Modulation of HDL metabolism : studies in APOE*3-Leiden.CETP mice

Haan, W. de

Chapter 6

PXR AGONISM DECREASES PLASMA HDL LEVELS IN APOE*3-LEIDEN.CETP MICE

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Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids
In Press

Abstract

Pregnane X receptor (PXR) agonism has been shown to affect multiple steps in both the synthesis and catabolism of HDL, but its integrated effect on HDL metabolism in vivo remains unclear. The aim of this study was to evaluate the net effect of PXR agonism on HDL metabolism in APOE*3-Leiden (E3L) and E3L.CETP mice, well-established models for human-like lipoprotein metabolism. Female mice were fed a diet with increasing amounts of the potent PXR agonist 5-pregnen-3β-ol-20-one-16α-carbonitrile (PCN). In E3L and E3L.CETP mice, PCN increased liver lipids as well as plasma cholesterol and triglycerides. However, whereas PCN increased cholesterol contained in large HDL-1 particles in E3L mice, it dose-dependently decreased HDL-cholesterol in E3L.CETP mice, indicating that CETP expression dominates the effect of PCN on HDL metabolism. Analysis of the hepatic expression of genes involved in HDL metabolism showed that PCN decreased expression of genes involved in HDL synthesis (Abca1, Apoa1), maturation (Lcat, Pltp) and clearance (Sr-b1). The HDL-increasing effect of PCN, observed in E3L mice, is likely caused by a marked decrease in hepatic SR-BI protein expression, and completely reversed by CETP expression. We conclude that chronic PXR agonism dose-dependently reduces plasma HDL-cholesterol in the presence of CETP.

Introduction

Since low HDL-cholesterol is a strong and independent risk factor for cardiovascular disease,[1] pharmacological approaches aimed at raising HDL are generally seen as a novel therapeutic strategy to reduce atherosclerosis. However, the recent large phase III trials assessing the effect of the CETP inhibitor torcetrapib in combination with atorvastatin failed, despite achieving a 60% increase in HDL-cholesterol.[2-4] Torcetrapib not only failed to reduce atherosclerosis, as assessed by coronary intima-media thickness (IMT) and intravascular ultrasonography (IVUS) measurements,[2-4] but also increased the risk of cardiovascular events and death rate.[5] Although these data question the therapeutic significance of raising HDL, the adverse effects of torcetrapib may well be compound-specific and related to increased inflammation.[6] Therefore, the search for additional strategies aimed at raising HDL, e.g. via increasing the expression of apoAI, is warranted.

The pregnane X receptor (PXR) may be a novel suitable target to raise HDL. PXR agonism has been shown to increase plasma apoAI and HDL-cholesterol in wild-type mice, but not in PXR-knockout mice, suggesting that PXR agonism may be a new strategy to increase HDL by enhancing apoAI expression.[7] In addition, PXR expression in mice antagonizes the cholic acid-mediated downregulation of plasma HDL-cholesterol and apoAI.[8] PXR activation may also increase HDL formation by the intestine by increasing ABCA1 and ABCG1 expression and protein levels in intestinal cells, which results in an increased cholesterol efflux from intestinal cells to apoAI and HDL in vitro.[9] PXR activation also decreased SR-BI expression in HepG2 cells and primary rat hepatocytes in vitro,[10] which may add to a potential HDL-increasing effect in vivo.

However, some data indicate that PXR agonism may also negatively affect HDL levels. For example, PXR activation decreases the expression of ABCA1 in hepatocytes in vitro,[10] which would reduce HDL formation in an in vivo setting. PXR also increases lipogenesis in the liver leading to an increased hepatic triglyceride (TG) content and increased plasma VLDL-TG levels,[11,12] which may result in reduced HDL-cholesterol levels via CETP-mediated exchange of neutral lipids. An increased hepatic lipid content may increase CETP expression, and increased VLDL-TG will result in a higher rate of cholesteryl ester transfer from HDL to VLDL with a higher reciprocal rate of TG transfer from VLDL to HDL, resulting in a relatively TG-rich HDL that is more rapidly remodeled and cleared via hepatic lipase.[13]

In this study we aimed to examine the integrated effect of PXR agonism by the established PXR agonist PCN[14,15] on HDL metabolism in vivo. Hereto, we used the APOE*3-Leiden (E3L) mouse, a well-established model for human-like lipoprotein metabolism.[16] In addition, we used the E3L.CETP mouse[6,17-19] to

assess the specific contribution of CETP in the PXR-mediated effects on HDL metabolism.

