Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Expression of the Pregnane X Receptor in Mice Antagonizes the Cholic Acid–Mediated Changes in Plasma Lipoprotein Profile

David Masson, Laurent Lagrost, Anne Athias, Philippe Gambert, Cynthia Brimer-Cline, Lubin Lan, John D. Schuetz, Erin G. Schuetz and Mahfoud Assem

Arterioscler. Thromb. Vasc. Biol. 2005;25;2164-2169; originally published online Aug 25, 2005;
DOI: 10.1161/01.ATV.0000183674.88817.fb
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
Copyright © 2005 American Heart Association. All rights reserved. Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/cgi/content/full/25/10/2164

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at
http://atvb.ahajournals.org/subscriptions/

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
http://www.lww.com/reprints

Downloaded from atvb.ahajournals.org by on October 31, 2007

Expression of the Pregnane X Receptor in Mice Antagonizes the Cholic Acid–Mediated Changes in Plasma Lipoprotein Profile

David Masson, Laurent Lagrost, Anne Athias, Philippe Gambert, Cynthia Brimer-Cline, Lubin Lan, John D. Schuetz, Erin G. Schuetz, Mahfoud Assem

Objective —Modification of lipoprotein metabolism by bile acids has been mainly explained by activation of the farnesyl X receptor (FXR). The aim of the present study was to determine the relative contribution of the pregnane X receptor (PXR), another bile acid–activated nuclear receptor to changes in plasma lipoprotein profile.

Methods and Results —Wild-type mice, Pxr-deficient mice, and Pxr-null mice expressing human PXR (Pxr-null SXR-Tg mice) were fed a cholic acid–containing diet, and consequences on plasma lipoprotein profiles and target gene expression were assessed. Cholic acid produced significant decreases in high-density lipoprotein (HDL) cholesterol, plasma apolipoprotein (apo)A-I and hepatic apoA-I mRNA in wild-type mice. Interestingly, the effect of cholic acid was significantly more pronounced in Pxr-deficient mice, indicating that PXR contributes to the weakening of the effect of bile acids on lipoprotein metabolism. Reciprocally, changes in HDL/apoA-I profiles were abolished in Pxr-null SXR-Tg mice in which PXR-responsive genes, particularly those involved in bile acid detoxification were readily activated after cholic acid treatment.

Conclusion —PXR expression in mice antagonizes the cholic acid–mediated downregulation of plasma HDL cholesterol and apoA-I, and magnification of PXR/SXR-mediated changes may constitute a new mean to counteract the effects of bile acids on plasma lipoproteins. (***Arterioscler Thromb Vasc Biol*. 2005;25:2164-2169.**)

Key Words: apolipoprotein A-I ■ bile acids ■ farnesyl X receptor ■ high-density lipoproteins ■ pregnane X receptor ■ steroid and xenobiotic receptor

High-density lipoproteins (HDL) and their major protein component, apolipoprotein A-I (apoA-I), are protective factors against the development of atherosclerosis.[1–4] These observations raised a considerable interest in the development of new therapeutic approaches that aim at increasing plasma HDL cholesterol and apoA-I levels. ApoA-I gene expression is under the control of multiple factors, including several nuclear receptors. ApoA-I gene expression is negatively regulated by the apolipoprotein regulatory protein-I (ARP-1) (NR2F2) and the farnesyl X receptor (FXR) (NR1H4),[5–7] whereas it is positively regulated by the hepatocyte nuclear factor-4α (HNF-4α) (NR2A1), the peroxisome proliferator receptor alpha (PPARα) (NR1C1), and the liver receptor homolog-1 (LRH-1) (NR5A2).[8–10]

See page 2016

Although several ligands of pregnane X receptor (PXR) (NR1I2) were reported to increase HDL cholesterol as well as apoA-I and apoA-I mRNA levels in rodents and humans,[11–15] the relative contribution of PXR to the regulation of plasma HDL levels in vivo remains to be ascertained. Interestingly, besides its role as a xenobiotic sensor,[16–17] PXR responds to bile acids that also constitute well-recognized FXR ligands.[18–22] Because PXR and FXR have opposite effects on apoA-I expression, it can be hypothesized that the final effect of bile acids on lipoprotein profile may actually reflect antagonistic contributions of PXR and FXR activations. To address the latter point in a comprehensive way, wild-type (WT) mice, PXR-deficient mice (Pxr-null mice),[23] and mice expressing human PXR but not mouse PXR (Pxr-null SXR-Tg mice)[23] were fed a 1% cholic acid (CA)-containing diet in the present study. We report here that PXR expression in mice antagonizes the CA-mediated downregulation of plasma HDL cholesterol and apoA-I. Interestingly, the negative effect of CA on plasma HDL profile could be completely reversed by elevated expression of human PXR in Pxr-null SXR-Tg mice, indicating that a magnification of PXR/SXR-mediated changes may constitute a new mean to counteract the negative effects of bile acids on lipoprotein profile.

Materials and Methods

Animals

WT mice, age-matched mice deficient for murine Pxr (Pxr-null),[23] and age-matched mice expressing human PXR (Pxr-null SXR-Tg)

Original received March 11, 2005; final version accepted August 2, 2005.

