Developmental and Pharmacological Regulation of Apolipoprotein C-II Gene Expression
Comparison With Apo C-I and Apo C-III Gene Regulation

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Abstract—Increased plasma triglyceride concentrations are often observed in metabolic disorders predisposing to coronary heart disease. Among the major determinants of plasma triglyceride metabolism are the apolipoproteins (apos) of the C class, C-I, C-II, and C-III. Whereas physiological concentrations of apo C-II are required for lipolysis of triglycerides by lipoprotein lipase (LPL), overexpression of all 3 C apolipoproteins leads to hypertriglyceridemia. In the present study, we investigated apo C-II gene regulation under conditions associated with profound changes in plasma triglyceride metabolism, ie, during postnatal development and after treatment with the triglyceride-lowering fibrate drugs, and compared its expression to that of apo C-I and apo C-III. Whereas the expression of both apo C-I and apo C-III is low in fetal liver, increases gradually after birth, and attains maximal levels after weaning, apo C-II gene expression is already detectable in the fetal liver, increases rapidly immediately after birth, and remains elevated throughout suckling. Thus, the increased ingestion of lipids during suckling is met by an earlier induction of apo C-II, the obligatory activator for LPL, compared with apo C-III and apo C-I, which antagonize triglyceride catabolism. Treatment of rats with fibrates decreased apo C-II gene expression in the liver, but not in the intestine, whereas apo C-I gene expression did not change. The decrease of liver apo C-II mRNA levels after fenofibrate occurred in a time- and dose-dependent manner and was reversible but appeared less pronounced than the decrease of apo C-III mRNA. Apo C-II mRNA levels were not affected after treatment with BRL49653, a peroxisome proliferator–activated receptor (PPAR)γ-specific ligand, suggesting that fibrates act on apo C-II expression via PPARγ. Addition of fenofibric acid to primary rat and human hepatocytes resulted in a decrease of apo C-II expression. In conclusion, fibrates decrease gene expression of apo C-II and apo C-III, but not apo C-I, in rat and human hepatocytes. This decrease of apo C-II and apo C-III gene expression, together with a lowered apo C-III to apo C-II ratio, should result in an improved clearance of triglyceride-rich remnant lipoproteins from plasma, without hampering triglyceride lipolysis by LPL. (Arterioscler Thromb Vasc Biol. 1999;19:115-121.)

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Hypertriglyceridemia, a common disorder predisposing to atherosclerosis and, in severe cases, to pancreatitis,1 is caused by overproduction and/or clearance defects of triglyceride-rich lipoproteins. Apolipoproteins (Apos) C-I, C-II, and C-III, which are protein components of the triglyceride-rich chylomicrons and VLDLs, are key players in plasma triglyceride metabolism. The initial step in the catabolism of plasma triglyceride-rich lipoproteins occurs through hydrolysis of the triglycerides by the enzyme lipoprotein lipase (LPL), which is located on the vascular endothelium.2 However, proper hydrolysis of these triglyceride-rich particles depends not only on LPL but also on their relative contents of the different C apolipoproteins. In contrast to apo C-I and apo C-III, which can be considered antagonists, apo C-II is a protagonist of plasma triglyceride catabolism under physiological conditions. Apo C-II is mainly synthesized and secreted by the liver as a component of nascent VLDL, but it is also produced to a lesser extent by the intestine as a component of chylomicrons.3,4 Apo C-II plays an important role in the hydrolysis of lipids as an obligatory activator for LPL.1,2 This function of apo C-II is illustrated in patients with homozygous deficiency of apo C-II, who display a massive hypertriglyceridemia comparable to the one observed in patients homozygous for LPL deficiency.1,5 Paradoxically and in contrast to its role as an activator of triglyceride hydrolysis under physiological conditions, at
high concentrations apo C-II displays antagonistic functions on plasma triglyceride metabolism, a property that it shares with the other C apolipoproteins. In all types of hypertriglyceridemia except for type I (familial apo C-II deficiency), positive correlations between plasma apo C-II and apo C-III, on the one hand, and triglyceride concentrations on the other, have been observed.4–8 In addition, a negative correlation between LPL activity and levels of apo C-II and apo C-III in plasma have been demonstrated in hypertriglyceridemic patients.9 Furthermore, in transgenic mice overexpressing either human apo C-I,10,11 apo C-II,12 or apo C-III,13 an accumulation of triglyceride-rich lipoproteins in plasma occurs, which suggests a causal relationship. These observations are mechanistically supported by results from in vitro studies showing that an excess of C apolipoproteins on triglyceride-rich lipoprotein particles interferes with their binding to cell surface receptors and subsequent clearance.14–17 As such they interfere with the function of apo E, which is a ligand of different lipoprotein receptors facilitating clearance of these remnant particles from plasma.15–17 Furthermore, apo C-III as well as apo C-II at high concentrations have, at least in vitro, an inhibiting effect on LPL lipolytic activity.9,18–20 Therefore, both the absolute level of the different C apolipoproteins as well as their respective ratios on the lipoprotein particle are important determinants of its metabolic fate.21,22 Indeed, in hypertriglyceridemic patients, both the absolute concentrations of the different C apolipoproteins as well as the apo C-III to apo C-II ratio are increased,26–30 both factors that may contribute to less efficient lipolysis by LPL as well as reduced apo E–dependent clearance.14,17