Materials and Methods

Animals and diets
Female APOE*3-Leiden (E3L) and E3L.CETP transgenic mice that express human CETP under control of its natural flanking regions[17] were housed under standard conditions with access to water and food ad libitum. Mice were fed a diet enriched with 15% cacao butter (Diet T; AB Diet Services, Woerden, The Netherlands) for 3 weeks to increase plasma cholesterol levels from 2 mM to ~6 mM. Blood was collected after a 4 h fast from the tail vein into EDTA-containing cups, and both E3L and E3L.CETP mice were randomized according to their plasma total cholesterol, TG and HDL-cholesterol. Subsequently, mice were fed control diet (diet T) or the same diet with 5-pregnen-3β-ol-20-one-16α-carbonitrile (PCN; Sigma) at increasing doses of 0.01%, 0.03% and 0.1% (corresponding with 11, 33 and 110 mg/kg/day) for three weeks each. After each treatment period, blood was drawn after 4 h of fasting into EDTA-containing cups via tail bleeding. After the last treatment period with the highest dosage, mice were sacrificed and livers were isolated. All experiments were approved by the Institutional Committee on Animal Care and Experimentation.

Plasma lipids and lipoprotein profiles
Plasma total cholesterol and triglycerides (TG) were measured using commercially available enzymatic kits (236691 and 1488872, respectively, Roche Molecular Biochemicals, Indianapolis IN, USA) according to the manufacturer's instructions. Phospholipids were determined using an enzymatic Phospholipids kit (Spinreact, Sant Esteve de Bas, Spain). To determine the lipid distribution over plasma lipoproteins, lipoproteins were separated using FPLC. Plasma was pooled per group, and 50 µL of each pool was injected onto a Superose 6 HR 10/30 column (Äkta System, Amersham Pharmacia Biotech, Piscataway, NJ, USA) and eluted at a constant flow rate of 50 µL/min in PBS, 1 mM EDTA, pH 7.4. Fractions of 50 µL were collected and assayed for cholesterol as described above. In E3L.CETP mice, plasma HDL-cholesterol was measured after precipitation of the apoB-containing lipoproteins from 20 µL EDTA plasma by adding 10 µL heparin (LEO Pharma, The Netherlands; 500 U/mL) and 10 µL 0.2 M $MnCl_2$. Mixtures were incubated during 20 min at room temperature and centrifuged for 15 min at 13,000 rpm at 4°C. In the supernatant HDL-C was measured.

Hepatic lipid levels
Liver samples (~50 mg) were vigorously shaken (20 sec at 4800 rpm) in ice-cold methanol (10 µL/mg tissue) using a Mini Bead Beater (BioSpec Products,

Bartlesville, USA). Tissue homogenates (45 µL~4.5 mg tissue) were diluted with ice-cold methanol (450 µL) and ice-cold chloroform (1350 µL), and further shaken (20 sec at 4800 rpm) to extract lipids from the tissue samples. Mixtures were centrifuged (15 min at 14,000 rpm; 4°C) and supernatant was transferred into a new tube, dried under nitrogen gas. Lipids were dissolved in 100 µL 2% Triton-X100. Total cholesterol, TG and phospholipid levels were assayed as described above.

Plasma CETP activity

Total (lipoprotein-independent) CETP activity was measured as the transfer of [^{3}H]cholesteryl oleate (CO) from LDL to HD,[20] exactly as described.[6] Endogenous (lipoprotein-dependent) CETP activity was determined by a fluorescent method using donor liposomes enriched with nitrobenzoxadiazole-labeled cholesteryl esters (RB-CETP, Roar Biomedical, New York),[6] as described.[21]

Table 1. Primers used for rtPCR

Gene	Forward primer	Reverse primer
Abca1	CCCAGAGCAAAAAGCGACTC	GGTCATCATCACTTTGGTCCTTG
Apoa1	GGAGCTGCAAGGGAGACTGT	TGCGCAGAGAGTCTACGTGTGT
CETP	CAGATCAGCCACTTGTCCAT	CAGCTGTGTGTTGATCTGGA
Cyp3A11	CTTTCCTTCACCCTGCATTCC	CTCATCCTGCAGTTTTTTCTGGAT
Cyp7a1	CAGGGAGATGCTCTGTGTTCA	AGGCATACATCCCTTCCGTGA
Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
Hl	CAGCCTGGGAGCGCAC	CAATCTTGTTCTTCCCGTCCA
Hprt	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAG
Lcat	GGCAAGACCGAATCTGTTGAG	ACCAGATTCTGCACCAGTGTGT
Cyclo	CAAATGCTGGACCAAACACAA	GCCATCCAGCCATTCAGTCT
Pltp	TCAGTCTGCGCTGGAGTCTCT	AAGGCATCACTCCGATTTGC
Sr-b1	GTTGGTCACCATGGGCCA	CGTAGCCCCACAGGATCTCA

Abca1, ATP-binding cassette transporter A1; Apoa1, apolipoprotein AI; CETP, human cholesteryl ester transfer protein; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Hl, hepatic lipase; Hprt, hypoxanthine-guanine phosphoribosyl transferase; Lcat, lecithin: cholesterol acyltransferase; Cyclo, cyclophilin; Pltp, phospholipid transfer protein; Sr-b1, scavenger receptor class B type I.

