Differential regulation of cytosolic and peroxisomal bile acid amidation by PPARα activation favors the formation of unconjugated bile acids

Karianne Solaas,* B. Frode Kase,[1,†] Viet Pham,[†] Krister Bamberg,[§] Mary C. Hunt,* and Stefan E. H. Alexson*

Division of Clinical Chemistry,* Karolinska Institutet, Karolinska University Hospital at Huddinge, SE-141 86 Stockholm, Sweden; Department of Pediatric Research,[†] Rikshospitalet, NO-0027 Oslo, Norway; and Department of Molecular Biology,[§] AstraZeneca R&D Mölndal, SE-431 83 Mölndal, Sweden

Abstract **In human liver, unconjugated bile acids can be formed by the action of bile acid-CoA thioesterases (BACTEs), whereas bile acid conjugation with taurine or glycine (amidation) is catalyzed by bile acid-CoA:amino acid *N*-acyltransferases (BACATs). Both pathways exist in peroxisomes and cytosol. Bile acid amidation facilitates biliary excretion, whereas the accumulation of unconjugated bile acids may become hepatotoxic. We hypothesized that the formation of unconjugated and conjugated bile acids from their common substrate bile acid-CoA thioesters by BACTE and BACAT is regulated via the peroxisome proliferator-activated receptor α (PPARα). Livers from wild-type and PPARα-null mice either untreated or treated with the PPARα activator WY-14,643 were analyzed for BACTE and BACAT expression. The total liver capacity of taurochenodeoxycholate and taurocholate formation was decreased in WY-14,643-treated wild-type mice by 60% and 40%, respectively, but not in PPARα-null mice. Suppression of the peroxisomal BACAT activity was responsible for the decrease in liver capacity, whereas cytosolic BACAT activity was essentially unchanged by the treatment. In both cytosol and peroxisomes, the BACTE activities and protein levels were upregulated 5- to 10-fold by the treatment. These effects caused by WY-14,643 treatment were abolished in PPARα-null mice. The results from this study suggest that an increased formation of unconjugated bile acids occurs during PPARα activation.**—Solaas, K., B. F. Kase, V. Pham, K. Bamberg, M. C. Hunt, and S. E. H. Alexson. **Differential regulation of cytosolic and peroxisomal bile acid amidation by PPARα activation favors the formation of unconjugated bile acids. *J. Lipid Res.* 2004.** 45: **1051–1060.**

Supplementary key words bile acid-coenzyme A:amino acid *N*-acyltransferase • bile acid-coenzyme thioesterase • peroxisome proliferator-activated receptor α • farnesoid X receptor • cholestyramine

The major metabolic pathway for the elimination of cholesterol from the body is its conversion to bile acids in the liver followed by excretion into bile (1). Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the primary bile acids synthesized in human; in addition to these bile acids, β-muricholic acid is formed in mouse liver. Before secretion into bile, the bile acids are converted to bile salts by conjugation to taurine or glycine in human and almost exclusively to taurine in mouse. The formation of bile salts serves several purposes: it greatly facilitates their secretion into bile (2), the lower pK_a of bile acid conjugates prevents their precipitation in the upper intestinal environment, and it enables bile acids to act as detergents in the intestine. In addition, the amino acid conjugation is important for the detoxification of hydrophobic bile acids.

The formation of bile salts is catalyzed by the bile acid-CoA:amino acid *N*-acyltransferase (BACAT) localized in the peroxisomes and the cytosol of liver cells (3). The substrate for BACAT is CoA-activated bile acids, otherwise known as bile acid-CoA thioesters. They are formed within two compartments of the hepatocyte by two different mechanisms: the ATP-dependent microsomal bile acid-CoA synthetase (ligase) (BACS), and the peroxisomal β-oxidative cleavage of the 5β-cholestanoic acid side chain, the final step in bile acid synthesis (4–6). These reactions are considered to be rate limiting in the cytosolic and peroxisomal conjugation pathways, respectively. The conjugation capacity of the liver is thought to be very high be-

Abbreviations: BACAT, bile acid-CoA:amino acid *N*-acyltransferase; BACS, bile acid-CoA synthetase; BACTE, bile acid-CoA thioesterase; CA, cholic acid; CDCA, chenodeoxycholic acid; FXR, farnesoid X receptor; HNF-4α, hepatocyte nuclear factor 4α; LCA, lithocholic acid; PPARα, peroxisome proliferator-activated receptor α; PTE-2, peroxisomal acyl-CoA thioesterase 2; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid.

[1] To whom correspondence should be addressed.
e-mail: b.f.kase@klinmed.uio.no

Manuscript received 1 July 2003, in revised form 23 December 2003, and in re-revised form 5 March 2004.

Published, JLR Papers in Press, March 16, 2004.
DOI 10.1194/jlr.M300291-JLR200

cause almost all bile acids secreted into bile are conjugated, even in liver disease (7). In the gut, the bile salts are oxidized or deconjugated to varying extents by intestinal microbes. A high proportion is recirculated back to the liver via the portal vein, and deconjugated bile acids are reconjugated, probably by the microsomal-cytosolic pathway, before entering another cycle of the enterohepatic circulation.

