Bile acids facilitate the absorption of dietary lipids and fat-soluble vitamins and are physiological ligands for the farnesoid X-activated receptor (FXR), a member of the nuclear hormone receptor superfamily. FXR functions as a heterodimer with the retinoid X receptor and in the presence of ligand, the heterodimer binds to specific DNA sequences in the promoters of target genes to regulate gene transcription. Phospholipid transfer protein (PLTP) has been identified as a possible target gene for FXR because the human promoter contains a potential FXR response element, an inverted repeat in which consensus receptor-binding hexamers are separated by one nucleotide (inverted repeat-1). PLTP is essential in the transfer of very low density lipoprotein phospholipids into high density lipoprotein (Jiang, X. C., Bruce, C., Mar, J., Lin, M., Ji, Y., Francione, O. L., and Tall, A. R. (1999) J. Clin. Invest. 103, 907–914). Here we report the regulation of PLTP gene expression by FXR and bile acids. In CV-1 cells, cotransfection of FXR and the retinoid X receptor resulted in bile acid-dependent transactivation of a luciferase reporter construct containing the human PLTP promoter. Mutation analysis demonstrated that the inverted repeat-1 (IR-1) in the PLTP promoter is required for this transactivation. Finally, we demonstrate that bile acids are able to regulate PLTP gene expression in vivo. Mice fed a chow diet supplemented with bile acid showed increased hepatic PLTP mRNA levels. These results suggest that FXR may play a role in high density lipoprotein metabolism via the regulation of PLTP gene expression.

The major metabolic pathway for the elimination of cholesterol is its conversion into bile acids in the liver. Bile acids, in the form of bile, are secreted from the gallbladder into the small intestine, where they act as detergents to emulsify dietary lipids and fat-soluble vitamins. Approximately 95% of bile acids are recycled in the small intestine by enterocytes and returned to the liver via the enterohepatic circulation, whereas 5% escape into the colon and are excreted from the body. Bile acids also play a role in the regulation of gene transcription, as they were recently shown to be physiological ligands for the farnesoid X-activated receptor (FXR) (2–4). Of the bile acids, the most potent activator of FXR is chenodeoxycholic acid (CDCA), which is able to both mediate FXR transcriptional activation and recruit steroid receptor coactivator-1 to the ligand-binding domain of FXR (3).

FXR (NR1H4 (5), originally isolated as RIP14 (retinoid X receptor-interacting protein-14) (6)) is a member of the nuclear hormone receptor superfamily and is primarily expressed in the liver, kidney, and intestine (6, 7). It functions as a heterodimer with the retinoid X receptor (RXR) and binds to response elements in the promoters of target genes to regulate gene transcription. The FXR-RXR heterodimer binds with highest affinity to an inverted repeat-1 (IR-1) response element, in which consensus receptor-binding hexamers are separated by one nucleotide. Intestinal bile acid-binding protein (I-BABP) was the first identified direct target of FXR (2, 8). I-BABP is a cytosolic protein that mediates bile acid uptake in the ileum and through its regulation FXR is able to modulate the enterohepatic circulation of bile acids. We have identified PLTP as a possible target for FXR because its promoter contains one copy of a potential FXR response element, IR-1 (9).

PLTP is a member of the lipid transfer/lipopolysaccharide-binding protein gene family (10). Other members in the family include the cholesteryl ester transfer protein, the lipopolysaccharide-binding protein gene, and the bactericidal permeability-increasing protein (10). PLTP is present in the plasma as a free or HDL-bound protein and is expressed at the highest levels in the placenta, pancreas, adipose tissue, and lung, with intermediate levels in the liver, kidney, and heart (11). Although the function of PLTP is still unclear, several studies, including results with PLTP knockout mice, indicate that PLTP has a role in HDL metabolism (11). The physiological function of PLTP in HDL metabolism is important because of the inverse relationship between atherosclerosis and the level of HDL (12). The PLTP knockout mice confirmed previous in vivo studies that suggested a role for PLTP in the transfer of phospholipids from large-sized triglyceride-rich lipoproteins to HDL. An in vivo lipid transfer activity assay showed that the transfer of plasma phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and sphingomyelin) and free cholesterol from very low density lipoprotein to HDL is absent in PLTP knockout mice (1). In addition, the knockout mice showed a 60–70% reduction in HDL levels, possibly the result of an increase in the catabolism of HDL protein and cholesteryl ester (13).