From INSERM U498 (D.M., L.L., A.A., P.G.), Faculté de Médecine, Dijon, France; Pharmaceutical Sciences (C.B.-C., L.L., J.D.S., E.G.S., M.A.), St. Jude Children's Research Hospital, Memphis, Tenn.

Correspondence to Dr Mahfoud Assem, Department of Pharmaceutical Sciences, St Jude Children's Research Hospital, 332 N. Lauderdale Ave, Memphis, TN 38105-2794. E-mail Mahfoud.Assem@stjude.org

© 2005 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000183674.88817.fb**

Downloaded from atvb.ahajournals.org by on October 31, 2007

TABLE 1. Effect of 1% CA Diet on Plasma Lipid Parameters

	Wild-Type		Pxr-Null		Pxr-Null SXR-Tg	
	Control	CA 1%	Control	CA 1%	Control	CA 1%
Total cholesterol, mmol/L	2.43±0.38	1.84±0.30*	2.54±0.34	1.16±0.24*†	2.98±0.19	2.65±0.53†
HDL cholesterol, mmol/L	1.77±0.41	1.13±0.27*	1.84±0.25	0.48±0.17*†	2.22±0.25	1.94±0.40†
Non-HDL cholesterol, mmol/L	0.66±0.19	0.71±0.16	0.70±0.48	0.68±0.33	0.77±0.20	0.71±0.14
Free cholesterol, mmol/L	0.62±0.12	0.83±0.47	0.64±0.09	0.29±0.08*†	0.86±0.27	0.63±0.24
Esterified cholesterol, mmol/L	1.81±0.33	1.01±0.13*	1.90±0.37	0.87±0.27*	2.12±0.24	2.02±0.57
Triglycerides, mmol/L	0.97±0.27	0.55±0.15*	1.28±0.15	0.19±0.09*†	0.99±0.26	0.90±0.46
Phospholipids, mmol/L	2.47±0.27	1.77±0.33*	2.54±0.34	1.43±0.20*	2.67±0.44	2.13±0.37*

Values represent the mean±SD.
*$P<0.05$ vs homologous mice under control diet.
†$P<0.05$ vs WT mice under homologous diet (Mann–Whitney test).

under the control of the albumin promoter[23] were used in the present study. The Pxr-null and Pxr-null SXR-Tg mice were generously provided by Dr Ronald Evans. WT, Pxr-null, SXR-Tg mice and Pxr-null mice were all of mixed genetic background (129/C57BL6). Mice were housed in a pathogen-free animal facility under a standard 12-hour light/12-hour dark cycle. All diets were prepared by Bioserv (Frenchtown, NJ) and were based on a standard AIN-93G rodent diet. The CA diet was identical to the control diet but supplemented with 1% (w/w) CA. Male mice were 8 to 12 weeks of age at the start of the study. Five to 6 animals per group were used in the present study. All protocols and procedures were approved by the NCI Division of Basic Sciences Animal Care and Use Committee and are in accordance with the National Institutes of Health guidelines.

Plasma and Liver Tissue Sampling

After 5 days of feeding the indicated diet, animals were anesthetized with isoflurane and blood samples were collected by intra-cardiac puncture in heparin-containing tubes that were centrifuged at 5000 rpm for 10 minutes. Plasmas were harvested and stored at −80°C. Livers were excised, weighed, immediately snap-frozen in liquid nitrogen (LN_2), and stored at −80°C before mRNA isolation and biochemical analysis.

Hepatic Lipid Analysis

Qualitative analysis of hepatic bile acids was performed by capillary gas-liquid chromatography as described previously.[24,25] Quantitative determination of total hepatic bile acids was performed by an enzymatic assay (Colorimetric Total Bile Acid Assay Kit; Bio Quant, San Diego, Calif) on hepatic alcoholic extract obtained as previously described.[26] Total cholesterol and triglycerides were determined as described previously.[25,27]

Plasma Lipid and Apolipoprotein Analysis

All assays were performed on Victor² 1420 Multilabel Counter (Perkin Elmer life Science, Boston, Mass). Cholesterol, phospholipids, and triglycerides were determined by enzymatic methods as described previously.[25] HDL and non-HDL cholesterol plasma fractions were determined as cholesterol concentration in d>1.07 and d<1.07 plasma fractions respectively. Mouse apoA-I concentration was determined by a nonimmunologic method. Briefly, plasma samples (0.25 μL) were submitted to electrophoresis on 5% to 15% polyacrylamide gradient gel (Invitrogen, Carlsbad, Calif). Gel were subsequently stained by Coomassie brilliant blue and scanned on a GS-800 densitometer (Biorad, Hercules, Calif). The amount of apoA-I per sample was determined quantitatively by comparison with apoA-I standards that were performed in parallel with the samples.

