Fibrates Down-regulate Hepatic Scavenger Receptor Class B Type I Protein Expression in Mice*

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Fibrates are normolipidemic drugs used in atherogenic dyslipidemia because of their ability to raise high density lipoprotein (HDL) and decrease triglyceride levels. They exert multiple effects on lipid metabolism by activating the peroxisome proliferator-activated receptor-α (PPAR-α), which controls the transcriptional regulation of genes involved in hepatic fatty acid, cholesterol, and lipoprotein metabolism. The hepatic expression of the scavenger receptor class B type I (SR-BI) plays a critical role in lipoprotein metabolism, mainly due to its ability to mediate selective cholesterol uptake. Because fibrates and PPAR-α agonists up-regulate SR-BI expression in human and murine macrophages, we tested whether fibrates raise a similar regulatory response on hepatic SR-BI expression in mice. Surprisingly, fibrate treatment suppressed SR-BI protein expression in the liver without changing steady state SR-BI mRNA levels. Decreased hepatic SR-BI protein expression correlated with enlarged HDL particle size. This effect was concomitant with down-regulation of CLAMP, a putative SR-BI-stabilizing protein found in the hepatic plasma membrane, which was also not associated to changes in CLAMP mRNA levels. The post-transcriptional regulatory effect of fibrates over hepatic SR-BI protein levels was dependent on PPAR-α expression, because it was absent in PPAR-α-deficient mice. Restoring hepatic SR-BI expression in fibrate-treated mice by recombinant adenoviral gene transfer abolished fibrate-mediated HDL particle size enlargement. This study describes a novel effect of fibrates on hepatic SR-BI expression providing an alternative mechanism by which this drug family modulates HDL metabolism in vivo.

The long standing epidemiological evidence correlating increased plasma high density lipoprotein cholesterol (HDL-C) with protection against atherosclerotic cardiovascular disease (reviewed in Refs. 1 and 2) has attracted significant attention toward the regulation of HDL-C homeostasis and the development of drugs directed to beneficially modulate HDL-C metabolism. Plasma levels of HDL-C are determined by a complex network of interactions between lipids (e.g. fatty acids and cholesterol), circulating apolipoproteins (e.g. apoA-I and apoA-II), and lipid transfer proteins (e.g. phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein), extracellular lipases (e.g. hepatic lipase), cell surface receptors, and plasma membrane lipid transporters (e.g. ATP-binding cassette transporter A1). The scavenger receptor class B type I (SR-BI), a cell surface HDL receptor capable of mediating selective HDL cholesterol uptake (reviewed in Refs. 3–5), plays a distinct role in HDL metabolism in mice by modulating plasma HDL-C levels and HDL particle size and composition (6). Manipulations of hepatic SR-BI expression levels by adenoviral gene transfer (7), transgenesis (8, 9), and targeted gene ablation (6) have profound influence on HDL-C levels and its availability for biliary cholesterol secretion in mice. Furthermore, additional studies have established an inverse correlation between murine hepatic SR-BI expression and atherosclerotic ischemic heart disease (10–13). Taken together, these studies strongly suggest that pharmacological agents that modulate SR-BI expression and/or activity in the liver may have significant impact on HDL metabolism, reverse cholesterol transport, and atherosclerotic cardiovascular disease.

Fibrates are commonly used normolipidemic drugs that efficiently decrease triglycerides and raise HDL levels in humans (14), resulting in an overall reduction of coronary heart disease risk and events (15–17). Several studies in animal models and cultured cells have established that the normolipidemic effects of fibrates occur mainly through transcriptional modulation of target genes involved in fatty acid, triglyceride, and cholesterol metabolism and also in lipoprotein formation and remodeling (18). This fibrate-mediated transcriptional regulation is caused by binding and activation of a specific nuclear receptor termed peroxisome proliferator-activated receptor-α (PPAR-α) (reviewed in Refs. 19 and 20). Indeed, fibrate-induced effects on gene transcription depend on the presence of functional PPAR-

poprotein; CETP, cholesteryl ester transfer protein; CLAMP, carboxyl-terminal linking and modulator protein; FPLC, fast performance liquid chromatography; HDL, high density lipoproteins; LDL, low density lipoproteins; PPAR-α, peroxisome proliferator-activated receptor-α; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor class B type I; VLDL, very low density lipoproteins; PBS, phosphate-buffered saline.
α-response elements in the promoter region of target genes (14, 19). Furthermore, PPAR-α knockout mice lack the above-mentioned lipid metabolism-related responses associated with fibrate treatment (21), indicating the essential role of this receptor in mediating fibrate action.