The liver is at the center of cholesterol homeostasis, as it is the site where cholesterol is biosynthesized from precursor molecules and catabolized into bile acids. Maintaining cholesterol homeostasis includes the regulation of gene expression by both cholesterol and bile acids. The physiological role of PLTP in the maintenance of HDL metabolism makes PLTP a good target for studying the regulation of gene expression by bile acids in vivo.
candidate for cholesterol and/or bile acid regulation. Except for a response element for the peroxisome proliferator-activated receptor in the human promoter (14), not much is known about the regulation of hepatic PLTP gene expression. Since FXR and PLTP are coexpressed in the liver, where bile acids are present at concentrations required to activate FXR, and because the human PLTP promoter has a potential FXR response element, we decided to study the role of FXR and its ligand CDCA in the regulation of PLTP gene expression. Part of the human PLTP gene promoter, including the PLTP IR-1, was linked to the luciferase reporter gene. In monkey kidney CV-1 cells, cotransfection of FXR and RXR resulted in transactivation of the PLTP promoter in the presence of the ligand CDCA, and mutation of the PLTP IR-1 eliminated this response. As expected, the FXR-RXR heterodimer bound specifically to the PLTP IR-1, but not to the mutant form, as shown by a gel mobility shift assay. Finally, the physiological role of FXR and bile acids in the regulation of PLTP gene expression was studied in mice. In the liver, PLTP gene expression was significantly increased when mice were fed a chow diet supplemented with 1% cholic acid.

EXPERIMENTAL PROCEDURES

**Plasmid Constructions**—The pPLTP−2800/+15 (15) plasmid was used as a template for the polymerase chain reaction to generate 955-, 385-, and 335-base pair fragments of the 5′-flanking region of the human PLTP promoter. These fragments were cloned into the pGL3-Basic vector (Promega) upstream of the luciferase gene to generate the PLTP940, PLTP370, and PLTP320 reporter constructs, respectively. The polymerase chain reaction was performed using PLu DNA polymerase (Stratagene), and the constructs were confirmed by DNA sequencing and restriction digests. The PLTP IR-1 of the PLTP940 reporter construct was mutated using the Chameleon™ double-stranded, site-directed mutagenesis kit (Stratagene). Three different mutants were generated using the following oligonucleotides: MUT1, gctgctgagaaactgattatatagtgacccaagtgaagtgacttgcccaagatcatgcagg; MUT2, ggaggaanaagctgcacggcaagtgaagtgacttgc; and MUT3, gtaagtgaaggtcaccagctgcacggcaagtgaagtgac. Mutations were confirmed by DNA sequencing.

**Cotransfection Assays**—CV-1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% charcoal-stripped serum at 37 °C and 5% CO2. Transfections were carried out in Dulbecco’s modified Eagle’s medium supplemented with 10% charcoal-stripped serum using calcium phosphate precipitation. The cells were plated in 24-well multiwell dishes to ~80% confluence and transfected either with 137.5 ng of pCDM8 or with 125 ng of pCDM8-FX or CDCA and/or 1 μM CDCA, and/or 1 μM 9-cis-retinoic acid (9-cis-RA) for 36 h. Cells were then transfected with 50 ng of pTKGH per well using a phosphoror saline calcium phosphate precipitation method (17) full-length cDNA. The following day, the cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% charcoal-stripped serum and vehicle alone (ethanol) or 25, 50, or 100 μM CDCA. After a 36-h incubation, cells were harvested, and luciferase was assayed as described previously (16). Growth hormone was measured using the human growth hormone transient gene expression kit (Nichols Institute).