Patients with hypertriglyceridemia are often treated with hypolipidemic drugs belonging to the class of fibrates.23 Recent studies from our and other laboratories on the mechanisms behind the triglyceride-lowering action of fibrates indicate that these drugs act primarily on the liver by modifying the expression level of key genes involved in lipoprotein metabolism (reviewed in References 23 and 24). For instance, fibrate treatment results in the induction of liver LPL expression25 and a downregulation of apo C-III expression,26 whereas apo E expression is not affected.26,27 These changes undoubtedly contribute to the increase in both lipolysis and receptor-mediated reduction of remnant lipoproteins, which result in the clinical effects observed after fibrate treatment. Fibrates act by modifying the transcription of specific target genes through activation of the peroxisome proliferator–activated receptors (PPARs). PPARs are transcription factors that belong to the superfamily of nuclear hormone receptors (reviewed in Reference 24). These receptors bind to response elements in the regulatory regions of target genes and thereby regulate their expression. So far, 3 different PPAR forms, α, β(δ), and γ, have been identified. Whereas the antidiabetic thiazolidinediones are specific ligands for PPARγ, fibrates appear to act primarily through the PPARα isoform.24–30

In view of the important role of the different C apolipoproteins in triglyceride metabolism, we studied their regulation under conditions known to profoundly affect plasma triglycerides: during development and after fibrate treatment. During postnatal development rats change their diet from a carbohydrate-rich one during the suckling period to a carbohydrate-rich diet after weaning. Our results show that apo C-II gene expression increases rapidly after birth and remains elevated throughout the suckling period, whereas apo C-I and apo C-III gene expression appear much more gradually thereafter. This expression pattern of apo C-II is reminiscent of that of the LPL gene, which is only expressed in the fetal and neonatal rat liver.23 Furthermore, we show that fibrates downregulate apo C-II gene expression, albeit to a lesser extent compared with apo C-III. In contrast, apo C-I gene expression is not affected by fibrates. Interestingly, the downregulation of apo C-II expression by fibrates occurs not only in rat but also in human hepatocytes. In contrast to fibrates, treatment with BRL49653, a synthetic PPARγ ligand with hypolipidemic activity,26 does not affect apo C gene expression, indicating that fibrates act through the PPARα form.

Methods

Animals and Treatments

Adult male rats were treated for different periods of time with fenofibrate, ciprofibrate, bezafibrate, or clofibrate either mixed in standard rat chow or given intragastrically at the indicated concentrations. BRL49653 suspended in 1% carboxymethyl cellulose was administered intragastrically once daily at the indicated doses. At the end of the experiments, the animals were killed by exsanguination under ether anesthesia. Liver and intestinal epithelia were removed immediately and frozen in LN2. To determine the developmental expression patterns, male rats of different ages (at least 4 per age group) were killed and the livers pooled. Pups born between the morning of 1 day and the next morning were considered 0 days old. Animal studies were performed according to the Guidelines for the Care and Use of Experimental Animals.