Native Polyacrylamide Gradient Gel Electrophoresis

Total lipoproteins were separated by ultra centrifugation as the d<1.21 g/mL plasma fraction with one 5.5 hour, 100 000-rpm spin in a TLA100 rotor in a TLX ultracentrifuge (Beckman, Krefeld, Germany). Lipoproteins were then applied to a 1.5% to 25% polyacrylamide gradient gel (Spiragel 1.5 to 25.0; Spiral, Couternon, France), and electrophoresis was conducted as recommended by the manufacturer. Gels were subsequently Coomassie stained and the distribution profiles of lipoprotein were determined by comparison with protein standards (HMW kit; Amersham Bioscience, Uppsala, Sweden) using a GS 800 densitometer.

RNA Isolation and Polymerase Chain Reaction Methods

Total RNA was extracted using Trizol reagent (Life technologies, Carlsbad, Calif). Specific mRNAs were analyzed by quantitative real-time reverse-transcription polymerase chain reaction using the ABI Prism 7900HT (Applied Biosystems, Foster City, Calif). Briefly, 5 μg of RNA were reverse-transcribed into cDNA using MuMLV retrotranscriptase and oligo dT (Life technologies); 50 ng of the cDNA mixture were used. Specific cDNAs were amplified using specific primers (see supplemental data at http://atvb.ahajournals.org).Values were normalized to Gapdh levels. Relative mRNA levels were evaluated using ΔΔCt method.

Western Blot

The preparation of liver homogenates and Western blot analysis were performed as described elsewhere.[19,28] Anti-Bsep, Mrp4, and cyp 3a11 antibodies were described previously.[28–30] Rabbit anti-Mrp2 was provided by Dr Bruno Stieger, Zurich, Switzerland.

Statistical Analysis

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

Results

Cholic Acid Feeding Reduces Cholesterol and ApoA-I Levels in WT and Pxr-Null Mice, but not in Pxr-Null SXR-Tg Mice

In agreement with previous studies,[31] total cholesterol, esterified cholesterol, phospholipid, and HDL cholesterol were significantly lower in WT mice fed a 1% cholic acid diet than in WT mice fed the standard chow (Table 1). Nondenaturing polyacrylamide gradient gel electrophoresis confirmed that the HDL fraction was mostly and selectively affected by the 1% CA treatment (Figure 1), which was accompanied by a significant reduction in plasma apoA-I concentration (0.56 g/L in WT mice fed 1% CA versus 1.18 g/L in WT mice fed the control diet; $P<0.05$) (Figure 1).

Downloaded from atvb.ahajournals.org by on October 31, 2007

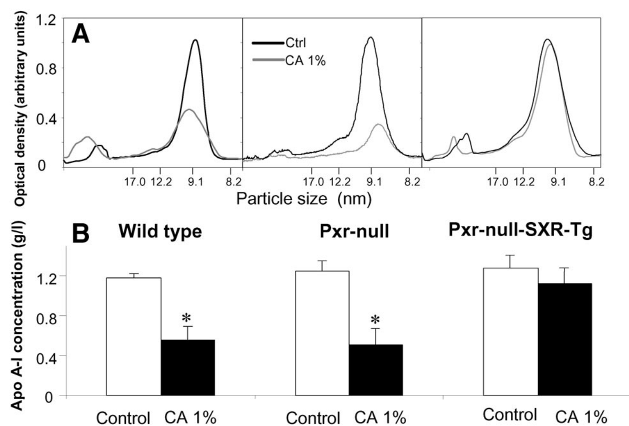

Figure 1. Plasma ApoA-I concentration and lipoprotein profile in WT, Pxr-null, and Pxr-null SXR-Tg mice fed either control or a 1% CA diet. A, Total plasma lipoproteins were submitted to electrophoresis on 1.5% to 25% polyacrylamide gradient gels that were stained for proteins as described in Materials and Methods. B, Plasma apoA-I concentrations were determined as described in Materials and Methods. (*) indicates a significant difference from homologous mice fed the control diet and Pxr-null SXR-Tg mice fed the 1% CA diet ($P<0.05$ in both cases; Mann–Whitney test).

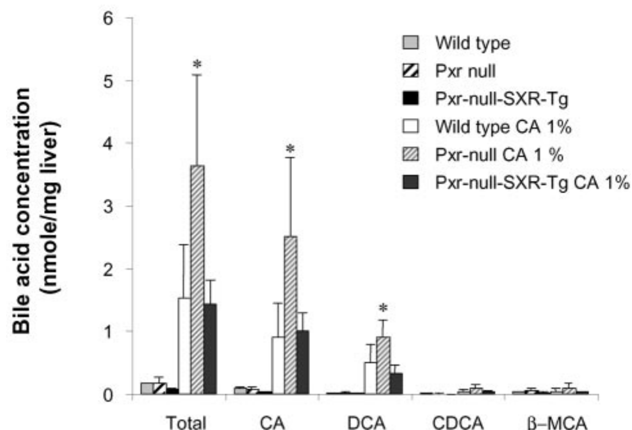

Figure 2. Hepatic bile acid content in WT, Pxr-null and Pxr-null SXR-Tg mice fed a 1% CA diet. Hepatic bile acid content was determined as described in Materials and Methods. *Significant difference from WT and Pxr-null SXR-Tg mice fed the 1% CA diet ($P<0.05$; Mann–Whitney test).

