Cholesteryl ester transfer protein modulates the effect of liver X receptor agonists on cholesterol transport and excretion in the mouse

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Abstract Human plasma, unlike mouse plasma, contains the cholesteryl ester transfer protein (CETP) that may influence the reverse cholesterol transport. Liver X receptor (LXR), an oxysterol-activated nuclear receptor induces CETP transcription via a direct repeat 4 element in the CETP gene promoter. The aim of the study was to assess in vivo the impact of LXR activation on CETP expression and its consequences on plasma lipid metabolism and hepatic and bile lipid content. Wild-type and humanized mice expressing CETP were treated for five days with T0901317 LXR agonist. This treatment produced marked rises in both hepatic CETP mRNA and plasma CETP activity levels. Interestingly, the LXR agonist-mediated, 2-fold rise in both total and HDL cholesterol levels in treated wild-type mice was not observed in CETPTg mice, and the accumulation of cholesterol in the liver of CETPTg mice was reversed by LXR agonist treatment. Moreover, LXR activation induced a 2-fold increase in hepatic LDL-receptor expression in wild-type and CETPTg mice, and it produced a significantly greater rise in biliary cholesterol concentration in CETPTg mice as compared with wild-type mice. In conclusion, induction of CETP constitutes a major determinant of the effect of LXR agonists on cholesterol transport and excretion.—Masson, D., B. Staels, T. Gautier, C. Desrumaux, A. Athias, N. Le Guern, M. Schneider, Z. Zak, L. Dumont, V. Deckert, A. Tall, X-C. Jiang, and L. Lagrost. **Cholesteryl ester transfer protein modulates the effect of liver X receptor agonists on cholesterol transport and excretion in the mouse.** ***J. Lipid Res.*** **2004.** 45: **543–550.**

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Recently, the use of synthetic liver X receptor (LXR) agonists appeared as a potential new pharmacological approach to stimulating reverse cholesterol transport (RCT) in vivo (1, 2). LXRs α and β are nuclear receptors that are activated by oxysterols (3, 4). They are involved in the regulation of cholesterol homeostasis, lipogenesis, glucose metabolism, and inflammation (4–9). Moreover, LXR agonist administration inhibits the development of atherosclerosis in LDL receptor (LDLR)-deficient and apolipoprotein E (apoE)-deficient mouse models, an effect that probably results from the modulation of the expression of both metabolic and inflammatory genes (10, 11). With regard to cholesterol metabolism, LXRs regulate RCT by inducing the expression of genes that are involved in cellular efflux, plasma transport, and biliary excretion of cholesterol. In animal models, the activation of LXR by synthetic agonists was shown to increase plasma HDL concentration, probably as the result of the induction of phospholipid transfer protein (PLTP), and ATP binding cassette (ABC) transporters A1 and G1 (12–14). LXR activation is also associated with a decrease in cholesterol content of the liver, an increase in biliary cholesterol secretion, and an increase in fecal neutral sterol excretion (5, 15, 16). Expression of the transporters ABCG5/G8 is known to be required for the latter phenomena to occur (15).

It is worth noting that most of the previous studies with LXR agonists were conducted in the mouse, an animal model having no plasma cholesteryl ester transfer protein (CETP) activity (17). CETP is a plasma glycoprotein that promotes the exchange of neutral lipids, i.e., cholesteryl

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Abbreviations: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; LXR, liver X receptor; PLTP, phospholipid transfer protein; RCT, reverse cholesterol transport; SREBP, sterol-responsive element binding protein.

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esters (CEs) and triglycerides, between different lipoprotein classes (18). Through its action, CETP is likely to influence both the atherogenicity of the lipoprotein profile and the centripetal flux of cholesterol from peripheral tissues toward the liver, a process also called reverse cholesterol transport. In particular, the rise in plasma CETP level in CETP transgenic mice is associated with the redistribution of CEs from the antiatherogenic HDLs to the proatherogenic VLDLs and LDLs (19–23). The lack of active CETP in the mouse may be a major limitation to the study of cholesterol homeostasis after LXR agonist administration, because CETP, one key factor in RCT, is a well-known LXR target (24, 25). Although CETP was proposed to exert a beneficial action in RCT by offering an alternative pathway for cholesterol to be brought back to the liver through the apoB-containing lipoprotein pathway, its potential beneficial action is counterbalanced by the concomitant downregulation of the LDLR in the liver that results from increased hepatic cholesterol content in CETPTg mice (23). This increased hepatic cholesterol content is likely to be modified upon LXR agonist administration, because many genes controlling cholesterol homeostasis, in addition to CETP, were shown to be stimulated by pharmacological LXR agonists.