Isolation and Culture of Rat and Human Hepatocytes

Rat hepatocytes were isolated by collagenase perfusion of livers from male rats weighing between 150 and 250 g (cell viability >85% by the trypan blue exclusion test). The hepatocytes were cultured in monolayer (1.5×104 cells/cm2) as previously described.26 Treatment with fenofibric acid (in dimethyl sulfoxide [DMSO]; 0.1% vol/vol final concentration) at the indicated concentrations and periods of time was started immediately after seeding. Human liver specimens were collected and hepatocytes isolated as previously described.26 Fenofibric acid (in DMSO; final concentration 0.5% vol/vol) was added at the indicated concentration in serum-free medium. At the end of the experiments, cells were washed 3 times with ice-cold PBS and solubilized by addition of 1 mL of 4 mol/L guanidinium isothiocyanate solution.

RNA Analysis

Total cellular RNA was prepared by the guanidinium isothiocyanate/ CsCl procedure (liver and intestine) or by the acid guanidinium isothiocyanate/phenol/chloroform method (primary hepatocyte cultures). Northern and dot-blot hybridizations of total cellular RNA were performed as described previously.27 The human apo C-II cDNA probe was the truncated form inserted in pUC18 and digested with EcoRI as described.32 The rat apo C-II cDNA probe was prepared by digestion with EcoRI, yielding a 600-bp fragment, as previously described.32 The mouse apo C-I probe, a kind gift from Dr M. Hofker, was prepared by EcoRI and HindIII digestion, yielding a 700-bp fragment as described.32 The rat and human apo C-III cDNA probes were exactly as described previously.27 As controls, rat apo E or 36B4, encoding for the human acidic ribosomal phosphoprotein P0, cDNA probes were used.23,31 All cDNA probes were labeled by random-primed labeling (Boehringer Mannheim). Filters were hybridized to 1.5×106 cpm/mL of each probe. The filters were washed twice in 0.5× SSC and 0.1% SDS for 10 minutes at 42°C and twice for 30 minutes at 65°C and subsequently exposed to x-ray film.
Figure 1. Developmental regulation of hepatic apo C-I, apo C-II, and apo C-III gene expression. Total RNA was prepared from pooled livers (n=4) of rats of different ages (at 17 and 19 days of gestation; 21 days of gestation = birth [day 0]; and 1, 3, 5, 20, and 80 days after birth). Apo C-I, apo C-II, and apo C-III mRNA levels were determined by dot blot analysis as described in Methods. Values are expressed relative to the levels in 80-day-old adult rats.

Antigen Isolation and Antibody Preparation
Apo C-II was isolated from VLDL of hypertriglyceridemic subjects fasted for 12 to 16 hours. VLDL was isolated by preparative ultracentrifugation, and the apo VLDLs were isolated by selective extraction with organic solvents. Apo C-II was purified from apo VLDL by anion-exchange fast-protein liquid chromatography. Antisera against apo C-II were prepared by injecting New Zealand White rabbits with purified apo C-II protein, and anti-apo C-II antibodies were isolated by immunoaffinity chromatography using an apo C-II immunosorbent.

Apo C-II and Apo C-III ELISAs
Apo C-II was measured by ELISA essentially as described. Purified rabbit anti-apolipoprotein C-II polyclonal antibody (10 μg/mL) was used as the capture antibody. The same antibody conjugated to horseradish peroxidase was used for detection with a chromogen (3,3′-diaminobenzidine dihydrochloride; Sigma Chemical Co). Pure apo C-II protein was used as a standard for determining the concentration of apo C-II in the culture medium. Apo C-III was measured by ELISA exactly as described.

Statistical Methods
Statistical analyses of treatments were performed using the Kruskal-Wallis test, followed by comparison of differences between separate groups by the Mann-Whitney U test.