Whereas lipoprotein phenotypes of Pxr-null mice and WT mice were similar under standard diet, CA induced significantly greater reductions in total cholesterol, esterified cholesterol, phospholipids, triglycerides, and HDL cholesterol in Pxr-null mice after treatment (Table 1). The CA-mediated decreases in plasma apoA-I concentration were of similar magnitude in Pxr-null and WT mice (Figure 1). Strikingly, the CA-mediated changes in lipid concentration and HDL distribution no longer appeared in Pxr-null SXR-Tg mice, with the exception of a reduction in plasma phospholipids (Table 1 and Figure 1).

The Accumulation of Bile Acids in the Liver Is Higher in Pxr-Null Mice Than in WT and Pxr-Null SXR-Tg Mice Under CA Diet

In all mouse lines, levels of cholic, deoxycholic, and chenodeoxycholic acids were increased by 5- to 10-fold by the CA diet, whereas the concentrations of 6-OH bile acids (ie, muricholic acids) remained unchanged (Figure 2). Interestingly, the increase in total bile acids, CA, and deoxycholic acid were significantly higher in the liver of Pxr-null mice than in the liver of WT and Pxr-null SXR-Tg mice (Figure 2). As shown in Table 2, hepatic triglyceride levels remain unchanged in all mouse lines whether they received the control chow or the CA-enriched diet. Hepatic triglyceride concentration was significantly higher in Pxr-null-SXR-Tg mice as compared with WT or Pxr-null mice under control and CA diets. Interestingly, the CA diet induced a significant increase in hepatic cholesterol concentration in WT and Pxr-null mice but not in Pxr-null SXR-Tg mice.

CA Feeding Activates FXR Target Genes in WT, Pxr-Null, and Pxr-Null SXR-Tg Mice

Expression of Cyp7a1 and Cyp8b1, ie, 2 genes indirectly regulated by FXR,[32] were dramatically reduced after CA treatment in all cases, indicating an efficient suppression of bile acid synthesis (Figure 3A). The expression of small heterodimeric partner (Shp) and bile salt export pump (Bsep), ie, 2 direct FXR targets,[32,33] was significantly increased after cholic treatment. Bsep mRNA levels were increased by 7-fold in the liver of CA-treated Pxr-null SXR-Tg mice, but only by 2-fold in CA-treated WT and Pxr-null liver ($P<0.05$) (Figure 3A). Induction of Bsep mRNA levels was accompanied by a proportional increase in Bsep protein (data not shown).

CA Feeding Activates PXR Target Genes Only in Pxr-Null SXR-Tg Mice

As shown in Figure 3B, PXR target genes were minimally affected by the 1% CA diet in WT and Pxr-null mice. In contrast, mRNA levels of Cyp3a11, Mrp2, and Mrp4 were markedly increased (4- to 6-fold increases) in Pxr-null SXR-Tg mice receiving the 1% CA diet as compared with control diet (Figure 3B). Activation patterns were confirmed by hepatic protein analysis (data not shown).

CA Feeding Decreases Hepatic ApoA-I mRNA Levels in WT and Pxr-Null Mice, but not in Pxr-Null SXR-Tg Mice

As shown in Figure 4A, no significant difference in hepatic apoA-I mRNA levels was observed between WT, Pxr-null,

TABLE 2. Effect of 1% CA Diet on Hepatic Lipid Concentrations

	Total Cholesterol, mmol/g tissue	Triglycerides, mmol/g tissue
Wild-type	6.7±1.1	6.9±1.5
Pxr-null	7.2±1.2	12.4±6.0
Pxr-null SXR-Tg	6.2±0.56	18.0±6.1†
Wild-type CA 1 %	9.3±3.0*	9.8±4.6
Pxr-null CA 1%	11.2±4.0*	9.8±5.8
Pxr-null SXR-Tg CA 1%	6.6±1.42†	18.4±5.6†

Values represent the mean±SD.
*$P<0.05$ vs homologous line under control diet.
†$P<0.05$ vs wild-type under same diet (Mann–Whitney test).