Whereas the role of fibrates in the regulation of plasma HDL-C levels through changes in expression of plasma apoprotein A-I (22, 23), apoA-II (24), PLTP (25), lipoprotein lipase (26), and macrophage ATP-binding cassette transporter A1 transporter (22, 23), apoA-II (24), PLTP (25), lipoprotein lipase (26), and PPAR-α/H9251 (27) has been studied extensively, much less is known about macrophage ATP-binding cassette transporter A1 transporter (22, 23), apoA-II (24), PLTP (25), lipoprotein lipase (26), and PPAR-α/H9251 (27) has been studied extensively, much less is known about fibrate-dependent regulation of SR-BI. Recently, Chinetti et al. (28) have shown that PPAR-α activation increased SR-BI protein but not mRNA levels in human monocytes as well as in fully differentiated macrophages. In addition, fenofibrate treatment elevated SR-BI protein content in macrophages of atherosclerotic lesions in apolipoprotein E (apoE) knockout mice (28). These findings suggested that fibrates might modulate HDL metabolism by increasing SR-BI expression in peripheral tissues.

Despite the importance of hepatic SR-BI expression for HDL metabolism and the pleiotropic effects of fibrates on HDL metabolism-related proteins in the liver, the consequences of fibrate administration on hepatic SR-BI expression have not been reported. In this study, we determined hepatic SR-BI protein and mRNA levels in fibrate-treated mice, and we correlated these findings with plasma lipoprotein cholesterol profiles. We also examined the effect of fibrates on hepatic protein and mRNA levels of CLAMP, a putative SR-BI-stabilizing protein expressed in the liver (29). The PPAR-α dependence of changes in hepatic SR-BI protein expression induced by fibrates was evaluated in fibrate-treated PPAR-α knockout mice. Finally, we tested the physiological relevance of the fibrate-induced SR-BI deficiency in the liver by restoring hepatic SR-BI expression through recombinant adenoaviral gene transfer in fibrate-treated mice.

**EXPERIMENTAL PROCEDURES**

**Animals and Fibrate Treatment**—Control male C57BL/6 mice (2–3 months old), originally obtained from The Jackson Laboratory (Bar Harbor, ME), were housed in a temperature- and humidity-controlled room with reverse light cycling and fed a low cholesterol-containing chow diet (ProLab RMH 3000; PMI Feeds, St. Louis, MO), with food and water available ad libitum. For plasma cholesterol and hepatic SR-BI and CLAMP expression studies, mice were switched to diets containing 0.2% ciprofibrate (Sanofi, Gentilly, France) or 0.2% fenofibrate (Sigma) for 7 days. For dose- and time-response studies, mice were fed with diets supplemented with ciprofibrate at the indicated doses and for the indicated times. To test the PPAR-α dependence of SR-BI regulation by fibrates, control and PPAR-α knockout mice (30) were fed with chow diet supplemented with 0.2% fenofibrate for 14 days.

**Recombinant Adenovirus Infection in Fibrate-treated Mice**—At day 0, control C57BL/6 mice (2–3 months old) were separated in six groups of four animals each and were switched to experimental diets as follows: one group was kept in control diet, three groups were switched to 0.2% ciprofibrate diet, and two groups were switched to 0.2% fenofibrate diet. At day 4, one group of mice treated with either ciprofibrate or fenofibrate were injected via the femoral vein with 5 × 1011 particles of recombinant adenoaviral adenovirus (Ad.mSR-B, see Ref. 7). Another group of ciprofibrate-treated mice was injected with 1 × 1011 particles of lacZ recombinant adenovirus (Ad.lacZ, see Ref. 7) as control for the adenoviral infection. Regardless of the use of adenoviruses, all groups were maintained until day 9 in their respective fibrate treatments.