Results
Developmental Regulation of Liver Apo C-I, Apo C-II, and Apo C-III Gene Expression in Rats
Apo C-II mRNA levels are already detectable in the fetal rat liver, being ≈50% of the levels in the adult rat liver (80 days of age; Figure 1). Around the time of birth there is a sharp increase in apo C-II mRNA levels, reaching a maximum at day 5 of age and declining thereafter to reach adult levels at ≈day 20. In contrast, both apo C-I and apo C-III mRNA levels are low in fetal and neonatal rat liver and increase gradually after birth, reaching adult levels at the suckling-weaning transition period (day 20). These observations indicate that expression of the LPL activator apo C-II closely follows liver LPL expression in the rat liver, being highest during the suckling period, whereas expression of the apo C-I and apo C-III genes is highest after weaning when rats start consuming a diet low in lipids.

Comparison of the Influence of Fenofibrate on Apo C-I, Apo C-II, and Apo C-III Gene Expression in Rats
Next the influence of fibrates was studied on apo C-I and apo C-II gene expression and compared with their effects on apo C-III. Therefore, adult male rats were treated for 7 days with the indicated doses of fenofibrate (wt/wt, mixed in rat chow) and killed after an overnight fast. Total RNA was extracted and apo C-I, apo C-II, apo C-III, and apo E mRNA levels were measured by dot blot (A) and Northern blot (B) analyses as described under Methods. Values represent the mean±SD of 4 animals. Statistically (Mann-Whitney U test, P<0.05) significant differences were observed between values followed by different letters.

Downregulation of Apo C-II Gene Expression by Fenofibrate Is Time Dependent, Tissue Specific, and Reversible
Apo C-II mRNA levels tended to decrease after 1 day of fenofibrate treatment, and a significant reduction was already observed after 3 days of treatment (Figure 3A). In contrast to those in the liver, intestinal apo C-II mRNA levels did not change significantly after treatment with fenofibrate, either at different doses or after several days of administration of the highest dose of fenofibrate used (0.5%; data not shown). These results indicate that apo C-II gene regulation is regulated in a tissue-specific manner by fibrates.

Because fibrates induce peroxisome proliferation and hepatomegaly in rodents, we next investigated whether the decreased liver apo C-II gene expression was associated with general, irreversible changes in liver structure and function or...
Gene Expression

Does Not Influence Rat Liver Apo C-II

triglycerides, 29, 40 we next investigated whether apo C-II expression by fenofibrate. A, Adult male rats were treated for the indicated number of days with fenofibrate (0.5%, wt/wt, mixed in rat chow), fasted overnight, and killed as described under Methods. Total RNA was extracted and apo C-II mRNA levels were measured as described in Methods. Values represent mean ± SD of 3 animals. A, Adult male rats (n=3) were given fenofibrate (0.5% wt/wt, mixed in rat chow) for 14 days, fasted overnight, and killed as described under Methods. Treatment with fenofibrate was stopped on day 0. Apo C-II mRNA levels were measured in livers of rats on 0, 1, 3, 7, 14, and 28 days after cessation of fenofibrate treatment and compared with the levels in untreated controls (C). Statistically (Mann-Whitney U test, P<0.05) significant differences are observed between values followed by different letters. Whether the downregulation of apo C-II expression was reversible on cessation of therapy. Therefore, rats were treated for 14 days with 0.5% fenofibrate, after which time fenofibrate was withdrawn (Figure 3B). Compared with the levels in untreated control rats, apo C-II mRNA levels dropped to ≈35% after 14 days of fenofibrate administration (cf C in Figure 3B with day 0). Apo C-II mRNA levels returned to control levels within 7 days after interruption of fenofibrate treatment, indicating that the repressive activity of fenofibrate on apo C-II gene expression is reversible (Figure 3B).

Downregulation of Apo C-II Gene Expression Is a General Characteristic of Fibrates

To investigate whether downregulation of apo C-II gene expression is a general property of fibrates, rats (n=4 per group) were treated with different fibrates for 14 days, and liver apo C-II mRNA levels were analyzed subsequently. Treatment with clofibrate (0.3% wt/wt), ciprofibrate (0.05% wt/wt), and bezafibrate (0.5% wt/wt), respectively, decreased liver apo C-II mRNA levels to 61±18%, 17±5%, and 25±4% of controls (100±2%), indicating that all fibrates tested reduce liver apo C-II gene expression in rats.