Downloaded from atvb.ahajournals.org by on October 31, 2007

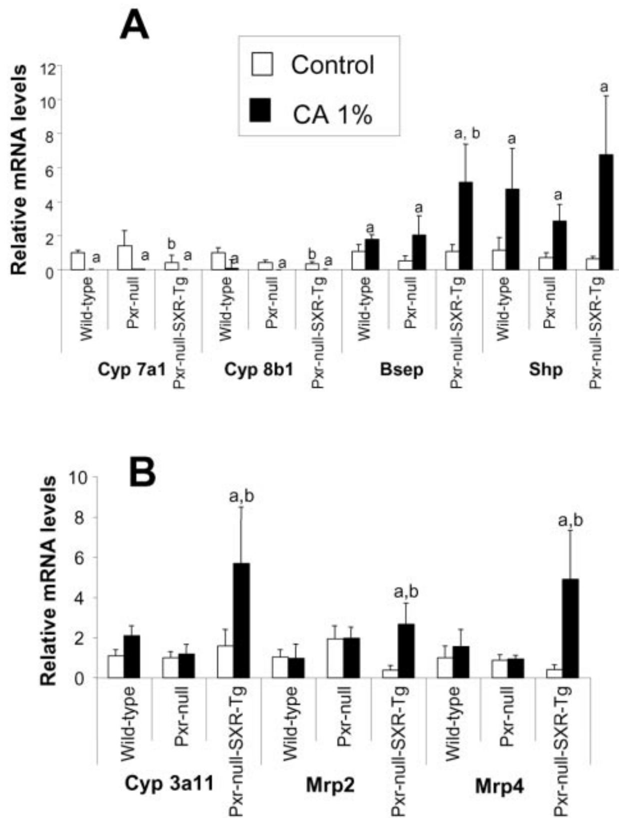

Figure 3. Relative changes in hepatic mRNA levels of FXR target genes (A) and PXR target genes (B) in WT, Pxr-null, and Pxr-null SXR-Tg mice fed a 1% CA diet. Total RNA was extracted from the liver and real time quantitative polymerase chain reaction was performed. Data were standardized for Gapdh mRNA, and mRNA levels in WT mice receiving the standard diet was set at 1.00. (a) indicates a significant difference from homologous mice fed the control diet; (b) indicates a significant difference from WT mice fed the 1% CA diet ($P<0.05$; Mann–Whitney test).

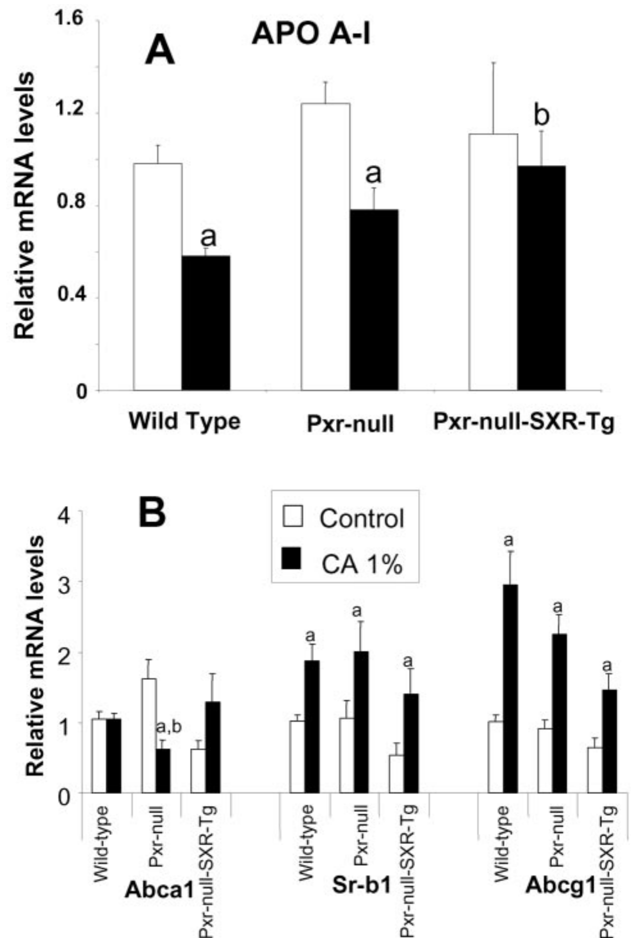

Figure 4. Abundance of hepatic ApoA-I mRNA (A) and Sr-b1, Abcg1, and Abca1 (B) in WT, Pxr-null, and Pxr-null SXR-Tg mice fed either control or 1% CA diet. Total RNA was extracted from the liver and real-time quantitative polymerase chain reaction was performed and analyzed as described in Figure 3 legend. (a) indicates a significant difference from homologous mice fed the control diet; (b) indicates a significant difference from WT mice fed the 1% CA diet ($P<0.05$; Mann–Whitney test).

and Pxr-null SXR-Tg mice when fed the standard diet. In WT mice, and as previously reported,[31] the 1% CA diet led to a significant 40% reduction in hepatic apoA-I mRNA levels as compared with WT mice fed the control diet. Similar reduction in hepatic apoA-I mRNA levels was also observed in Pxr-null mice under the CA diet. In contrast, in Pxr-null SXR-Tg mice, hepatic apoA-I mRNA levels remained constant under the CA-containing diet (Figure 4A), as were plasma apoA-I concentrations (Figure 1).

CA Feeding Reduces Hepatic Abca1 mRNA Levels in Pxr-Null Mice, but not in WT and Pxr-Null SXR-Tg Mice

As shown in Figure 4B, the mRNA levels of Sr-b1 and Abcg1, 2 genes involved in HDL metabolism, were significantly increased by 2- to 3-fold in the 3 mouse lines by the CA diet. In addition, a significant reduction of Abca1 mRNA level was observed in Pxr-null mice receiving the CA diet but not in WT mice and Pxr-null SXR-Tg mice.