**Hepatocyte Culture and Fibrate Treatment**—Rat hepatocytes were isolated and cultured as described previously (31). Briefly, hepatocytes were prepared with collagenase perfusion from liver of male Sprague-Dawley rats. Isolated hepatocytes were seeded (105 cells/well) in 8-well plates for 24 h in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 1% glucose without or with supplementation with 100 μM fenofibrate. Cells were lysed with 0.2% SDS, 2% Nonidet P-40, 2 mM 2-mercaptoethanol, and protease inhibitors (ICN Biomedicals mixture kit) in 0.1% phosphate buffer, and post-nuclear extracts were prepared by centrifugation at 10,000 × g for 15 min at 4 °C.

**Blood and Liver Sampling and Processing**—After fibrate treatment with or without associated adenoviral infections, mice were anesthetized with pentobarbital (4.5 mg/100 g body weight) by intraperitoneal injection. The abdomen was opened and blood was removed by puncture of the inferior vena cava with a heparinized syringe; mice were euthanized, and livers were removed. Plasma was separated by low speed centrifugation for 10 min at 4 °C and kept at −20 °C, whereas liver was stored at −70 °C for further biochemical analyses. Total membrane extracts (postnuclear 100,000 × g membrane pellets) from individual liver samples were prepared as described (32).

**Plasma Lipoprotein Analysis**—Size fractionation of plasma lipoproteins was performed by fast performance liquid chromatography (FPLC) of pooled plasma samples, and total cholesterol content on each fraction was assayed enzymatically (33). Results are expressed as micrograms of total cholesterol per FPLC fraction.

**Immunoblotting Analysis**—Hepatocyte post-nuclear lysates (60 μg of proteins/sample) or total liver membranes (40–50 μg of protein/sample) were size-fractionated by 10% SDS-PAGE and immunoblotted on nitrocellulose with either a polyclonal antipeptide antibody against murine SR-BI protein (34), a polyclonal antibody generated against the entire SR-BI protein (35), or a monoclonal antibody against recombinant CLAMP (29). Polyacrylamide anti-e-COP (36) or anti-actin (Santa Cruz Bio-technology, Santa Cruz, CA) antibodies were used as protein loading control. Antibody binding to protein samples was visualized by the enhanced chemiluminescence procedure (Amersham Biosciences) and quantified with a Macintosh Color One scanner (Apple, Cupertino, CA) and NIH imaging software version 1.6. SR-BI and CLAMP expression studies were normalized to the signal of e-COP or actin proteins detected on the same nitrocellulose membrane.

**Immunofluorescence Analysis**—Mice were anesthetized by intraperitoneal injection of pentobarbital; the abdominal cavity was opened, and livers were perfused with cold phosphate-buffered saline (PBS) through the portal vein. Livers were then excised and frozen under 2-methylbutane in liquid nitrogen. Cryosections (4 μm thick) were fixed in 7% formaldehyde solution in PBS for 15 min, rinsed 3 times in PBS, and permeabilized with 1% Triton X-100, blocked overnight in 10% goat serum in PBS, and incubated for 2 h at room temperature with a polyclonal antibody generated against the entire SR-BI protein (35) (dilution 1:80). As secondary antibody, fluorescein-isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) (dilution 1:100) was used. Negative controls were performed by omitting the primary antibody. After washing in PBS, samples were mounted with coverslips using Fluoromount-G (Electron Microscopy Sciences, Fort Washington, PA). Stained sections were examined by immunofluorescence microscopy.

**RNA Analysis**—Total RNA was prepared from mouse liver by the acid guanidinium thiocyanate-phenol-chloroform method (37). RNA samples (20 μg/lane) were size fractionated by formaldehyde-agarose gel electrophoresis and transferred to nylon. cDNA probes for SR-BI, CLAMP, and 18 S rRNA were prepared by standard reverse transcriptase-PCR using primers designed on cDNA sequences available through GenBank® DNA databases. All probes were labeled by the random primer method (Promega, Madison, WI) and used for Northern blot hybridization as described previously (38). Quantification was performed by PhosphorImaging with the GS-525 Molecular Image System (Bio-Rad). The relative levels of SR-BI and CLAMP mRNAs were determined by normalizing in the same filter to the levels of 18 S rRNA.