Treatment With the PPARγ Agonist BRL49653 Does Not Influence Rat Liver Apo C-II Gene Expression

Because both PPARα and PPARγ activators decrease plasma triglycerides, 29, 40 we next investigated whether apo C-II expression was also under control of PPARγ activators. Therefore, rats were treated with the thiazolidinedione BRL49653, a high-affinity PPARγ ligand, at a concentration previously shown to reduce plasma triglycerides, 40 and its effects were compared with those of fenofibrate. BRL49653 treatment did not change rat liver apo C-II or apo C-III gene expression, whereas fenofibrate decreased the expression of both genes (Figure 4). Combination treatment with both drugs resulted in a reduction comparable to that of fenofibrate alone. Under these conditions plasma triglyceride concentrations changed from 154±45 mg/dL in the control rats to 56±24, 102±27, and 37±14 mg/dL in the fenofibrate-, BRL49653-, and fenofibrate plus BRL49653-treated rats, respectively. Treatment with neither BRL49653 nor fenofibrate significantly influenced mRNA levels of apo C-I or apo E, the ligands for receptors mediating remnant particle clearance. These results further confirm that fibrates regulate triglyceride metabolism through distinct mechanism as thiazolidinediones and that this implicates PPARα, but not PPARγ, activation.

Fenofibric Acid Decreases Apo C-II Gene Expression in Primary Cultures of Rat and Human Hepatocytes

To study whether the regulation of apo C-II gene expression observed in vivo was due to a direct effect on the hepatocyte, primary cultures of adult rat hepatocytes were isolated and treated with fenofibric acid. Addition of fenofibric acid for 24 hours to the culture medium of primary rat hepatocytes resulted in a dose-dependent reduction of apo C-II mRNA levels to 58% and 42% of the untreated control value at 200 and 300 μmol/L of fenofibric acid, respectively.

Addition of fenofibric acid for 48 hours to the culture medium of primary hepatocytes isolated from human liver resulted in a decrease of human apo C-II mRNA levels to

![Figure 3](image_url)

**Figure 3.** Time-dependent and reversible regulation of rat liver apo C-II gene expression by fenofibrate. A, Adult male rats were treated for the indicated number of days with fenofibrate (0.5%, wt/wt, mixed in rat chow), fasted overnight, and killed as described under Methods. Total RNA was extracted and apo C-II mRNA levels were measured as described in Methods. Values represent mean ± SD of 3 animals. B, Adult male rats (n=3) were given fenofibrate (0.5% wt/wt, mixed in rat chow) for 14 days, fasted overnight, and killed as described under Methods. Treatment with fenofibrate was stopped on day 0. Apo C-II mRNA levels were measured in livers of rats on 0, 1, 3, 7, 14, and 28 days after cessation of fenofibrate treatment and compared with the levels in untreated controls (C). Statistically (Mann-Whitney U test, P<0.05) significant differences are observed between values followed by different letters.

![Figure 4](image_url)

**Figure 4.** Comparison of the effects of BRL49653 and fenofibrate on liver apo C-I, apo C-II, and apo C-III mRNA levels. Rats (n=3) were treated for 14 days with BRL49653 (5 mg · kg⁻¹ · day⁻¹), fenofibrate (40 mg · kg⁻¹ · day⁻¹, which corresponds to a dose of ≈0.05% wt/wt when mixed in rat chow), or both as described under Methods. Control rats received vehicle only. Animals were not fasted before they were killed. Statistically (Mann-Whitney U test, P<0.05) significant differences are observed between values followed by different letters.

![Figure 5](image_url)

**Figure 5.** Regulation of apo C-II expression by fenofibric acid in primary cultures of adult human hepatocytes. Human hepatocytes were isolated and incubated for 48 hours with fenofibric acid (250 μmol/L) or vehicle (DMSO). Apo C-II, apo C-III, and 36B4 mRNA levels and apo C-II and apo C-III protein levels were measured as described under Methods. B, Values are expressed relative to controls and represent mean ± SD of 3 points. Statistically (Mann-Whitney U test, P<0.05) significant differences are indicated by an asterisk.
C-II acts predominantly as an obligatory activator of LPL,1,2,41 thus allowing a more efficient catabolism of the fat.