Discussion

Bile acids modulate the atherogenicity of the lipoprotein profile.[34] In particular, they act as negative regulators of hepatic apoA-I transcription;[7] this downregulation has been explained either in terms of a direct effect of activated FXR at the apoA-I gene promoter,[7] or in terms of an indirect effect of FXR through the SHP/LRH-I pathway.[9] Activation of FXR by bile acids also reduces plasma triglyceride concentration by decreasing both hepatic lipogenesis and secretion of very-low-density lipoprotein.[35,36] Besides FXR, bile acids are also potent activators of another nuclear receptor, ie, PXR.[18–22] Interestingly, several studies have reported that administration of pharmacological PXR agonists is associated with significant increases in both hepatic apoA-I transcription and circulating levels of apoA-I and HDL cholesterol in rats, mice, and humans.[11–15, 37–39] The purposes of the present study were: (1) to establish the relative contribution of PXR to the effect of bile acids on plasma lipoprotein profile through the comparison of WT and Pxr-null mice; and (2) to determine whether overexpression of human PXR in Pxr-null SXR-Tg mice was able to counteract the deleterious, FXR-mediated effects of bile acids.

In agreement with previous observations,[31] the CA-containing diet significantly reduced hepatic apoA-I expres-

Downloaded from atvb.ahajournals.org by on October 31, 2007

sion and HDL cholesterol levels in WT mice. Although the effects of CA were reported to be exacerbated in PXR-deficient mice, in particular with more pronounced effects on total cholesterol and triglyceride levels as compared with WT mice,[40] consequences of PXR-deficiency in terms of HDL structure and composition had not been specifically addressed. The present studies demonstrate for the first time that CA treatment of PXR-deficient mice produced more profound effects on plasma HDL cholesterol as compared with WT counterparts. Because exacerbation of the effect of CA on HDL cholesterol in PXR-deficient mice was not accompanied by further decrease in apoA-I gene expression in the present study, we thought further for differential expressions of specific genes that are known to affect lipidation (ie, ABCA1 and ABCG1)[41–42] or catabolism (ie, SR-BI)[43] of HDL. While induction of SR-BI by bile acids in WT mice confirmed previous studies,[44] it is reported for the first time that bile acids positively regulate hepatic ABCG1 mRNA levels. Because hepatic overexpression of either Sr-b1 or Abcg1 are known to induce a reduction of HDL cholesterol levels in mice, caused by an increase in hepatic HDL catabolism,[41,45] it is possible that these two proteins contribute in addition to apoA-I to the bile acid-mediated reduction of HDL. More importantly, and in contrast to Sr-b1 and Abcg1 mRNA levels that were induced by the CA treatment in the 3 mouse lines studied, Abca1 mRNA levels were shown in the present study to be significantly reduced by CA in Pxr-null mice only. Recent observations have demonstrated that hepatic ABCA1 is a key factor contributing to lipidation and formation of nascent HDL [42]; therefore, down-regulation of Abca1 expression arises as a possible mechanism for the greater effect of the CA diet on HDL cholesterol levels in Pxr-null mice as compared with WT mice.

In contrast to observations made with WT and PXR-deficient mice, CA-mediated changes in HDL/apoA-I profiles were completely abolished in mice expressing human PXR (Pxr-null SXR-Tg mice). Moreover, typical PXR-responsive genes were readily activated after CA treatment in this latter mouse line only. Different mechanisms that are not mutually exclusive may explain the positive effect of PXR. First, a direct transactivating effect of PXR on the apoA-I promoter is possible, and it is consistent with the positive effect of phenobarbital on apoA-I transcription as already reported.[38–39] Alternatively, it is possible that PXR interferes with some FXR-mediated pathways by inducing qualitative and quantitative changes in hepatic bile acid composition.[46] This hypothesis is supported by significant changes in hepatic bile acid composition in Pxr-null mice treated with CA and is susceptible to explain the exacerbation of the effect of the CA diet on lipoprotein profile in Pxr-null mice. However, no qualitative or quantitative differences in hepatic bile acid profiles were observed between Pxr-null SXR-Tg and WT mice fed the CA-enriched diet. Finally, PXR might interfere directly with FXR by targeting their common coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (Pgc-1α) [47] as proposed recently to explain the inhibition of HNF4α signaling pathway by PXR. However, and in contrast to apoA-I, the expression of specific targets genes that are highly responsive to FXR (ie, SHP or CYP7A1) was not affected by PXR in the present study. Finally, although greater induction of PXR responsive genes in Pxr-null SXR-Tg mice under CA diet might be related in part to the highly active albumin promoter,[23] it is well-known that mouse and human PXR exhibit marked differences with regard to their ligand specificity[48] and lithoCA, the most potent PXR activator among bile acids, is more efficient in activating human PXR than mouse Pxr in vitro.[19]

In conclusion, our results indicate that FXR and PXR exert distinct, but complementary roles in HDL metabolism, as they actually do in the protection against bile acid toxicity.[19,40,49] Additional investigations will be necessary to confirm the pathophysiological relevance of our study. Because of the peculiar sensitivity of SXR to bile acids, the existence of a compensation by PXR of the FXR-mediated changes in lipoprotein metabolism deserves further attention in humans. Overall, magnification of the beneficial effects of PXR in Pxr-null SXRTg mice under CA treatment suggest that beside reduction of bile acid–mediated liver toxicity, PXR agonist may also be useful to counteract the deleterious effects of bile acids on plasma lipoproteins.