The aim of the present study was to determine the impact of the synthetic T0901317 LXR agonist on the lipoprotein profile, hepatic lipid metabolism, and biliary cholesterol secretion in the absence or in the presence of CETP, an LXR target gene that plays a major role in HDL metabolism. To this end, C57Bl6 wild-type and CETPTg mice expressing human CETP under the control of its natural flanking regions containing an LXR-responsive element (20) were treated for 5 days with a synthetic LXR agonist at a dose of 10 mg/kg/day, shown to be efficient in increasing HDL cholesterol. After treatment with the agonist, lipid content of plasma, liver, and bile was determined, and resulting data were analyzed in the context of concomitant changes in the expression of several genes, i.e., CETP, ABCG5, LDLR, and sterol-responsive element binding proteins (SREBPs), that are known to be involved in cholesterol metabolism. The present study using the synthetic LXR agonist demonstrated that expression of CETP in the mouse modulates the biological response to LXR activation. The T0901317 LXR agonist produced a marked rise in hepatic CETP mRNA and plasma CETP activity levels. The LXR agonist-mediated, 2-fold rise in plasma HDL cholesterol levels in treated wild-type mice was not observed in mice expressing human CETP. The CETP-mediated accumulation of cholesterol in the liver of CETPTg mice was fully reversed by administration of the LXR agonist, which produced a significantly greater rise in biliary cholesterol secretion in CETPTg mice, as compared with wild-type mice.

MATERIALS AND METHODS

Animals

Wild-type mice (4–6 month-old) and age-matched C57Bl6 mice expressing the human CETP under the control of its natural flanking regions (CETPTg) (20) were used in the present study. Both male and female mice were used for the experiments. The mice had free access to water and food, and they were placed on a standard chow diet. All experimental procedures were in accordance with the local guidelines for animal experimentation. The synthetic LXR agonist T0901317 was solubilized in DMSO (100 mg/ml). The stock solution was further diluted 1:100 (v/v) with water containing 1% carboxy-methyl-cellulose. Animals received 10 mg/kg/day of the agonist by gavage for 5 days. Control groups were treated with the vehicle solution only.

Plasma, bile, and liver tissue sampling

At day 6, mice were anesthetized by intraperitoneal injection of pentobarbital. Blood samples were collected by intracardiac puncture in heparin-containing tubes that were centrifuged at 5,000 rpm for 10 min and stored at −80°C. Bile was collected by puncture of the gallbladder and stored at −80°C. Livers were excised, weighed, and divided into three parts that were immediately snap frozen in liquid nitrogen and stored at −80°C before mRNA isolation and biochemical analysis.

Plasma lipid analysis

All assays were performed on a Victor^2 1420 Multilabel Counter (Perkin Elmer Life Sciences, Boston, MA). Total cholesterol was measured by the enzymatic method, using Cholesterol 100 reagent (ABX Diagnostics, Montpellier, France), and unesterified cholesterol concentration was determined by the CHOD-PAP method (Sigma, St. Louis, MO). Esterified cholesterol concentration was calculated as the difference between total and free cholesterol. Phospholipids and triglycerides were determined by enzymatic methods, as described previously (26). HDL and non-HDL cholesterol fractions were determined by fast-protein liquid chromatography (FPLC) analysis.

Hepatic lipid analysis

Hepatic lipids were extracted according to the method of Bligh and Dyer (27), and cholesterol and phospholipid levels were determined by enzymatic methods, as described above. Triglycerides were determined by the method of Danno et al. (28).

Bile lipid analysis

Biliary acids in the bile were determined by capillary gas-liquid chromatography as described previously (29), except for the detection step, which was performed by mass spectrometry determination (MSD). *N*-butylester trimethylsilane (TMS) ether derivatives of biliary acids and TMS ether derivatives of sterols were detected by selected ion monitoring at $m/z = 253$ for cholic acid, 255 for desoxycholic acid and chenodesoxycholic acid, and 257 for lithocholic acid. A 6890 Gas Chromatograph coupled with a 7673 MSD (Agilent Technologies, Palo Alto, CA) was used. The column was a 30 m × 0.25 mm HP-5MS (Agilent Technologies), with helium as the carrier gas. The conditions were as follows: injector temperature 250°C, oven temperature programmed after injection at a rate of 10°C/min from 150 to 260°C then at a rate of 2°C/min to 280°C. The MSD operating conditions for electron impact ionization mass spectrometry were: source temperature, 230°C, and ionizing voltage, 70 eV. Biliary cholesterol was quantitated by an enzymatic method using bilirubin oxidase to remove interference by bilirubin, as previously described (30).