Hepatic mRNA expression

Total mRNA extraction from liver tissue samples was performed using TRIzol (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. mRNA quality was confirmed with lab-on-a-chip (Bio-Rad Laboratories, Hercules, CA, USA), and mRNA was converted to single-stranded cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Ontario, Canada). RT-PCR was performed using the IQ5 multicolor real-time PCR detection system using the SYBR Green RT-PCR mix (Bio-Rad Laboratories, Hercules,

CA, USA). mRNA levels were normalized to mRNA levels of hypoxanthine-guanine phosphoribosyl transferase (HPRT), cyclophilin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers are listed in Table 1.

HDL apolipoprotein composition
Plasma was pooled per group and lipoproteins were separated using FPLC. HDL fractions (7.5 μL) were run on a 4-20% SDS-PAGE gel (Bio-Rad Laboratories, Hercules CA, USA). Gels were stained with Coomassie brilliant blue.

Hepatic ABCA1 and SR-BI protein
Immunoblot analysis of hepatic ABCA1 and SR-BI was performed as described.[22] In short, liver samples were lysed, cell debris was removed, and protein concentration was determined. Equal amounts of protein (20 μg) were separated on 7.5% SDS-PAGE gels and transferred to nitrocellulose membrane. Loading of equal amounts of cell protein was confirmed with Ponceau S staining of the resulting blots. Immunolabeling was performed using murine monoclonal αABCA1 (AC-10) or rabbit polyclonal αSRBI (anti-BI[495]) as primary antibody and goat-anti-mouse IgG and goat-anti-rabbit IgG, respectively, as secondary antibodies. Immunolabeling was detected by enhanced chemiluminescence.

Statistical analysis
Data are presented as means ± SD. Statistical differences were assessed using the Student T Test (hepatic mRNA expression) or the Mann Whitney U test (all other analyses). SPSS 14.0 was used for statistical analysis and $p<0.05$ was regarded as statistically significant.

Results

PXR agonism affects plasma lipid levels
E3L and E3L.CETP mice were fed a control diet or a diet with increasing doses of the PXR agonist PCN (0, 0.01, 0.03 and 0.1%), and plasma TG and cholesterol were determined (Fig. 1). PCN dose-dependently increased plasma TG in both E3L mice (up to + 218%; $p<0.01$) (Fig. 1A) and E3L.CETP mice (up to + 185%; $p<0.05$) (Fig. 1B), indicating that the effect of PCN on plasma TG is independent of CETP expression. However, whereas PCN significantly increased plasma cholesterol in E3L mice (up to +19%; $p<0.01$) (Fig. 1C), PCN only tended to increase plasma cholesterol in E3L.CETP mice (Fig. 1D).