**Statistical Analysis**—Results of hepatic protein and mRNA levels are expressed as fold change relative to the control group. The statistical significance of the differences between the means of the experimental groups was tested by the Student's t test or variance analysis. A difference was considered statistically significant when p < 0.05.

**RESULTS**

**Hepatic SR-BI Protein Levels in Fibrate-treated Mice**—In a previous study, Chinetti et al. (28) have shown that treatment with different PPAR-α agonists increased SR-BI protein mass in human macrophages and in atherosclerotic lesions of apoE-deficient mice. Here we tested two commonly used fibrates, ciprofibrate and fenofibrate, for the ability to modulate SR-BI protein levels in the murine liver. Treatment of mice with either 0.2% ciprofibrate or 0.2% fenofibrate drastically decreased SR-BI protein to undetectable levels when analyzed by immunoblotting in total liver membranes (Fig. 1A). The reduc-
Experimental Procedures.

0.2% ciprofibrate for 7 days. Liver samples were removed and processed for immunoblotting and immunofluorescence as described under "Experimental Procedures." Primary antibody binding was detected by appropriate secondary antibodies for chemiluminescence (A–C) or fluorescence microscopy (D). SR-BI protein levels are expressed as fold change relative to chow-fed mice after correction for 

Wild-type C57BL/6 mice were fed with standard chow diet without (control) and with supplementation with fibrates as follows: A, 0.2% ciprofibrate (CF) or 0.2% fenofibrate (FF) for 7 days; B, ciprofibrate at increasing doses for 7 days; C, 0.02% ciprofibrate for increasing times; D, 0.2% ciprofibrate for 7 days. Liver samples were removed and processed for immunoblotting and immunofluorescence as described under "Experimental Procedures." Primary antibody binding was detected by appropriate secondary antibodies for chemiluminescence (A–C) or fluorescence microscopy (D). SR-BI protein levels are expressed as fold change relative to chow-fed mice after correction for e-COP level (n = 4; *, p < 0.01). The results of this figure are representative of at least 3 independent experiments.

Fig. 1. Hepatic SR-BI protein expression analysis by immunoblotting and immunofluorescence in control and fibrate-treated mice. Wild-type C57BL/6 mice were fed with standard chow diet without (control) and with supplementation with fibrates as follows: A, 0.2% ciprofibrate (CF) or 0.2% fenofibrate (FF) for 7 days; B, ciprofibrate at increasing doses for 7 days; C, 0.02% ciprofibrate for increasing times; D, 0.2% ciprofibrate for 7 days. Liver samples were removed and processed for immunoblotting and immunofluorescence as described under "Experimental Procedures." Primary antibody binding was detected by appropriate secondary antibodies for chemiluminescence (A–C) or fluorescence microscopy (D). SR-BI protein levels are expressed as fold change relative to chow-fed mice after correction for e-COP level (n = 4; *, p < 0.01). The results of this figure are representative of at least 3 independent experiments.

Fig. 2. SR-BI protein expression in fibrate-treated cultured rat hepatocytes. Isolated rat hepatocytes were cultured for 24 h in the absence (control) or presence of 100 μM fenofibric acid (FF). Hepatocyte post-nuclear lysates were prepared, subjected to electrophoresis, and immunoblotted with anti-SR-BI and anti-actin antibodies. Primary antibody binding was detected by chemiluminescence. SR-BI levels are expressed as fold change relative to control cells after normalization for actin levels (*, p < 0.05). The results of this figure are representative of three independent experiments.

SR-BI Protein Expression in Fibrate-treated Hepatocytes—To evaluate whether fibrate treatment alters liver SR-BI protein expression through a direct action on the hepatocyte, the effect of 100 μM fenofibric acid, the active form of fenofibrate, on SR-BI protein levels was tested in cultured rat hepatocytes (Fig. 2). Treatment of rat hepatocytes with fenofibric acid reduced SR-BI protein expression by ~50% compared with nontreated hepatocytes, indicating that fibrates suppress hepatic SR-BI protein expression acting in a direct manner on liver parenchymal cells.