Fractionation of plasma lipoproteins

Individual plasma samples (200 μl) were injected on a Superose 6 HR 10/30 column (Amersham-Pharmacia Biosciences, Freiburg, Germany) connected to an FPLC (Amersham-Pharmacia Biosciences). Lipoproteins were eluted at a constant 0.3 ml/min flow rate with Tris-buffered saline (Tris 10 mmol/l, NaCl 150 mmol/l, pH 7.4) containing 0.074% EDTA and 0.02% sodium

azide. Total cholesterol concentrations were assayed in individual 0.3 ml fractions (26). VLDLs and LDLs were contained in fractions 1–25; HDLs were contained in fractions 26–45.

CETP activity

CE transfer activity was measured with a commercially available fluorescence assay using synthetic liposomes enriched with nitrobenz-oxadiazol (NBD)-labeled CEs as donors and VLDLs as acceptors (Roar Biomedical, New York, NY). The fluorescent CE is present in a self-quenched state in the core of the donor. The CETP-mediated transfer is determined by the increase in fluorescence intensity as the fluorescent CE is removed from the self-quenched donor and transferred to the acceptor particle. Briefly, plasma samples (5 μl), fluorescent-CE-labeled liposomes (4 μl), and unlabeled VLDL acceptors (4 μl) were incubated at 37°C in a final volume of 200 μl Tris-buffered saline in 96-well microplates. Changes in fluorescence were monitored for a 3 h period using a Victor$^{2\ TM}$ fluorescent counter (Perkin Elmer Life Sciences) with 465 nm excitation and 535 nm emission wavelengths.

RNA isolation and PCR methods

Total RNA was isolated with Trizol (Eurogentec). The following specific primer pairs were used: mouse SREBP1c sense: 5′-GTTACTCGAGCCTGCCTTCAGG-3′, mouse SREBP1c antisense: 5′-CAAGCTTTGGACCTGGGTGTG-3′; mouse LDLR sense: 5′-CAGGCAGCAGGAACGAGTTC-3′, mouse LDLR antisense: 5′-GGAGTCAGGAATGCATCGGC-3′; mouse angiopoietin-like protein 3 (Angptl3) sense: 5′-AGGGCTTTGGAGGAGCAGCTAACC-3′; mouse Angptl3 antisense: 5′-GCAGTCGGCAGGAAGGTCATCTTG-3′; 28S sense: 5′-AAACTCTGGTGGAGGTCCGT-3′, 28S antisense: 5′-CTTACCAAAAGTGGCCCACTA-3′; CETP sense: 5′-CAGATCAGCCACTTGTCCAT-3′, CETP antisense: 5′-CAGCTGTGTGTTGATCTGGA-3′. Other primers [fatty acid synthase (FAS), ABCG5, ABCA1, SREBP2] have been described previously (6, 31). CETP, FAS, ABCG5, SREBP2, and 18S RNA levels were quantified by reverse transcription followed by real-time PCR using an ABI PRISM 7700 Sequence Detection System instrument (Applied Biosystems, Courtaboeuf, France). Reactions were carried out with 200 nM of both sense and antisense primers using the Quantitect SYBR Green amplification kit (Quiagen S.A., Courtaboeuf, France) following the instructions provided by the manufacturer. CETP, FAS, ABCG5, and SREBP2 mRNA levels were normalized to the 18S internal control and expressed as fold induction over the untreated wild-type group. SREBP1c, LDLR, Angptl3, and 28S RNA levels were quantified by reverse transcription followed by real-time PCR using a MX4000 apparatus (Stratagene, La Jolla, CA). Reactions were carried out with 100 nM of each primer using the Brilliant SybR Green QPCR Master Mix as recommended by the manufacturer. SREBP1c, LDLR, and Angptl3 mRNA levels were normalized to the 28S internal control and expressed as fold induction over the untreated control group.

Statistical analyses

Mann-Whitney U test was used to determine the significance between the data means.