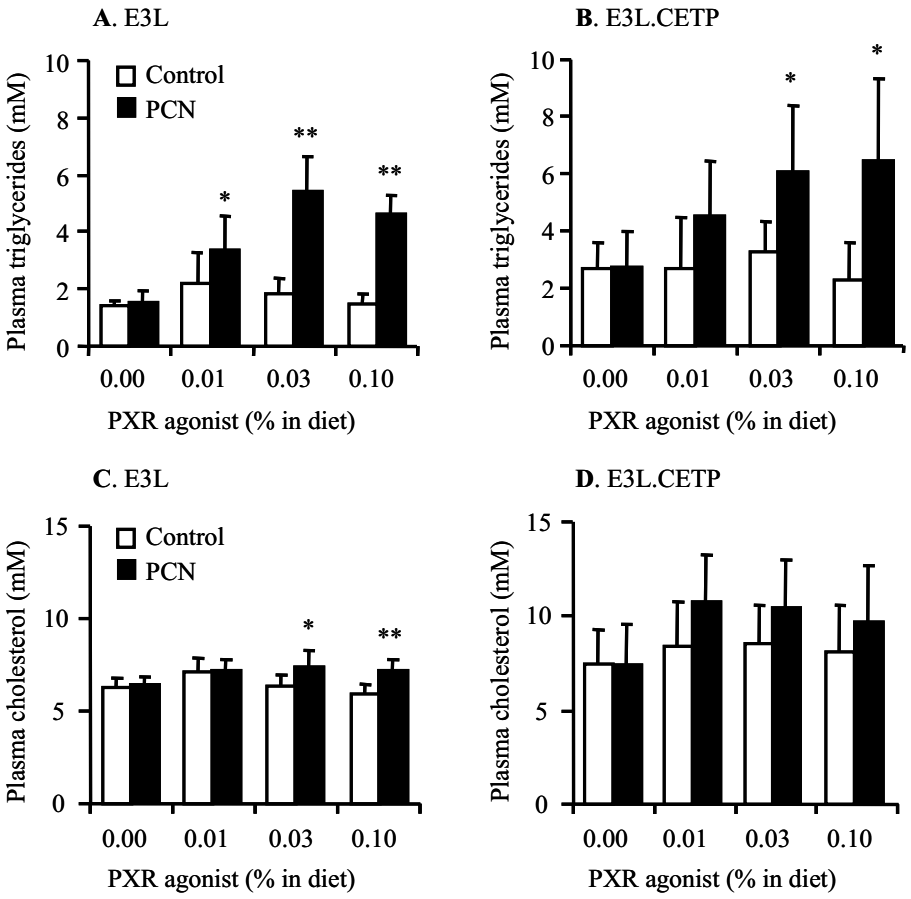

Figure 1. PXR agonism dose-dependently increases plasma cholesterol and triglycerides. APOE*3-Leiden (E3L) mice (A, C) and E3L.CETP mice (B, D) were fed a control diet (time-matched control group) or a diet with increasing doses of 5-pregnen-3β-ol-20-one-16α-carbonitrile (PCN) (0, 0.01, 0.03, and 0.10%) for three weeks each. Before treatment and at the end of the 3 week periods, blood was drawn from both PCN-treated and time-matched control mice and plasma was assayed for triglycerides (A, B) and cholesterol (C, D). Values are means ± SD (n= 6-7 per group); * $p<0.05$, ** $p<0.01$ versus control group.

PXR agonism increases hepatic lipid levels

Since the effects of the PXR agonist on plasma lipids may be caused by an altered hepatic lipid homeostasis, the effect of PCN on hepatic lipid composition was determined (Fig. 2). In E3L mice, PCN increased the levels of TG (+342%; $p<0.01$), total cholesterol (+159%; $p<0.01$) and phospholipids (+100%; $p<0.01$) (Fig. 2A). Similar effects of PCN were observed in E3L.CETP mice (Fig. 2B), indicating that the effect of PCN on hepatic lipid levels is also independent from CETP expression.

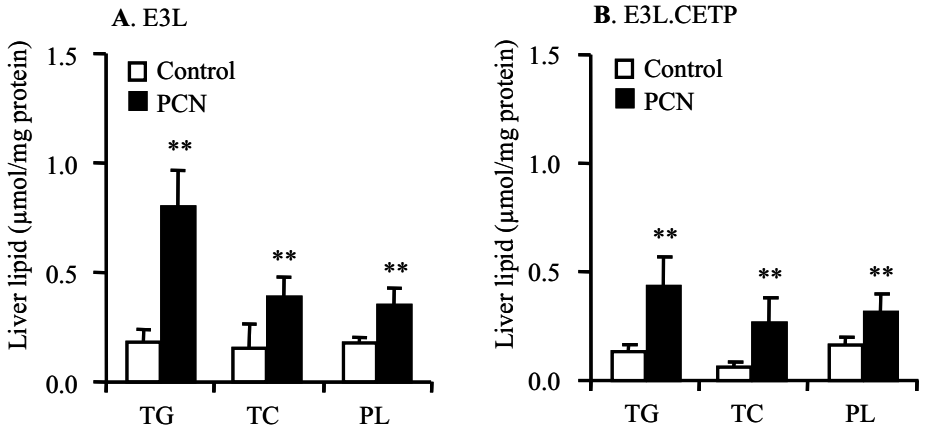

Figure 2. PXR agonism increases hepatic lipid levels. E3L mice (A) and E3L.CETP mice (B) were fed a control diet (time-matched control group) or a diet with increasing doses of PCN for three weeks each. After the last treatment period (0.10% PCN and time-matched control), mice were sacrificed and livers were isolated. Liver were homogenized, lipids were extracted, and triglycerides (TG), total cholesterol (TC) and phospholipids (PL) were quantified. Values are means ± SD (n= 6-7 per group); ** $p<0.01$ versus control group.