Hepatic SR-BI mRNA Levels in Mice Treated with Cipriofibrate—Fibrate-mediated changes in protein expression in the liver have been shown so far to be due to changes in target gene transcriptional activity, secondary to activation of the nuclear receptor PPAR-α (reviewed in Refs. 19 and 20). Therefore, we tested whether lowered hepatic SR-BI protein expression induced by fibrates was also due to decreased gene expression by analyzing SR-BI mRNA levels in livers from control and ciprofibrate-treated animals. Surprisingly, ciprofibrate treatment was not associated with changes in steady state hepatic SR-BI mRNA levels (Fig. 3), suggesting a post-transcriptional mechanism for fibrate-dependent regulation of SR-BI protein expression in the liver.

Fibrate Responsiveness of Hepatic SR-BI Expression in PPAR-α-deficient Mice—The next important question to address was whether the down-regulation of hepatic SR-BI expression by fibrates required the presence of PPAR-α or whether this effect was PPAR-α-independent. Thus, fibrate regulation of hepatic SR-BI protein expression was analyzed in mice lacking PPAR-α. Wild-type and PPAR-α-deficient mice were treated with 0.2% fenofibrate for 14 days, and hepatic SR-BI protein levels were analyzed by immunoblot (Fig. 4). Fibrate administration decreased SR-BI protein in livers of wild-type mice by 72%, whereas no change was observed in PPAR-α-deficient animals. The lack of a regulatory effect on hepatic protein SR-BI levels in fibrate-treated PPAR-α-deficient mice shows unequivocally that PPAR-α is both involved and required for the fibrate responsiveness of SR-BI protein expression.

Hepatic CLAMP Protein and mRNA Levels in Fibrate-treated Mice—CLAMP (for carboxyl-terminal linking and modulator protein, also known as PDZk1) is a multiple PDZ domain-
containing protein, which has recently been shown to interact through its most amino-terminal PDZ domain with the cytoplasmic carboxyl-terminal tail of SR-BI (29). By coexpressing SR-BI and CLAMP in Chinese hamster ovary cells, steady state levels of SR-BI protein were significantly higher in the presence of CLAMP (29), suggesting its role in stabilizing SR-BI protein expression.

Next, we evaluated whether changes in CLAMP expression may provide a potential mechanism for the fibrate-dependent post-transcriptional regulation of SR-BI protein expression in murine liver. As was the case for SR-BI, CLAMP protein levels were drastically decreased in the liver by fibrate administration (Fig. 5A), following dose and time pattern of responses (Fig. 5B and C). To evaluate further whether decreased hepatic CLAMP protein levels were due to transcriptional repression of the CLAMP gene by fibrates, steady state CLAMP mRNA levels in livers of control and fibrate-treated mice were measured by Northern blot analysis (Fig. 5C). Fibrates did not produce any alteration in hepatic CLAMP mRNA levels, indicating that neither SR-BI nor CLAMP were subjected to transcriptional regulation as a result of PPAR-α activation by fibrates.

**Plasma Lipoprotein Analysis and Recombinant Adenoviral Transfer of SR-BI to Fibrate-treated Mice**—Due to its ability to mediate selective HDL cholesterol uptake, the experimental manipulation of hepatic SR-BI levels in mice is tightly associ-

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formed FPLC lipoprotein cholesterol analyses in mice fed fibrate diets. Fig. 6 shows that ciprofibrate (A) and fenofibrate (B) treatment led to the appearance of a broader HDL cholesterol peak shifted to earlier FPLC fractions, indicating the presence of more disperse and larger HDL particles. In fact, the HDL-C peak of ciprofibrate (Fig. 6A, gray circles) and fenofibrate (Fig. 6B, gray circles) groups eluted one fraction ahead of the HDL peak detected in the control group (Fig. 6, open circles). Besides an altered HDL size distribution, there was a substantial reduction in VLDL cholesterol in fibrate-treated animals reflecting the well known effect of PPAR-α activation on triglyceride-rich lipoprotein synthesis and catabolism (18).