RESULTS

LXR agonist treatment induces hepatic CETP expression and plasma CETP activity in CETPTg mice

To assess the effect of LXR agonist treatment on CETP expression in CETPTg mice, both hepatic CETP mRNA and plasma CETP activity levels were compared in animals that were treated or not with T0901317. CETPTg mice receiving only the solvent vehicle displayed moderate CE transfer activity that was in the same range as that of a pool of normolipidemic human plasmas (~17 pmol NBD-CE transferred per μl plasma per h in both cases). As shown by real-time quantitative PCR, the 5 day treatment with LXR agonist induced an 8-fold increase in the level of hepatic CETP mRNA (**Fig. 1**) that was associated with a significant but less-pronounced 3-fold rise in plasma CETP activity, as compared with CETPTg mice receiving the vehicle only (**Fig. 2**). As expected, neither CETP mRNA nor CETP activity was detected in wild-type mice, whether they were treated or not with the agonist (data not shown).

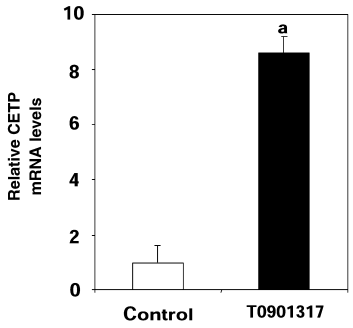

Fig. 1. Hepatic cholesteryl ester transfer protein (CETP) mRNA levels in CETPTg mice with or without liver X receptor (LXR) agonist treatment. Over 5 days, CETPTg mice received either the T0901317 LXR agonist (10 mg/kg/day) or the solvent vehicle only (n = 6 per group). CETP mRNA levels were determined by real-time quantitative PCR as described in Materials and Methods. Data were standardized for 18S RNA, and mRNA level in CETPTg mice receiving the solvent was set at 1.00. Values are means ± SEM. a indicates the significant difference from control CETPTg mice ($P < 0.001$; Mann-Whitney test).

LXR agonist treatment does not increase plasma HDL levels in CETPTg mice

CETPTg mice receiving only the solvent vehicle displayed moderate but significant differences in plasma

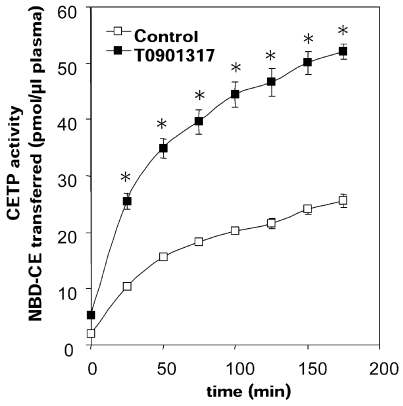

Fig. 2. Plasma cholesteryl ester (CE) transfer activity in CETPTg mice receiving or not the LXR agonist. Over 5 days, CETPTg mice received either the T0901317 LXR agonist (10 mg/kg/day) or solvent vehicle only (n = 6 per group). Plasma CE transfer activity was determined in each group as described in Materials and Methods. Values are means ± SEM. Asterisks indicate significance of the difference from untreated CETPTg mice ($P < 0.001$; Mann-Whitney test).

TABLE 1. Effect of T0901317 LXR agonist treatment on plasma lipid levels in C57Bl6 wild-type and CETPTg mice

	C57Bl6 Control (n = 8)	CETPTg Control (n = 8)	C57Bl6 T0901317 (n = 6)	CETPTg T0901317 (n = 6)
		mmol/l		
Total cholesterol	1.79 ± 0.25	2.22 ± 0.50[a]	3.37 ± 0.69[a]	2.08 ± 0.46[b]
HDL cholesterol	1.49 ± 0.32	1.62 ± 0.28	3.00 ± 0.62[a]	1.46 ± 0.33[b]
Non-HDL cholesterol	0.30 ± 0.14	0.61 + 0.39[a]	0.37 ± 0.11	0.62 ± 0.18[b]
Free cholesterol	0.44 ± 0.15	0.51 ± 0.31	0.97 ± 0.39[a]	0.43 ± 0.20[b]
Esterified cholesterol	1.35 ± 0.18	1.71 ± 0.27[a]	2.39 ± 0.54[a]	1.65 ± 0.27[b]
Triglycerides	0.51 ± 0.18	0.49 ± 0.10	0.62 ± 0.18	0.58 ± 0.20
HDL triglycerides	0.05 ± 0.04	0.10 ± 0.02[a]	0.08 ± 0.04	0.15 ± 0.05
Phospholipids	1.45 ± 0.39	1.50 ± 0.39	2.29 ± 1.01[a]	1.57 ± 0.49[b]

LXR, liver X receptor. Mice were treated for 5 days either with the LXR agonist (10 mg/kg/day) or with the solvent vehicle only. Blood samples were collected by cardiac puncture. Values are mean ± SD.

[a] $P < 0.05$ versus C57Bl6-control.