Acknowledgments

This work was supported by National Institutes of Health Research grants CA77545, GM60904, ES058571, GM60346, P30 CA21745, the Cancer Center support grant, the American Lebanese Syrian Associated Charities (ALSAC), Conseil Régional de Bourgogne, Fondation de France, Université de Bourgogne, and INSERM U498.

References

1. Miller NE. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. *Am Heart J*. 1987;113:589–597.
2. Gordon DJ, Rifkind BM. High-density lipoprotein-the clinical implications of recent studies. *N Engl J Med*. 1989;321:1311–1316.
3. Castelli WP, Anderson K, Wilson PW, Levy D. Lipids and risk of coronary heart disease. The Framingham Study. *Ann Epidemiol*. 1992;2:23–28.
4. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature*. 1991;353:265–267.
5. Zannis VI, Kan HY, Kritis A, Zanni EE, Kardassis D. Transcriptional regulatory mechanisms of the human apolipoprotein genes in vitro and in vivo. *Curr Opin Lipidol*. 2001;12:181–207.
6. Ladias JAA, Karathanasis SK. Regulation of the apolipoprotein AI gene by ARP-1, a novel member of the steroid receptor superfamily. *Science*. 1991;251:561–565.
7. Claudel T, Sturm E, Duez H, Torra IP, Sirvent A, Kosykh V, Fruchart JC, Dallongeville J, Hum DW, Kuipers F, Staels B. (2002) Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. *J Clin Invest*. 2002;109:961–971.
8. Sladek FM, Zhong WM, Lai E, Darnell JE. Liver-enriched transcription factor HNF4 is a novel member of the steroid hormone receptor superfamilly. *Genes Dev*. 1990;4:2353–2365.
9. Delerive P, Galardi CM, Bisi JE, Nicodeme E, Goodwin B. Identification of liver receptor homolog-1 as a novel regulator of apolipoprotein AI gene transcription. *Mol Endocrinol*. 2004;18:2378–2387.
10. Berthou L, Duverger N, Emmanuel F, Langouet S, Auwerx J, Guillouzo A, Fruchart JC, Rubin E, Denefle P, Staels B, Branellec D. Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J Clin Invest*. 1996;97:2408–2416.
11. Bachmann K, Patel H, Batayneh Z, Slama J, White D, Posey J, Ekins S, Gold D, Sambucetti L. PXR and the regulation of apoA1 and HDL-cholesterol in rodents. *Pharmacol Res*. 2004;50:237–246.
12. Jürgen M, Lehmann DD, McKee MA, Watson TMW, Moore JT, Kliewer SA. The human orphan nuclear receptor PXR is activated by compounds