By using molecular size monitoring by a non-denaturing electrophoretic mobility assay of density gradient-purified HDL, similar effects of fibrates on HDL size were reported in wild-type and human apoA-I transgenic mice (25). That study later been reported in a variety of experimental models. In nephrotic rats, there was also a decrease in hepatic SR-BI protein, but SR-BI mRNA expression was originally suggested by the lack of effect of PPARE on hepatic SR-BI expression. However, the post-transcriptional regulation of SR-BI indeed required normal PPAR-α expression as shown by the lack of effect of fibrate treatment on hepatic SR-BI protein levels of PPAR-α-deficient mice (Fig. 4). Post-transcriptional control of SR-BI expression was originally suggested by the initial characterization of its expression pattern in different tissues (34) and has later been reported in a variety of experimental models. In apoE-deficient mice, there was a 2.3-fold induction of hepatic SR-BI protein without changes in SR-BI mRNA (39). Similarly, Witt et al. (40) demonstrated that hepatic SR-BI protein was strongly induced (11-fold) in rats fed a vitamin E-deficient diet and was down-regulated in HepG2 cells incubated with a vitamin E-enriched culture medium, in both cases without concomitant changes in SR-BI mRNA. In nephrotic rats, there was also a decrease in hepatic SR-BI protein, but SR-BI mRNA levels remained unaltered (41). Finally, intestinal SR-BI protein, but not mRNA, is down-regulated in several experimental models of impaired enterohepatic circulation, such as bile-diverted rats, Mdr2-deficient mice, and cholesterol-7a-hydroxylase-deficient mice (42). Whether altered PPAR-α signaling might be underlying the post-transcriptional regulation of hepatic SR-BI Down-regulation by Fibrates in Mice

consistently with a functionally relevant effect of fibrate-dependent hepatic SR-BI regulation on HDL metabolism in mice.

Even though SR-BI is a key regulator of HDL metabolism (reviewed in Refs. 3–5), the role of PPAR-α activation on controlling SR-BI expression in the liver, a major site of PPAR-α and SR-BI expression as well as selective HDL cholesterol uptake, had not been thoroughly investigated. In clear contrast to the effect observed in macrophages (28), ciprofibrate and fenofibrate led to a marked reduction of immunodetectable SR-BI in the liver of treated mice in a dose- and time-dependent manner (Fig. 1). More intriguingly, hepatic SR-BI mRNA levels remained unchanged (Fig. 3), suggesting a post-transcriptional mechanism for SR-BI protein down-regulation by fibrates. SR-BI protein was also reduced by fibrate treatment in isolated rat hepatocytes (Fig. 2), clearly showing that the in vivo data were not an indirect systemic effect of fibrates but rather involved a defined molecular mechanism of action of these drugs on the hepatic cells.

Because SR-BI was post-transcriptionally down-regulated, it was tempting to speculate that PPAR-α would not be required for the effect of fibrates on hepatic SR-BI expression. However, the post-transcriptional regulation of SR-BI indeed required normal PPAR-α expression as shown by the lack of effect of fibrate treatment on hepatic SR-BI protein levels of PPAR-α-deficient mice (Fig. 4). Post-transcriptional control of SR-BI expression was originally suggested by the initial characterization of its expression pattern in different tissues (34) and has later been reported in a variety of experimental models. In apoE-deficient mice, there was a 2.3-fold induction of hepatic SR-BI protein without changes in SR-BI mRNA (39). Similarly, Witt et al. (40) demonstrated that hepatic SR-BI protein was strongly induced (11-fold) in rats fed a vitamin E-deficient diet and was down-regulated in HepG2 cells incubated with a vitamin E-enriched culture medium, in both cases without concomitant changes in SR-BI mRNA. In nephrotic rats, there was also a decrease in hepatic SR-BI protein, but SR-BI mRNA levels remained unaltered (41). Finally, intestinal SR-BI protein, but not mRNA, is down-regulated in several experimental models of impaired enterohepatic circulation, such as bile-diverted rats, Mdr2-deficient mice, and cholesterol-7a-hydroxylase-deficient mice (42). Whether altered PPAR-α signaling might be underlying the post-transcriptional regulation of hepatic SR-BI Down-regulation by Fibrates in Mice

DISCUSSION

This study demonstrates that SR-BI and the SR-BI-interacting protein CLAMP are down-regulated by fibrates in the murine liver by a novel post-transcriptional PPAR-α-dependent mechanism. Fibrate-mediated reduced hepatic SR-BI expression correlated with enlarged HDL particle size, which was reverted by normalizing hepatic SR-BI levels by recombinant adenoaviral gene transfer. Taken together, these findings are
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SR-BI under some of these experimental conditions remains to be explored. However, the current work clearly indicates that hepatic SR-BI regulation is commanded by a PPAR-α-dependent fibrate-activated event possibly due to transcriptional modulation of a yet unrecognized gene that controls expression/stability of SR-BI protein itself and/or other proteins involved in the SR-BI pathway.