[b] $P < 0.05$ versus C57Bl6-T0901317.

lipid levels, as compared with wild-type mice, with higher non-HDL cholesterol and higher HDL triglyceride concentrations (significant 2-fold increases in both parameters in CETPTg mice vs wild-type mice; $P < 0.05$). As previously reported (5, 12, 15, 16), treatment with the LXR agonist T0901317 induced profound changes in plasma lipid parameters in wild-type mice (**Table 1**). After treatment, wild-type mice displayed significantly elevated plasma levels of phospholipids (+60%; $P < 0.05$), esterified cholesterol (+75%; $P < 0.05$), and free cholesterol (+160%; $P < 0.05$) but no significant variations in plasma triglyceride levels (Table 1). Gel permeation chromatography analysis revealed that plasma lipid changes were largely explained by an increase in the HDL fraction, in particular with the emergence of large-sized HDL1 (**Fig. 3**). In contrast to wild-type mice, CETPTg mice did not undergo significant changes in plasma lipid parameters after T0901317 treatment (Table 1). Only a nonsignificant tendency toward a shift of total cholesterol from the HDL- to the LDL-containing fractions was observed by FPLC analysis (Fig. 3). In agreement with previous studies (12, 32), no HDL1-like particles were detected in CETPTg mice, and the present results show that this phenotype was not modified by treatment with the LXR agonist (Fig. 3).

LXR agonist treatment normalizes hepatic cholesterol levels in CETPTg mice

As previously reported (23), CETPTg mice displayed significantly higher hepatic cholesterol content, as compared with wild-type mice (**Table 2**). As shown in Table 2, LXR agonist treatment produced a significant decrease in both free and esterified cholesterol concentrations in the livers of CETPTg mice. In contrast, LXR treatment did not modify hepatic cholesterol concentrations in wild-type animals, with the exception of a small but statistically significant reduction in esterified cholesterol content (Table 2). After treatment, the cholesterol content of CETPTg mouse livers was normalized, as compared with wild-type mice (Table 2). As expected from previous studies, LXR agonist treatment was associated with an increase in hepatic triglyceride levels, an effect that was identical in treated wild-type and CETPTg mice (Table 2).

LXR agonist treatment is associated with higher biliary cholesterol secretion in CETPTg mice compared with wild-type mice

A significant 2-fold increase in biliary cholesterol concentration was observed in wild-type mice treated with T0901317, as compared with untreated wild-type mice (**Fig. 4**). Under the same conditions, a significant increase

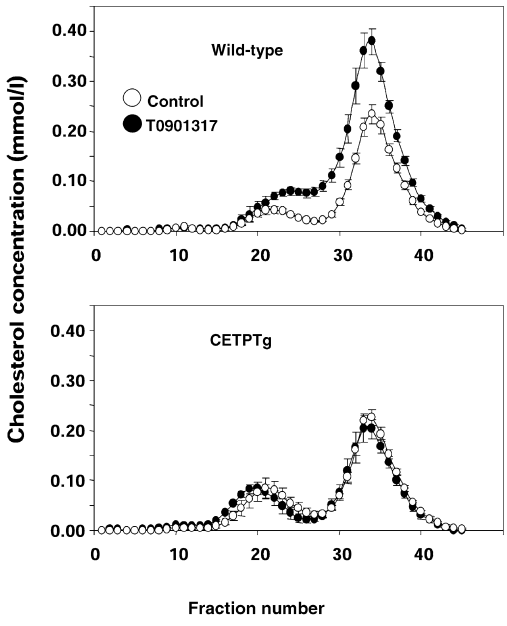

Fig. 3. Fast-protein liquid chromatography analysis of plasma cholesterol of C57Bl6 wild-type and CETPTg mice receiving or not the LXR agonist. Two hundred microliters of individual mouse plasma samples was passed through a Superose 6-HR column, and total cholesterol contents of individual fractions were determined as described in Materials and Methods. Each point is the mean ± SEM of eight distinct plasmas (CETPTg mice and wild-type mice receiving the solvent) or six plasmas (CETPTg mice and wild-type mice receiving the agonist). Closed circles: mice receiving the LXR agonist; open circles: mice receiving the solvent vehicle only.

