice are treated with PPAR α agonists, the expression of SR-BI protein in their livers is greatly suppressed. Whether the overaccumulation of remnants in the plasma of apoE-deficient mice reported in the present study is, in fact, caused by PPAR α -agonist-induced down-regulation of hepatic SR-BI remains to be explored.

The development of atherosclerotic lesions in apoE-deficient mice is known to be accelerated when the number of apoB-48-carrying remnants in the circulation is greatly increased, as, for example, when the animals are fed with diets that are high in fat and cholesterol [8]. It was not unexpected, therefore, that the greatly increased remnant accumulation in the plasma of apoE^{-/-} mice treated with ciprofibrate also resulted in the development of more severe atherosclerotic lesions. It is noteworthy that, very recently, Duez et al. [25] also examined the development of atherosclerotic lesions in apoE^{-/-} mice treated with another fibric acid derivative, namely fenofibrate. Unlike the results reported in the present study, however, they found no changes in the severity of the lesions in the aortic sinus and an actual decrease in atherosclerosis in the descending aorta of the treated animals. This discrepancy between the present results and those of Duez et al. [25] may be partly ascribed to differences in the methodology used. Apart from the fact that the two studies examined the effects of two different fibrates, in the present study the apoE^{-/-} mice were fed with a low-fat chow diet, whereas in the study of Duez et al. [25] the animals were fed with a high-fat, high-cholesterol diet that, by itself, causes a large plasma accumulation of remnants and a more rapid development of atherosclerotic lesions. In animals treated with fenofibrate, Duez et al. [25] observed higher plasma cholesterol levels, which were probably caused by a further accumulation of apoB-48-carrying remnants, as reported in the present study. However, the increase in hypercholesterolaemia was relatively small (approx. 30%) and, presumably, not sufficient to cause an obvious aggravation of the already advanced diet-induced lesions in the aortic sinus. The conclusion by Duez et al. [25] that fenofibrate treatment caused a decrease in atherosclerosis lesions, as determined by a decrease in the cholesterol content of the aortas, cannot be readily reconciled with the present findings, showing that the ciprofibrate-treated animals had more widespread lesions than the untreated animals.

In conclusion, we have demonstrated that treatment of chow-fed apoE-deficient mice with ciprofibrate and with other PPAR α agonists causes a large accumulation of lipoprotein remnants in the plasma and, consequently, a more rapid development of atherosclerotic lesions. It should be noted that many effects that fibrates have on rodents (e.g. hepatic peroxisome proliferation and tumourigenesis) are not observed in human subjects. The observations described here appear to be another instance in which these compounds affect rodents and humans differently. Available evidence indicates that the development of atherosclerotic lesions in human subjects can, in fact, be retarded by treatment with fibrates [3]. The apoE-deficient mouse treated with PPAR α agonists may be very useful, however, in providing further insights into the mechanisms that operate in the hepatic uptake of lipoprotein remnants in the absence of apoE, and in the processes of remnant-induced development of atherosclerotic lesions.

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