Downloaded from atvb.ahajournals.org by on October 31, 2007

that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest*. 1998;102:1016–1023.
13. Aynaci FM, Orhan F, Orem A, Yildirmis S, Cedik Y. Effect of Antiepileptic drugs on plasma lipoprotein (a) and other lipid levels in childhood. *J Child Neural*. 2001;16:367–369.
14. Romaschin AD, Goldberg DM. Effect of phenobarbital upon serum cholesterol lipoprotein fractions of three rodent species. *Clin Physiol Biochem*. 1987;5:77–84.
15. Gerhold D, Lu M, Xu J, Austin C, Caskey CT, Rushmore T. Monitoring expression of genes involved in drug metabolism and toxicology using DNA microarrays. *Physiol Genomics*. 2001;5:161–170.
16. Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, Perlmann T, Lehmann JM. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell*. 1998;92:73–82.
17. Xie W, Yeuh MF, Rodominska-Pandya A, Saini SPS, Negishi Y, Bottroff BS, Cabrera GY, Tukey RH, Evans RM. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. *Proc Natl Acad Sci U S A*. 2003;100:4150–4155.
18. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, Kliewer SA. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A*. 2001;98:33369–33374.
19. Schuetz EG, Strom S, Yasuda K, Lecureur V, Assem M, Brimer C, Lamba J, Kim RB, Ramachandran V, Komoroski BJ, Venkataramanan R, Cai H, Sinal CJ, Gonzalez FJ, Schuetz JD. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. *J Biol Chem*. 2001;276: 39411–39418.
20. Xie W, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, Evans RM. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci U S A*. 2001;98:3375–3380.
21. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM. Bile acids: natural ligands for an orphan nuclear receptor. *Science*. 1999; 284:1365–1825.
22. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. Identification of a nuclear receptor for bile acids. *Science*. 1999;284:1362–1365.
23. Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, Evans RM. Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature*. 2000;406:435–439.
24. Batta AK, Salen G, Rapole KR, Batta M, Earnest D, Alberts D. Capillary gas chromatographic analysis of serum bile acids as the n-butyl ester-trimethylsilyl ether derivatives. *J Chromatogr B Biomed Sci Appl*. 1998; 706:337–341.
25. Masson D, Staels B, Gautier T, Desrumaux C, Athias A, Le Guern N, Schneider M, Zak Z, Dumont L, Deckert V, Tall A, Jiang XC, Lagrost L. 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.
26. Wildgrube HJ, Stockhausen H, Metz P, Mauritz G, Mahdawi R. Radioimmunoassay of bile acids in tissue, bile, and urine *Clin Chem.* 1983;29: 494–498.
27. Danno H, Jincho Y, Budiyanto S, Furukawa Y, Kimura S. A simple enzymatic quantitative analysis of triglycerides in tissues. *J Nutr Sci Vitaminol (Tokyo).* 1992;38:517–521.
28. Assem M, Schuetz EG, Leggas M, Sun D, Yasuda K, Reid G, Zelcer N, Adachi M, Strom S, Evans RM, Moore DD, Borst P, Schuetz JD. Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. *J Biol Chem*. 2004; 279:22250–22257.
29. Hostetler KA, Wrighton SA, Kremers P, Guzelian PS. Immunochemical evidence for multiple steroid-inducible hepatic cytochromes P-450 in the rat. *Biochem J*. 1987;245:27–33.
30. Lecureur V, Sun D, Hargrove P, Schuetz EG, Kim R, Lan LB, Schuetz JD. Cloning and Expression of Murine Sister of P-Glycoprotein Reveals a More Discriminating Transporter Than MDR1/P-Glycoprotein. *Mol Pharmacol*. 2000;57:24–35.
31. Srivastava RAK, Srivastava N, Averna M. Dietary cholic acid lowers plasma levels of mouse and human apolipoprotein A-I primarily via a transcriptional mechanism. *Eur J Biochem*. 2000;267:4272–4280.
32. Goodwin B, Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell*. 2000;6:507–515.
33. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem*. 2001;276: 28857–28865.
34. Leiss O, von Bergmann K. Different effects of chenodeoxycholic acid and ursodeoxycholic acid on serum lipoprotein concentrations in patients with radiolucent gallstones. *Scand J Gastroenterol*. 1982;17:587–592.
35. Hirokane H, Nakahara M, Tachibana S, Shimizu M, Sato R. Bile acid reduces the secretion of very low density lipoprotein by repressing microsomal triglyceride transfer protein gene expression mediated by hepatocyte nuclear factor-4. *J Biol Chem*. 2004;279:45685–45692.
36. Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, Moore DD, Auwerx J. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest*. 2004;113:1408–1418.
37. Luoma P, Myllyla V, Hokkanen E. Relationship between plasma high-density lipoprotein cholesterol and anticonvulsant levels in epileptics. *J Cardiovasc Pharmacol*. 1982;4:1024–1027.
38. Chao YS, Pickett CB, Yamin TT, Guo LS, Alberts AW, Kroon PA. Phenobarbital induces rat liver apolipoprotein A-I mRNA. *Mol Pharmacol*. 1985;27:394–398.
39. Tam SP, Deeley RG. Regulation of apolipoprotein A-I gene expression by phenobarbital in the human hepatocarcinoma cell line, Hep3B. *Atherosclerosis*. 1994;105:235–243.
40. Guo GL, Lambert G, Negishi M, Ward JM, Brewer HB, Kliewer SA, Gonzalez FJ, Sinal CJ. Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. *J Biol Chem*. 2003;278:45062–45071.
41. Ji Y, Wang N, Ramakrishnan R, Sehayek E, Huszar D, Breslow JL, Tall AR. Hepatic scavenger receptor BI promotes rapid clearance of high density lipoprotein free cholesterol and its transport into bile. *J Biol Chem*. 1999;274:33398–33402.
42. Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, Gebre AK, Coutinho JM, Colvin PL, Smith TL, Hayden MR, Maeda N, Parks JS Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. *J Clin Invest.* 2005;Apr 7[Epub ahead of print].
43. Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci U S A*. 2004;101:9774–9779.
44. Lambert G, Amar MJ, Guo G, Brewer HB Jr., Gonzalez FJ, Sinal CJ. The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. *J Biol Chem*. 2003;278:2563–2570.
45. Ito T. Physiological function of ABCG1. *Drug News Perspect*. 2003;16: 490–492.
46. Stedman C, Robertson G, Coulter S, Liddle C. Feed-forward regulation of bile acid detoxification by CYP3A4: studies in humanized transgenic mice. *J Biol Chem*. 2004;279:11336–11343.
47. Bhalla S, Ozalp C, Fang S, Xiang L, Kemper JK. Ligand-activated PXR interferes with HNF-4 signaling by targeting a common coactivator PGC-1alpha: functional implications in hepatic cholesterol and glucose metabolism. *J Biol Chem*. 2004;279:45139–45147.
48. Krasowski MD, Yasuda K, Hagey LR, Schuetz EG. Evolution of the pregnane X receptor: adaptation to cross-species differences in biliary bile salts. *Mol Endocrinol*. 2005;19:1720–1739.
49. Zhang J, Huang W, Qatanani M, Evans RM, Moore DD. The constitutive androstane receptor and pregnane X receptor function coordinately to prevent bile acid-induced hepatotoxicity. *J Biol Chem*. 2004;279: 49517–49522.

Downloaded from atvb.ahajournals.org by on October 31, 2007