TABLE 2. Effect of T0901317 LXR agonist treatment on hepatic lipid parameters in C57Bl6 wild-type and CETPTg mice

	C57Bl6 Control (n = 6)	CETPTg Control (n = 5)	C57Bl6 T0901317 (n = 6)	CETPTg T0901317 (n = 6)
		nmol/mg liver		
Total cholesterol	4.94 ± 0.44	6.20 ± 0.47[a]	4.65 ± 0.66	4.95 ± 0.48[b]
Free cholesterol	3.53 ± 0.52	4.40 ± 0.46[a]	3.57 ± 0.61	3.81 ± 0.38[b]
Esterified cholesterol	1.41 ± 0.24	1.80 ± 0.26[a]	1.08 ± 0.23[a]	1.17 ± 0.24[b]
Phospholipids	28.7 ± 4.0	28.2 ± 4.9	26.6 ± 4.0	26.6 ± 5.2
Triglycerides	11.9 ± 3.7	11.5 ± 4.2	30.0 ± 14.4[a]	28.0 ± 14.8[b]

Mice were treated for 5 days either with the LXR agonist (10 mg/kg/day) or with the solvent vehicle only. Lipid parameters were determined as described in Materials and Methods. Values are mean ± SD.

[a] $P < 0.05$ versus C57Bl6-control.

[b] $P < 0.05$ versus CETPTg-control.

in biliary cholesterol concentration was also observed in treated CETPTg mice compared with untreated CETPTg mice (Fig. 4). Although biliary cholesterol levels were similar in untreated wild-type and CETPTg mice, a significant difference between the two groups appeared after treatment, with a significantly greater effect of LXR agonist in the CETPTg mice (Fig. 4). In contrast to biliary cholesterol, bile salt concentration did not undergo any significant changes in the present study (Fig. 4).

Effect of LXR agonist treatment on lipid-related gene expression in the liver of wild-type and CETPTg mice

mRNA levels of known LXR target genes were determined in the livers of wild-type and CETPTg mice (5, 6, 15, 33, 34). As shown in **Fig. 5**, expression of SREBP1c, FAS, ABCG5, and Angptl3 was significantly increased upon LXR agonist treatment in wild-type animals. In contrast, the expression of SREBP2 remained unchanged (Fig. 5). The T0901317-mediated effects were similar in CETPTg mice, with significant rises in the mRNA levels of SREBP1c, FAS, ABCG5, and Angptl3, and with no significant changes in SREBP2 mRNA levels (Fig. 5). Because LXR activation results in profound changes in lipid homeostasis in the liver, we measured the mRNA levels of LDLR, a gene not directly regulated by LXR but highly sensitive to alterations in the cholesterol content of the liver. As shown in **Fig. 6**, treatment with the LXR agonist was associated with a significant, 2-fold increase in hepatic LDLR mRNA expression in wild-type mice ($P < 0.05$). A similar upregulation of LDLR mRNA expression was maintained in treated CETPTg mice despite an 8-fold rise in CETP gene expression (Fig. 1) reaching high plasma CETP levels that were previously associated with a potent negative regulation of liver LDLR expression in high-expressor CETPTg mouse lines (23).

DISCUSSION

Recent studies demonstrated that pharmacological activation of the nuclear receptor LXR in vivo dramatically influences the control of cholesterol homeostasis (1–3). In particular, administration of the highly specific and potent synthetic LXR agonist T0901317 in mice induces an increase in plasma HDL cholesterol concentration, a decrease in hepatic cholesterol content, and an increase in cholesterol secretion in the bile and the feces (5, 12, 15, 16). These changes are mediated by direct LXR activation of key genes that control cholesterol metabolism, among them ABCA1, ABCG1, ABCG5, ABCG8, and PLTP (12–15). Most of these studies have been conducted in the mouse, a mammal species with no detectable plasma CE transfer activity. The latter point may be of particular importance, inasmuch as CETP per se can influence cholesterol homeostasis by transferring CE between HDL- and apoB-containing lipoproteins that are catabolized by distinct metabolic pathways. Moreover, the human CETP gene has been characterized as an LXR target gene (24, 25), and therefore must be considered when attempting to determine the effect of LXR agonists on cholesterol homeostasis in human, a mammal species with substantial

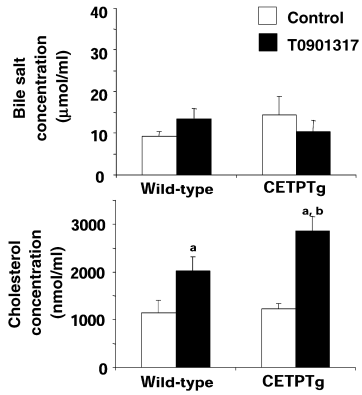

Fig. 4. Cholesterol and bile salt concentrations in the bile of C57Bl6 wild-type and CETPTg mice with or without LXR agonist treatment. Gallbladder bile was collected from C57Bl6 wild-type and CETPTg mice receiving the T0901317 treatment or solvent vehicle only (n = 6 per group). The concentrations of cholesterol and bile acids were subsequently measured as described in Materials and Methods. Values are means ± SEM. a indicates the significance of the difference from homologous mice receiving the solvent vehicle only; b indicates the significance of the difference from C57Bl6 wild-type mice treated with the T0901317 LXR agonist ($P < 0.05$; Mann-Whitney test).