C-I and apo C-III mRNA levels increase gradually during days after birth. Thereafter, apo C-II gene expression decreases, reaching a maximum a few days after birth. Immediately after birth gene expression is barely detectable. Immediately after birth marked changes during this period of development, roughly following 2 distinct patterns. The apo C-II gene is already highly expressed in fetal liver, whereas apo C-I and apo C-III gene expression is barely detectable. Immediately after birth apo C-II mRNA levels increase, reaching a maximum a few days after birth. Thereafter, apo C-II gene expression decreases slightly, reaching adult rat liver levels around the suckling-weaning transition (day 20 of age). By contrast, apo C-I and apo C-III mRNA levels increase gradually during suckling, reaching adult levels around weaning. This differential expression pattern of genes with opposing functions in triglyceride metabolism is certainly of physiological importance, thus allowing a more efficient catabolism of the fat load from milk. Whereas under physiological conditions apo C-II acts predominantly as an obligatory activator of LPL,1,12,41 apo C-III and possibly apo C-I act as inhibitors of LPL activity.9,18 The apo C-III to apo C-II ratio has been implicated as a determinant for efficient lipid metabolism, with a low ratio favoring lipolysis.51,12 Interestingly, LPL gene expression in rat liver follows a pattern similar to that of apo C-II, although after weaning LPL expression is extinguished in the liver, presumably because at that time LPL expression is sufficiently high in other tissues to metabolize fatty acids.31,42 Furthermore, the liver, which starts secreting triglycerides in VLDL particles after weaning, no longer requires a high import of fatty acids. The increased expression of the LPL and apo C-II genes probably reflects the increased need to handle the huge amount of ingested fat in the milk during suckling, whereas to improve triglyceride catabolism, the inhibitors apo C-I and apo C-III are most likely regulated in such a way to keep them at low concentrations.

Although the postnatal period is accompanied by a variety of hormonal and nutritional changes, it is tempting to speculate that it is the high-fat diet itself that stimulates the transcription of LPL and apo C-II. Such a mechanism may implicate activation of specific transcription factors of the PPAR family. The PPARs belong to the nuclear hormone receptor gene superfamily and are activated by peroxisome proliferators, among which are fatty acids and fibrate hypolipidemic drugs.23 In rodents the PPARs isoform mediates the induction, by these agents, of several other genes coding for peroxisomal enzymes, which results in a strong proliferation of peroxisomes and an extreme hepatomegaly.24 Although this phenomenon does not occur in humans, fibrates are also implicated in the regulation of several human genes involved in lipoprotein metabolism, such as apo A-I,43 apo A-II,44 apo C-III,26 and LPL,29 all of which carry PPAR-responsive elements in their promoter regions. Because fibrates are highly efficient lipid-lowering drugs used in the treatment of hypertriglyceridemia,23,44 we further studied their influence on apo C-I and apo C-II expression and compared their effects to those on apo C-III. Our results show that fenofibrate treatment decreases rat hepatic apo C-II gene expression in a dose- and time-dependent manner, which is already significant after 3 days and maximal within 7 days of treatment. After withdrawal of fenofibrate, apo C-II expression returns toward normal within several days, indicating that the reduction in apo C-II expression is reversible and not linked to irreversible changes in liver structure, morphology, or function. This decrease in apo C-II expression after fenofibrate treatment is a result of a direct action on the hepatocyte and not an indirect effect due to alterations in plasma lipid and lipoprotein concentrations, since treatment of isolated rat hepatocytes with fenofibrate, the active form of fenofibrate, results in a downregulation of apo C-II expression. Because all fibrates tested lower hepatic apo C-II mRNA levels in the rat, it appears to be a general effect of fibrates.