Interestingly, CLAMP (a hepatic protein potentially involved in SR-BI stabilization in hepatocyte plasma membrane (29)) showed a similar regulated expression as SR-BI in fibrate-treated animals (Fig. 5). This is the first example of pharmacological regulation of CLAMP expression in vivo. By using site-directed mutagenesis of SR-BI within the CLAMP-interacting domain, Silver (43) has shown recently the critical requirement of SR-BI/CLAMP interaction for adequate expression of SR-BI in the cell surface of hepatocytes. On the other hand, SR-BI expression is not required for hepatic CLAMP expression, because CLAMP protein levels are normal in livers of SR-BI-deficient mice (data not shown). We speculate that the simultaneous post-transcriptional down-regulation of hepatic CLAMP and SR-BI expression by fibrates might have been caused by modulation of a yet undiscovered PPAR-α target gene that controls SR-BI and CLAMP protein synthesis, interaction, intracellular trafficking, or degradation as a protein-protein complex, or by two completely independent regulatory events on these proteins elicited by fibrates. As reported previously for estrogen and androgen receptors (44–46), PPAR-α could also be modulating a signal transduction pathway through a non-genomic activity that results in decreased SR-BI and CLAMP protein levels.

The effect of fibrates on hepatic SR-BI and CLAMP protein expression is indeed the first reported case in which fibrate treatment-induced changes in the levels of proteins expressed in the liver are not associated with concomitant variations in their corresponding mRNAs. In this regard, our study also indicates that gene expression analyses associated with PPAR-α activation might have been biased by only looking at steady state mRNA levels of target genes rather than protein levels or functional activities encoded by those genes. In fact, a preliminary study (47) has reported no effect of treatment with PPAR-α agonists on hepatic SR-BI mRNA levels in primary rat hepatocytes. Our analysis on SR-BI regulation induced by fibrates emphasizes the potential relevance of proteomic studies regarding regulation by pharmacological or physiological activators of PPAR-α in particular as well as other nuclear receptors and transcription factors.

Mice treated with the PPAR-α agonists cipriobafibrate or fenofibrate exhibited enlarged HDL particle sizes, which were normalized when hepatic SR-BI protein levels were restored by recombinant adenoviral SR-BI gene transfer in fibrate-treated mice (Fig. 6, A and B). These findings are consistent with a potential role of SR-BI in the altered HDL particle size distribution produced by fibrate treatment besides the up-regulation of PLTP (25). The absolute requirement of PLTP up-regulation for fibrate-induced HDL particle enlargement, as reported previously (25), does not discard the notion that this phenotype might also be due to the down-regulation of SR-BI, whose deficiency in mice caused the appearance of bigger and more heterogeneous HDL particles (6) similar to those found in fibrate-treated mice. We speculate that SR-BI may contribute to the appearance of abnormal HDL particles in fibrate-treated PLTP-expressing mice as a downstream element of the PLTP-mediated HDL remodeling. However, the hepatic SR-BI pathway for selective cholesterol uptake may be less relevant in PLTP-deficient mice because this receptor has higher affinity for lipid-rich large HDL (48). In fact, lipid-poor HDL from PLTP knockout mice are hypercatabolized most likely through an SR-BI-independent pathway (49). Taken together, these findings suggest that SR-BI and PLTP may act in concert to modulate HDL size distribution in wild-type mice, whereas SR-BI is less relevant in controlling HDL particle size in PLTP-deficient animals.

In summary, we have identified a novel PPAR-α-dependent, post-transcriptional effect of fibrates over SR-BI expression in murine liver, which may lead to a better understanding of how these drugs affect lipoprotein metabolism in vivo.

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