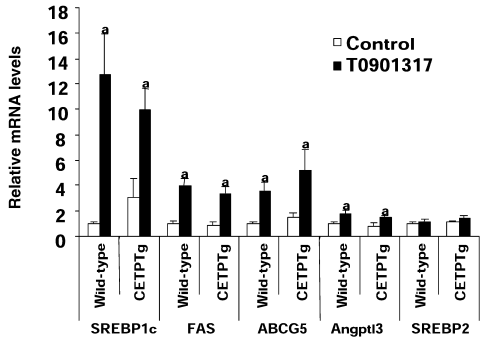

Fig. 5. Relative changes in hepatic mRNA levels in C57Bl6 wild-type and CETPTg mice upon LXR activation. Total RNA was extracted from the liver, and real-time quantitative PCR was performed as described in Materials and Methods. Data were standardized for 18S [fatty acid synthase, ATP binding cassette G5 (ABCG5), sterol-responsive element binding protein 2 (SREBP2)] or 28S [SREBP1c, angiopoietin-like protein 3 (Angptl3)] RNA, and mRNA level in C57Bl6 wild-type mice receiving the solvent vehicle was set at 1.00. a indicates the significance of the difference from homologous control groups ($P < 0.05$; Mann-Whitney test).

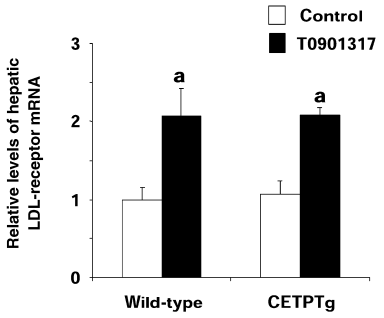

Fig. 6. Relative changes in LDL receptor (LDLR) gene expression in the liver from CETPTg and C57Bl6 wild-type mice upon LXR activation. Over 5 days, C57Bl6 and CETPTg mice received either the T0901317 LXR agonist (10 mg/kg/day) or the solvent vehicle only (n = 5 per group). Hepatic LDLR mRNA levels were subsequently determined by real-time quantitative PCR as described in Materials and Methods. Data were standardized for 28S RNA, and mRNA level in C57Bl6 mice receiving the solvent was set at 1.00. Values are means ± SEM. a indicates the significance of the difference from homologous control groups ($P < 0.05$; Mann-Whitney test).

plasma CE transfer activity. The present study, using a synthetic LXR agonist, demonstrated that expression of CETP in the mouse modulates the biological response to LXR activation.

In the present study, LXR agonist treatment produced a significant, 8-fold rise in hepatic CETP mRNA levels, with only a significant, 3-fold increase in plasma CETP activity (Figs. 1, 2). Interestingly, in the same mouse line, another CETP-raising treatment, i.e., feeding a high-cholesterol diet, was also proven to produce an effect on plasma CETP activity lower than that on hepatic levels of CETP mRNA (20). In both cases, differences in the magnitude of changes in hepatic CETP mRNA and plasma CETP activity levels might relate, at least in part, to a smaller induction of CETP expression in extrahepatic tissues, which contribute significantly to plasma CETP levels. In agreement with previous studies (5, 12, 16), LXR activation in wild-type mice induced pronounced rises in the size and cholesterol content of HDLs that were previously related to specific induction of ABCA1 and PLTP (12–16). In contrast to wild-type mice, treatment of CETPTg mice with T0901317 produced no significant alterations in the plasma lipid concentrations, indicating in this case that the known ABCA1- and PLTP-mediated rises in plasma HDL cholesterol are compensated for by another mechanism involving CETP. Indeed, CETP is recognized as a key factor in promoting in vivo the net transfer of CEs from HDL- to apoB-containing lipoproteins (19, 22, 23). In the present study, the appearance of large-sized HDL1 upon LXR activation in wild-type mice was completely abolished in CETPTg mice, again supporting the peculiar sensitivity of this HDL subpopulation to CETP-mediated remodeling (12, 32, 35). However, and in contrast to previous observations in other CETPTg mouse lines expressing high levels of plasma CETP (23), the marked induction of CETP activity by an LXR agonist in the present study was not accompanied by a significant accumulation of cholesterol in the apoB-containing lipoproteins, VLDL and LDL. In other words, and beyond CETP overexpression, the present observations suggest that coordinated regulation of other LXR target genes, such as those allowing metabolism and elimination of hepatic cholesterol, may have compensated for the CETP-mediated rise in apoB-containing lipoproteins, in particular VLDL (23).