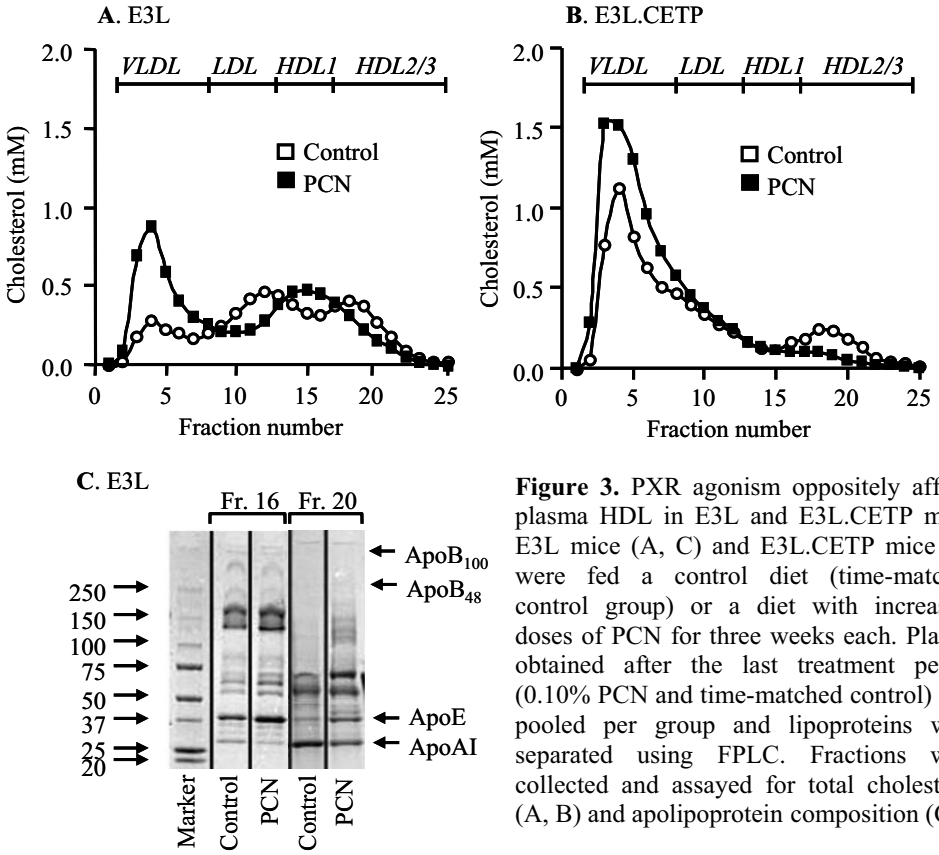

Figure 3. PXR agonism oppositely affects plasma HDL in E3L and E3L.CETP mice. E3L mice (A, C) and E3L.CETP mice (B) were fed a control diet (time-matched control group) or a diet with increasing doses of PCN for three weeks each. Plasma obtained after the last treatment period (0.10% PCN and time-matched control) was pooled per group and lipoproteins were separated using FPLC. Fractions were collected and assayed for total cholesterol (A, B) and apolipoprotein composition (C).

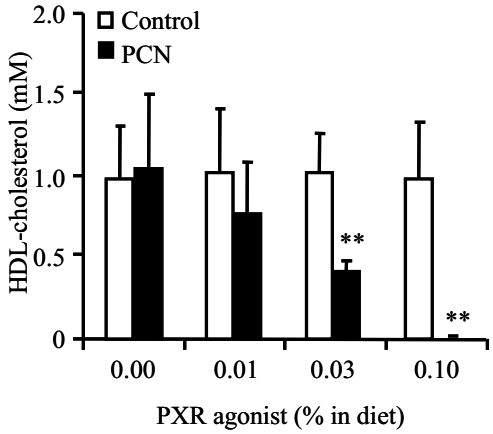

Figure 4. PXR agonism dose-dependently decreases plasma HDL in E3L.CETP mice. E3L.CETP mice were fed a control diet (time-matched control group) or a diet with increasing doses of PCN for 3 weeks each. After the last treatment period (0.10% PCN and time-matched control), blood was drawn and plasma was assayed for HDL-cholesterol after precipitation of apoB-containing lipoproteins. Values are means ± SD (n= 6-7 per group); ** p<0.01 versus control group.