**Gel Mobility Shift Assays**—Using the TNT-coupled reticulocyte lysate system (Promega), in vitro transcribed/translated RIP14 and human RXRα were generated from pT7-lac-His-Myc expression vectors. The cultured cell lines were transfected with the empty expression vector or with RXRα expression plasmids and the PLTP940 reporter construct. Cells were treated with vehicle alone or with 100 μM CDCA and/or 1 μM 9-cis-retinoic acid (9-cis-RA) for 36 h. The relative luciferase expression was normalized using the basal value for the reporter in the absence of receptors and ligand.

**Animal Treatments**—Mice were maintained on 12-h light/12-h dark cycle and fed ad libitum a standard mouse diet. To study the effect of bile acids on PLTP gene expression, mice (n = 3) were fed ad libitum the standard diet supplemented with 1% cholic acid (test diet). After 50 days, the mice were killed. Their livers were harvested, snap-frozen in liquid nitrogen, and stored at −80 °C until further processing. All procedures in this study were performed in accordance with guidelines set by the Center for Comparative Medicine at Baylor College of Medicine.

**Northern Blotting**—Total RNA was extracted from the liver using the acid guanidinium thiocyanate/phenol/chloroform extraction method as described (18) and mRNA was isolated using Oligotex suspension (QIA-GEN Inc.) according to the manufacturer’s instruction. The mRNA (5 μg) was resolved on a 2.2 M formaldehyde and 1% agarose gel and transferred to a nylon membrane using the Turboblotter Rapid Downward Transfer system (Schleicher & Schuell). A mouse cDNA probe for PLTP (1.7 kilobase pairs) was derived from the mouse PLTP cDNA (19). The membrane was stripped with 60% formamide and 0.1% SDS at 65 °C for 30 min and reprobed with human β-actin cDNA probe (CLON-TECH). Probes were labeled by random priming with [a-32P]dCTP (Amersham Pharmacia Biotech) using the Random Primers DNA labeling system kit (Life Technologies, Inc.). Bands were quantitated using a densitometer and ImageQuant (Molecular Dynamics, Inc.) and were standardized against β-actin controls.

**Statistical Analysis**—Values are expressed as means ± S.E. The statistical significance of the difference of the means was evaluated using the two-sample t test for independent samples with equal variances.

RESULTS

**The PLTP Promoter Is Activated by FXR and Bile Acids**—The FXR-RXRα heterodimer binds to specific DNA sequences in the promoters of target genes to regulate gene transcription. The DNA sequences recognized by the heterodimer include several direct and inverted repeats of the consensus receptor-binding hexamer, including an IR-1 (6). Recently, it was deduced that the FXR-RXRα heterodimer binds with highest affinity to the consensus sequence RGTTCA arranged as an

![Figure 1](http://www.jbc.org/) FXR and bile acids activate the PLTP promoter. A, CV-1 cells were cotransfected either with the empty expression vector (CDM8) or with FXR and FXRα expression plasmids and the PLTP940 reporter construct. Cells were treated with vehicle alone or with 100 μM CDCA and/or 1 μM 9-cis-retinoic acid (9-cis-RA) for 36 h. B, CV-1 cells were cotransfected either with the empty expression vector (CDM8) or with FXR and FXRα expression plasmids and the PLTP940 reporter construct. Cells were treated with vehicle alone or with increasing concentrations of CDCA for 36 h. The relative luciferase expression was normalized using the basal value for the reporter in the absence of receptors and ligand.
IR-1 (9). In addition, a data base search using the IR-1 consensus sequence identified an IR-1 with a perfect match to the consensus sequence in the promoter of the human PLTP gene (9).