As previously observed (23), and as compared with wild-type mice, CETPTg mice displayed higher hepatic content of cholesterol in both free and esterified forms. Interestingly, LXR agonist treatment normalized the hepatic cholesterol levels in CETPTg mice, which reached those of untreated wild-type mice. In direct explanation of these observations, biliary cholesterol secretion was significantly increased by LXR agonist treatment in both wild-type and CETPTg mice, but to a significantly greater extent in the latter group. This is attributable, at least in part, to the induction of the ABCG5/G8 transporters, which were recently shown to be required for the LXR-mediated oversecretion of cholesterol in the bile (15). The uptake and metabolism of apoB-containing lipoproteins in the liver are tightly dependent on the expression of hepatic lipoprotein receptors, in particular LDLR. Although the LDLR gene has not yet been reported as an LXR target, it is susceptible to being indirectly regulated by LXR agonists through the modulation of the hepatic cholesterol content. In both CETPTg mice and wild-type mice, LXR activation induced ~2-fold increases in the hepatic LDLR mRNA levels, an effect that might relate to the decreased hepatic esterified cholesterol concentration and the subsequent rise in SREBP2 maturation (36, 37). Given that the CE content of smooth endoplasmic reticulum in hepatocytes constitutes a major determinant of SREBP2 maturation (38), the significant and selective decrease in CE concentration in the liver of wild-type mice upon LXR ac-

tivation might account for the upregulation of LDLRs in the present study. Alternatively, it cannot be excluded that the induction of SREBP1c by LXR also contributes to LDLR induction (36, 37). It is worth noting that the 2-fold rise in LDLR expression occurred despite an 8-fold increase in hepatic CETP mRNA levels and a 3-fold increase in plasma CETP activity. In a different context, comparing several untreated CETPTg mouse lines, Jiang and colleagues (23) reported that a gradual CETP overexpression was accompanied by a concomitant and progressive downregulation of hepatic LDLRs, accounting for the accumulation of plasma apoB-containing lipoproteins, in particular, VLDL.

Taken together, the results of the present study support an important function of CETP in RCT, an effect that may well have been masked, at least in part, by the concomitant downregulation of LDLR expression in previous studies of CETPTg mice (39–41). These observations indicate that the metabolic context is a major determinant of the real impact of CETP on cholesterol homeostasis. In fact, apoB-containing lipoproteins have been proposed to constitute an alternative pathway for RCT, in addition to the HDL pathway. However, the selective, CETP-mediated enhancement of the apoB-containing pathway at the expense of the HDL pathway has been proven to exert a counter-regulatory effect on hepatic LDLR expression, leading secondarily to the accumulation of apoB-containing lipoproteins in the plasma of the CETPTg mouse lines expressing the highest levels of CETP (23). In the particular context of CETP upregulation in response to LXR activation, it can be hypothesized that the coordinated induction of hepatic ABCG5/G8 transporters could prevent the CETP-mediated cholesterol accumulation in the liver, maintaining elevated expression of hepatic LDLRs. Given that most of the cholesterol in apoB-containing lipoproteins is cleared from the circulation through the hepatic LDLR pathway, the previous mechanism might account for efficient clearance of apoB-containing lipoproteins (42).

Finally, the impact of LXR-mediated induction of CETP in term of atherogenicity remains an open question, and it will deserve further attention in future studies. In humans, CETP is now generally considered to be a proatherogenic factor, particularly under dyslipidemic conditions associated with a defective clearance of apoB-containing lipoproteins in the liver. Moreover, the CETP-inhibitory strategy appears to be a promising approach to reducing the atherogenic apoB-containing lipoprotein pathway in favor of the antiatherogenic HDL pathway (18, 43, 44). However, in the particular context of pharmacological LXR activation, it is possible that the potentially deleterious increase in CETP activity, precluding the rise in plasma HDL cholesterol, is counterbalanced by the concomitant, beneficial increase in the hepatic clearance of potentially atherogenic lipoproteins. Because CETP, as an LXR target, can influence both plasma HDL levels and biliary excretion of cholesterol, CETP-expressing animals may constitute relevant models for future studies of the effect of LXR agonists on the development of atherosclerosis.

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