PXR agonism decreases plasma HDL levels in presence of CETP

We next investigated the effect of PCN on the cholesterol distribution over lipoproteins after separation by FPLC (Fig. 3). In both E3L and E3L.CETP mice, PCN increased the amount of cholesterol in VLDL. HDL-cholesterol contained in large HDL particles increased in E3L mice (Fig. 3A). On the other hand, HDL-cholesterol of all sizes was markedly decreased in E3L.CETP mice upon PCN treatment (Fig. 3B). Analysis of the apolipoprotein composition of FPLC fractions 16 and 20 of plasma from E3L mice, showed that PCN induces the appearance of large apoE-rich HDL-1 as apparent from a high ratio of apoE to apoAI (fraction 16), and that PCN treatment reduced the amount of apoAI in HDL of regular size (fraction 20) (Fig. 3C). Analysis of HDL-cholesterol in plasma after precipitation of apoB-containing lipoproteins showed that the HDL-decreasing effect of PCN in E3L.CETP mice was dose-dependent (Fig. 4).

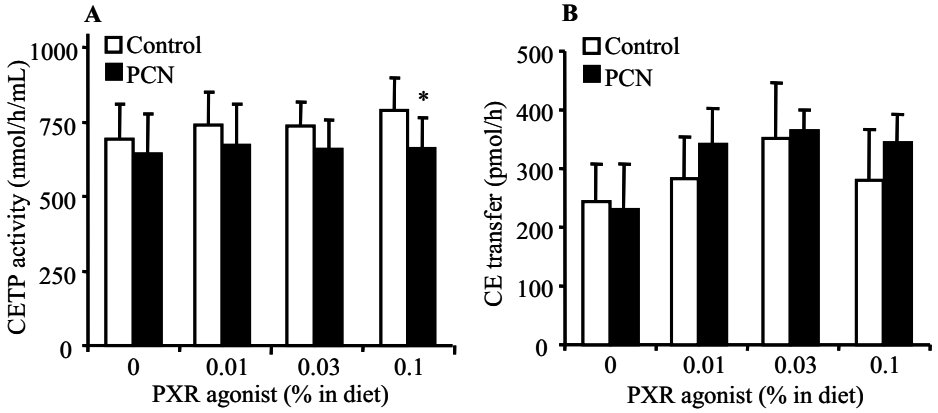

Figure 5. PXR agonism does not affect plasma CETP activity in E3L.CETP mice. E3L.CETP mice were fed a control diet (time-matched control group) or a diet with increasing doses of PCN for three weeks each. After the last treatment period (0.10% PCN and time-matched control), blood was drawn and plasma was assayed for total CETP activity (A) and endogenous CETP activity (B). Values are means ± SD (n= 6-7 per group); * p<0.05, ** p<0.01 versus control group.

PXR agonism does not affect plasma CETP activity

We have previously observed that a decrease in the hepatic cholesterol content of E3L.CETP mice e.g. by treatment with fenofibrate [18] or atorvastatin [19] decreases both the hepatic expression of CETP and the activity of CETP in plasma. Since PXR agonism strongly increases hepatic cholesterol content, we questioned whether the reduction in plasma HDL in E3L.CETP mice may be related to increased plasma CETP activity. Therefore, the effect of PCN was determined on hepatic CETP expression as well as on total and endogenous CETP activity in plasma of E3L.CETP mice (Fig. 5). Albeit that a small effect was observed on total plasma CETP activity at the highest dose, PCN in general did not affect either the total CETP activity (Fig. 5A) or endogenous (Fig. 5B) CETP activity, which is in line with an unaltered hepatic gene expression (Table 1). The reduction of HDL in E3L.CETP mice can thus not be explained by increased CETP activity.

Table 2. PXR agonism affects hepatic gene expression.

A. PXR targets

	E3L control	PCN	E3L.CETP control	PCN
Cyp3a11	1.00 ± 0.35	12.74 ± 5.05***	1.00 ± 0.38	13.37 ± 2.76***
Cyp7a1	1.00 ± 0.53	0.32 ± 0.15**	1.00 ± 0.41	0.59 ± 0.34

B. HDL metabolism

	E3L Control	PCN	E3L.CETP control	PCN
Abca1	1.00 ± 0.24	0.76 ± 0.23	1.00 ± 0.16	0.81 ± 0.31
Apoa1	1.00 ± 0.33	0.60 ± 0.20*	1.00 ± 0.38	0.50 ± 0.10*
Hl	1.00 ± 0.17	0.67 ± 0.16**	1.00 ± 0.28	0.73 ± 0.17
Lcat	1.00 ± 0.48	0.80 ± 0.18	1.00 ± 0.23	0.68 ± 0.13*
Pltp	1.00 ± 0.22	0.45 ± 0.12***	1.00 ± 0.29	0.54 ± 0.18**
Sr-b1	1.00 ± 0.16	0.45 ± 0.09***	1.00 ± 0.28	0.61 ± 0.16*
CETP	n.d.	n.d.	1.00 ± 0.53	1.77 ± 0.80

E3L and E3L.CETP mice were fed a control diet or a diet with increasing doses of PCN. After the last treatment period (0.10% PCN or time-matched control), mice were sacrificed and livers were isolated. mRNA was isolated and mRNA expression of the indicated genes was quantified by RT-PCR. Genes are grouped as established PXR targets (A) and genes involved in HDL metabolism (B). Data are calculated as fold difference as compared to the control group. Values are means ± SD (n= 6-7 per group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus control group. N.d., not detected.