To characterize the regulation of the human PLTP promoter by FXR via the IR-1, 955 base pairs of the 5'-flanking region of the human PLTP promoter were cloned upstream of the luciferase gene to generate PLTP940. Monkey kidney CV-1 cells were cotransfected with FXR and RXR expression plasmids plus the PLTP940 reporter construct in the absence of ligand and in the presence of 100 μM CDCA and/or 1 μM 9-cis-retinoic acid, an RXR ligand (Fig. 1A). Cells cotransfected with FXR and RXR showed an ∼10-fold induction of the PLTP940 promoter when treated with 100 μM CDCA relative to untreated cells. As expected from previous results (8), a weaker transactivation was observed with 9-cis-retinoic acid alone, and addition of 9-cis-retinoic acid augmented the CDCA response. The transactivation of the PLTP940 reporter construct by CDCA via FXR showed a dose-dependent effect (Fig. 1B). Although a plateau was not reached due to the toxicity of higher bile acid concentrations, CDCA activated the PLTP940 promoter with an estimated half-maximal effective concentration (EC₅₀) of 37 μM. This agrees well with the EC₅₀ of 35 μM estimated for the activation of the I-BABP promoter by CDCA (8).

To determine if the PLTP IR-1 is necessary for the transactivation of the PLTP940 promoter, two other reporter constructs were generated, PLTP370 and PLTP320 (Fig. 2A). Only the PLTP940 and PLTP370 reporter constructs contain the IR-1, as it is located between nucleotides −339 and −327. Both the PLTP940 and PLTP370 reporter constructs were induced ∼10- and 8-fold, respectively, in the presence of 100 μM CDCA (Fig. 2B), whereas the PLTP320 reporter construct lacking the IR-1 showed only minimal transactivation. The basis for this residual response is unclear, as no other candidate bile acid response elements are present in this region.

To confirm these 5'-deletion results in the context of the PLTP940 reporter construct, mutations were introduced into either one or both of the IR-1 half-sites (PLTP940 MUT1, MUT2, and MUT3) (Fig. 3A). In all three cases, FXR and CDCA had only a modest residual effect on luciferase gene expression (Fig. 3B).

The FXR-RXRα Heterodimer Binds Specifically to PLTP IR-1—As expected, the FXR-RXRα heterodimer is able to bind specifically to the IR-1 in a gel mobility shift assay (9), but neither FXR nor RXR could bind oligonucleotide alone (Fig. 4). Competition analysis showed that an unlabeled IR-1 oligonucleotide was able to compete for binding at a 100- or 1000-fold molar excess, whereas a 1000-fold molar excess of a nonspecific competitor, an Sp1-binding site, had no effect. Furthermore, the three different mutant versions of the IR-1 (PLTP940 MUT1, MUT2, and MUT3) were also unable to compete for binding at a 1-, 10-, 100-, or 1000-fold molar excess. These results demonstrate that the FXR-RXRα heterodimer is able to bind specifically to the IR-1 located between nucleotides −339 and −327 in the PLTP gene promoter and confirm that the introduced mutations block this binding. We conclude that the IR-1 is required for the FXR and CDCA activation of the PLTP promoter.

PLTP Gene Expression Is Regulated by Bile Acids—The physiological role of FXR and CDCA in the regulation of PLTP gene expression was studied in mouse liver because FXR and PLTP are coexpressed there. To test the effect of bile acids on the expression of PLTP, male mice were fed chow supplemented with 1% cholic acid. We chose to study the effects of a cholic acid diet on the expression of the PLTP gene since this diet was previously shown to activate FXR (20, 21). A modest but significant increase in the level of PLTP mRNA expression was seen in the mice fed the cholic acid diet (Fig. 5, A and B). This result demonstrates that bile acids can regulate the expression of the PLTP gene in vivo.