In both humans and rats, the main apo C-II–producing tissues are the liver and intestine,3,33 with the intestine producing 10% to 30% of that made by the liver. Interestingly, similar to the situation for apo C-III,26 fibrate treatment downregulates apo C-II mRNA levels in both human and rat hepatocytes but not in the intestines. These data extend previous observations that fibrates regulate genes involved in lipoprotein metabolism in a tissue-specific manner in the rat.26,27,46 Previous reports suggested that the downregulation of apo C-III gene expression by fibrates was due to a dual mechanism involving competition for binding and repression of expression of the hepatocyte-enriched transcription factor HNF-4.47 Although the apo C-II gene promoter has not yet been characterized and the factors driving its expression in liver are unknown, it is tempting to speculate that the negative effect of fibrates on apo C-II gene expression occurs through similar molecular mechanisms.

An increase of the apo C-II and apo C-III concentrations in plasma is observed in hypertriglyceridemic patients, and these concentrations are positively correlated to triglyceride level5–8 and negatively to LPL activity.1 Furthermore, hypertriglyceridemias also have increased synthesis rates of apo C-II and apo C-III.5,69 Although the mechanism behind these changes in expression in hypertriglyceridemic patients is unknown, fenofibrate treatment can normalize these abnormalities, at least in part, by lowering plasma apo C-III and apo C-II concentrations.49 These observations suggest an active role for apo C-II and apo C-III as factors participating in plasma lipoprotein clearance as well as in modulating LPL
activity. Indeed, high concentrations of apo C-II have been shown to inhibit LPL activity in vitro. Furthermore, all 3 C apolipoproteins interfere with the apo E–mediated hepatic uptake of lipoproteins in vitro. The mechanisms behind the triglyceride-lowering action of fibrates are multiple, including a lowered synthesis of VLDL triglycerides, an increased lipolysis, as well as increased receptor-mediated uptake of the remnant particles from plasma. The decrease of apo C-II and apo C-III gene expression by fibrates may contribute to all of these steps. Our results on rat and human primary hepatocytes indicate that fibrates decrease apo C-II and apo C-III gene expression through a direct action on the hepatocyte (this study and Reference 26). This leads to a decreased apo C to apo E ratio of triglyceride-rich particles, since fibrates do not influence apo E expression (this study and References 26 and 27). These triglyceride-rich particles could then be more efficiently cleared from the plasma via cellular receptor uptake. Furthermore, fibrate treatment also lowers the apo C-III to apo C-II ratio, thereby enhancing triglyceride lipolysis and clearance.

Similar to fibrates, which act through liver PPARα treatment with thiazolidinediones such as BRL49653, which are synthetic ligands for the adipose tissue–selective PPARγ form, also decrease triglyceride concentrations in plasma (this study and Reference 40). In contrast to fibrates, however, BRL49653 acts primarily by inducing LPL expression in adipose tissue. Interestingly, BRL49653 treatment does not influence apo C-II or apo C-III gene expression, nor does it change the lipid and apolipoprotein composition of the secreted VLDL particles. This may explain why, in contrast to fibrates, the triglyceride lowering after thiazolidinedione treatment is accompanied by an increased accumulation of remnant particles in plasma. Altogether these data further extend the concept that compounds with combined PPARα and PPARγ activation potential should yield more efficient drugs for the treatment of hypertriglyceridemia.

Interestingly, fenofibrate treatment did not influence apo C-I gene expression in rat liver. Although little is known on the exact functions of apo C-I, a recent study showed elevated plasma apo C-I concentrations in hypertriglyceridemic patients. Furthermore, as for apo C-II and apo C-III, transgenic mice overexpressing apo C-I10,11 develop severe hypertriglyceridemia. In addition, in vitro studies have demonstrated that of all 3 C apolipoproteins, apo C-I most efficiently interferes with receptor-mediated uptake. If apo C-I proves to be causally related to the development of hypertriglyceridemia in humans also, these results may incite efforts to find novel drugs that lower apo C-I gene expression.

In summary, we have shown that apo C-II gene expression is regulated during development and by fibrates, but not by thiazolidinediones. By contrast, fenofibrate treatment did not influence apo C-I gene expression. In the hypertriglyceridemic patients, this decrease of apo C-II expression by fibrates may be beneficial by favoring the otherwise-impaired lipolysis and particle clearance.

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