PXR agonism affects hepatic expression of genes involved in HDL metabolism

To get further insight into the mechanism(s) underlying the effects of PCN on HDL metabolism, we evaluated the hepatic expression of genes involved in HDL metabolism (Table 2).

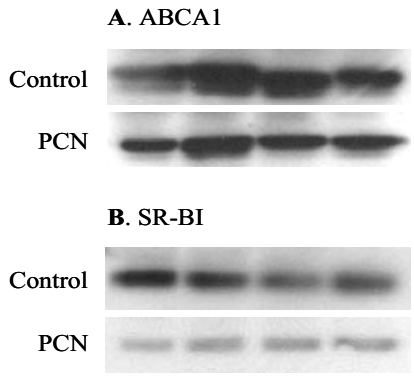

Figure 6. PXR agonism reduces hepatic SR-BI protein. E3L.CETP mice were fed a control diet (time-matched control group) or a diet with increasing doses of PCN for three weeks each. After the last treatment period (0.10% PCN and time-matched control), mice were sacrificed and livers were isolated. Livers were homogenized and equal amounts of hepatic proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane. ABCA1 (A) and SR-BI (B) were visualized by immunolabeling. Results of 4 individual mice per group are shown.

As a control for PXR agonism, we determined the effects of PCN on the expression of Cyp3a11, which is an important target gene of PXR,[23,24] and Cyp7a1, which is negatively regulated by PXR.[25] PCN strongly upregulated Cyp3a11 (13-14-fold) and down-regulated Cyp7a1 (-60 -70%) in E3L mice and E3L.CETP mice, which confirms that PCN is a potent PXR agonist in these mouse models.
PCN had similar effects on the hepatic expression of the various genes involved in HDL metabolism in E3L and E3L.CETP mice. PCN decreased proteins involved in HDL assembly including Abca1 (~20%, n.s.) and Apoa1 (~40-50%; $p<0.05$). In addition, PCN decreased the expression of genes involved in HDL maturation such as Lcat (~20-30%), Hl (~30%) and Pltp (~50%), as well as the gene involved in hepatic clearance of HDL-cholesterol, Sr-b1 (~40-50%).

PXR agonism decreases hepatic SR-BI protein levels
Since a decrease in SR-BI can explain the increase in HDL-cholesterol contained in large HDL-1 particles in E3L mice, we determined whether the relatively large effect of PCN on the hepatic expression of Sr-b1 was reflected by reduced hepatic protein levels. Western blot analysis of hepatic homogenates of E3L.CETP mice indicated that PCN did not substantially reduce hepatic ABCA1 protein (-21%, n.s.), which is in line with Abca1 expression analysis, but substantially reduced SR-BI protein (-77%, $p<0.05$) (Fig. 6). Similar results were obtained for E3L mice (data not shown).

Discussion

Studies on the effect of PXR activation on HDL metabolism have generated conflicting data with respect to their net effect on HDL levels. However, these data have been derived either from in vitro studies of from in vivo studies in wild-type mice that naturally have very low (V)LDL and high HDL levels, and do not express CETP. Therefore, we have evaluated the effect of PXR agonism

on HDL metabolism in E3L mice, which have a more favorable ratio of (V)LDL to HDL and is a well-established model for human-like lipoprotein metabolism, as well as E3L.CETP mice.

We have demonstrated that E3L and E3L.CETP mice respond well to PXR agonism. PCN not only considerably increased hepatic Cyp3a11 expression and reduced Cyp7a1 expression, but also induced fatty livers as judged from an increased liver weight, increased levels of hepatic TG, total cholesterol and phospholipid, as well as formation of lipid droplets (not shown). Hepatic steatosis appears to be a common effect of PXR agonism, since 1) expression of activated PXR in the livers of transgenic mice increases hepatic TG levels,[12] 2) PXR agonism in mice expressing the human PXR gene increases hepatic TG levels,[12] and 3) PCN increases hepatic TG levels in wild-type mice, but not in PXR knockout mice.[11]

We showed that PCN markedly increased plasma TG accompanied by a modest increase in plasma cholesterol, as reflected by increased (V)LDL levels. This is most probably a consequence of the increased hepatic lipid levels, which may result in an increased substrate-driven hepatic VLDL production.