DISCUSSION

We have characterized the role of FXR and bile acids in the regulation of PLTP gene expression. Our results show that FXR and bile acids can regulate the human PLTP promoter. CDCA induced the expression of the reporters containing the PLTP promoter −8−10-fold in the presence of the FXR-RXRα heterodimer, and this response required the PLTP IR-1 element. To determine whether bile acids could regulate PLTP gene expression in vivo, we fed mice a chow diet supplemented with 1% cholic acid and observed a 1.6-fold increase in the level of PLTP mRNA in the livers of mice fed the cholic acid diet.
Previously, it was shown that a taurocholic acid diet results in a similar increase in the expression of the murine I-BABP gene in the small intestine (8). The relatively modest magnitude of these responses may be due to the fact that both PLTP and I-BABP are expressed in tissues where the endogenous level of bile acids is already high.

The regulation of PLTP by FXR suggests a model in which cholesterol, in the form of bile acids, is able to indirectly regulate its return to the liver via HDL. HDL is thought to promote the delivery of cholesterol from peripheral tissues to the liver via the reverse cholesterol transport pathway, and numerous studies have shown it to have anti-atherogenic properties (12). In this scenario, an increase in bile acids due to a higher cholesterol concentration would result in increased HDL levels as a consequence of the induction of PLTP gene expression by FXR and bile acids. This model is consistent with what is

![Diagram of the FXR-RXR heterodimer binding to PLTP IR-1.](image1)

![Northern blot of liver mRNA isolated from male mice fed chow alone (Control) or supplemented with 1% cholic acid (CA). The blot was probed for PLTP mRNA and then stripped and reprobed for β-actin mRNA. Liver mRNA was isolated from three different mice in each group and analyzed separately. 5 μg of poly(A)+-enriched mRNAs were resolved on a 2.2 M formaldehyde and 1% agarose gel and transferred to a nylon membrane. B, quantitation of expression relative to the β-actin control by densitometric scanning and ImageQuant. p < 0.01.](image2)

![Graph showing the relative expression of PLTP in response to FXR activation.](image3)
known about the physiological functions of PLTP and FXR. Both PLTP and FXR are coexpressed in tissues where bile acids are present at concentrations that are required to activate FXR. Studies with PLTP knockout mouse revealed that HDL levels were decreased by 60–70% (1), demonstrating that PLTP is essential for maintaining HDL levels. The PLTP knockout study showed that PLTP is required for the transfer of very low density lipoprotein phospholipids into HDL. Thus, induction of PLTP expression by bile acid-activated FXR should result in increased HDL levels.

This model is also consistent with a number of human studies. High cholesterol diets have been reported to increase plasma HDL levels. In addition, in the initial stages of primary biliary cirrhosis, a cholestatic disease, serum HDL levels are increased as expected (22, 23). The initial rise in HDL levels could be the direct result of an increase in PLTP gene expression, as an increasing bile acid level in the liver activates FXR. Interestingly, several studies of patients with cholestatic diseases have also shown that HDL is enriched in surface lipids, phospholipids, and free cholesterol (24–27), perhaps as the result of an increase in PLTP gene expression, via FXR activation, by elevated bile acid levels in the liver.

Although this model implies that a bile acid diet should increase HDL levels, HDL metabolism is complex and involves many proteins, including apoA-I. A recent study in mice has shown that a cholic acid diet down-regulates hepatic apoA-I mRNA, resulting in decreased HDL levels (28). The mechanism for this down-regulation is not clear. Moreover, the PLTP promoter can also be regulated by fenofibrate via a peroxisome proliferator-activated receptor response element located upstream of the IR-1 (19). Therefore, although an increase in bile acid can result in higher HDL levels, a number of other factors clearly contribute to the overall regulation of cholesterol metabolism.

In conclusion, we have demonstrated that FXR and bile acids can regulate PLTP gene expression. This regulation is consistent with the physiological roles of FXR and PLTP and suggests that FXR may have a role in regulating HDL metabolism.

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