The effect of PXR agonism on plasma HDL levels appeared more complex. In E3L mice, PCN increased cholesterol contained in large HDL-1. Accumulation of apoE-rich large HDL-1 is a common characteristic of SR-BI deficient mice, since SR-BI appears solely responsible for the selective clearance of HDL-cholesteryl esters in mice.[26] Indeed, we observed that PCN largely decreased the hepatic expression of Sr-b1 as well as hepatic SR-BI protein in both E3L and E3L.CETP mice. This strongly suggests that the decrease in hepatic SR-BI may be a causal factor for the increase in large HDL. Based on these data we speculate that a decrease in hepatic SR-BI may also contribute to the increase in HDL-cholesterol and apoAI in wild-type mice as previously observed by Bachman et al.[7] In contrast, PXR agonism by PCN failed to increase cholesterol within large HDL-1 in E3L.CETP mice. This can be explained by the fact that large HDL-1 is a preferred substrate for CETP, since CETP expression in SR-BI-deficient mice normalized both the particle size and plasma levels of HDL.[27]

PXR agonism not only failed to increase the HDL particle size in E3L.CETP mice as compared to E3L mice, but even dose-dependently decreased the HDL-cholesterol level. We have previously shown that fenofibrate[18] and atorvastatin[19] increase HDL-cholesterol levels by decreasing hepatic CETP expression related to lower liver lipid levels, suggesting that the PXR-induced increased liver lipid levels may conversely reduce HDL levels by increased CETP expression. However, PCN did not affect hepatic CETP expression or total CETP activity in plasma in E3L.CETP mice. One could argue that the PCN-induced increase in VLDL-TG levels may result in a substrate-driven increase in CETP activity, resulting in a relatively TG-rich HDL that would be more rapidly remodeled and cleared via hepatic lipase.[13] However, such a mechanism is less plausible

since we were also unable to detect an increase in the endogenous (lipoprotein-dependent) CETP activity.
Albeit that CETP expression per se seems to be the main contributor to the PXR-induced decrease in HDL-cholesterol in E3L.CETP mice, other players involved in HDL metabolism may contribute as well. Previous studies showed that PXR agonism decreased ABCA1 expression in hepatocytes,[10] but we only observed a tendency towards reduced hepatic Abca1 mRNA (~20%) and protein (~20%). However, PCN markedly reduced hepatic Apoa1 mRNA (~40-50%). Given the fact that both apoAI and ABCA1 are important for the generation of discoidal HDL precursors (i.e. apoAI) and their subsequent lipidation (i.e. ABCA1), genetic deficiency for either apoAI[28] or ABCA1[29] dramatically decreases HDL levels. Therefore, a potential modest reduction of ABCA1 accompanied by the large reduction in apoAI may well have synergistically contributed to the dose-dependent marked decrease in HDL in our in vivo study in mice, but only in the mice that express CETP. In addition, PCN decreased the expression of genes involved in HDL maturation such as Hl (~30%), Lcat (~20-30%) and Pltp (~50%). Although HL-deficiency mildly increases HDL,[30] LCAT-deficiency[31] and PLTP-deficiency[32] both reduce plasma HDL levels. Therefore, the effects of PXR agonism on the hepatic expression of Lcat and Pltp, but not Hl, may also have contributed to some extent to the observed reduction in HDL.
Together, our data show that PXR agonism increases cholesterol contained in large HDL-1 particles in E3L mice, as related to decreased hepatic SR-BI levels, and decreases HDL-cholesterol in E3L.CETP mice primarily resulting from CETP expression per se. Since the E3L.CETP mouse has proven a valuable model to predict drug-induced responses in humans with respect to HDL metabolism[6,18,19] we anticipate that PXR agonism is not a valid strategy to raise HDL.

Acknowledgements

This work was performed in the framework of the Leiden Center for Cardiovascular Research LUMC-TNO, and supported by the Netherlands Heart Foundation (NHS grant 2003B136 to P.C.N.R.), the Netherlands Organization for Scientific Research (NWO grant 908-02-097 and NWO VIDI grant 917.36.351 to P.C.N.R.) and the Centre for Medical Systems Biology (CMSB) in the framework of the Netherlands Genomic Initiative (NGI) (project 115). J.W.J. is an established clinical investigator of the Netherlands Heart Foundation (2001D032).

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