The formation of unconjugated bile acids in liver may not be reflected in the bile, either because of impaired secretion into the bile canaliculi (2) or because the free bile acids may be reabsorbed in the biliary tree. Consequently, measurement of hepatic levels of unconjugated bile acids and estimation of the formation rate should be performed in liver tissue specimens. Only a few groups have performed such investigations by use of quantitative mass spectrometry (8, 9). In human liver, the levels of unconjugated bile acids and their physiological and pathological effects remain unknown. It has been shown in vitro, however, that unconjugated bile acids are formed by cleavage of the bile acid-CoA thioesters catalyzed by the bile acid-CoA thioesterases (BACTEs) (3, 10). The activities of BACAT and BACTE have been shown to be comparable in isolated peroxisomes, and these enzymes apparently compete for the bile acid-CoA thioesters slowly formed by the peroxisomal β-oxidation. We recently demonstrated that an acyl-CoA thioesterase in mouse liver peroxisomes, named peroxisomal acyl-CoA thioesterase 2 (PTE-2), is a major BACTE (10). Recombinant PTE-2 can compete with BACAT for the bile acid-CoA substrate in vitro, indicating that the formation of unconjugated bile acids could be high under certain conditions in vivo (10). There is recent evidence for a similar competition between the cytosolic BACAT and BACTE activities for the bile acid-CoA thioesters formed by the microsomal synthetase (3, 11). In vivo factors and conditions that influence the rates of bile acid versus bile salt formation remain unknown. High intrahepatic levels of unconjugated hydrophobic bile acids probably induce or aggravate parenchymal liver disease (12–16). Physiological levels of unconjugated bile acids, however, are purported to be important for cholesterol homeostasis by acting as potent ligands for the farnesoid X receptor (FXR), the bile acid-sensing nuclear receptor (17–21). Unconjugated CDCA has been shown to be the most potent activator of FXR in cell systems, followed by deoxycholic acid and lithocholic acid (LCA), whereas FXR activation by amidated bile acids and CA requires the expression of bile acid transporters. Recently, BACAT mRNA and BACS mRNA were shown to be upregulated by the FXR agonists GW 4064 and CDCA in rat liver hepatocytes. Functional FXR response elements were found in the proximal promoter of the BACS gene and in the first intron of the BACAT gene, presenting evidence for an FXR-mediated regulation of bile acid amidation (22).

Recently, the peroxisome proliferator-activated receptor α (PPARα) was shown to downregulate the expression of cholesterol 7α-hydroxylase (Cyp7A1) and bile acid secretion (23) and to change bile acid composition by regulating the expression of the sterol 12α-hydroxylase gene (Cyp8B1) (24). Here, we report a study of the regulation of the formation of unconjugated and conjugated bile acids from their common substrate, the bile acid-CoA thioesters, via PPARα. In mouse liver, the expression and activity of the peroxisomal and cytosolic BACAT enzyme are differentially regulated by PPARα, which may have implications for the liver content of bile acids and secretion into bile.

MATERIALS AND METHODS

Chemicals

[24-^{14}C]choloyl-CoA and [24-^{14}C]chenodeoxycholoyl-CoA were synthesized by the mixed anhydride procedure (25) and purified by HPLC using a Beckman ODS 5μ (10 mm × 25 cm) column. [24-^{14}C]CA and [24-^{14}C]CDCA were purchased from DuPont NEN. Optiprep and Maxidens were from Nycomed Pharma (Oslo, Norway). WY-14,643 was from Calbiochem-Novabiochem International. Taurine, CA, CDCA, CoA, and cholestyramine were from Sigma (St. Louis, MO). All other chemicals were commercial products of high purity.

Animals and treatment

Ten- to 12-week-old male wild-type (+/+) and PPARα-null (−/−) mice of a pure Sv/129 genetic background (derived from the original colony of mixed background mice) (26) were used throughout this study. The mice were housed in a temperature- and light-controlled environment and were maintained on a standard chow diet (R36; Lactamin, Vadstena, Sweden) before commencement of the treatment. The mice were treated with a diet containing either 0.1% (w/w) of the PPARα agonist WY-14,643 or 4% (w/w) cholestyramine for 1 week. All mice had access to water ad libitum. At the end of the treatment, the mice were euthanized by carbon dioxide asphyxiation, followed by cervical dislocation. The liver samples were weighed immediately and either frozen at −20°C and freshly homogenized in a Potter-Elvenhjelm homogenizer for fractionation experiments or snap-frozen in liquid nitrogen and stored at −70°C until further analysis.

Preparation and characterization of mouse liver subcellular fractions

Homogenates were prepared from frozen or fresh wild-type and PPARα-null mouse liver tissues as described (27). Fresh liver homogenates prepared from pools of eight untreated wild-type mice or three WY-14,643-treated wild-type mice were fractionated into postnuclear, nuclear, mitochondrial, "light-mitochondrial" (peroxisome-enriched), microsomal, and cytosolic fractions as described (3). Aliquots of the light-mitochondrial fractions were layered on top of 15–45% (w/v) Optiprep density gradients and centrifuged for 75 min at 20,000 rpm in a Sorvall TV-850 vertical rotor. Sixteen fractions of 2 ml each were collected from the bottom of the gradient tubes.

To characterize the subcellular fractions, the following marker enzyme activities were measured: cytochrome *c* oxidase for mitochondria (28), the microsomal marker *o*-nitrophenyl acetate esterase (29), the peroxisomal marker catalase (30), and the cytosolic marker lactate dehydrogenase (31).

Protein concentration was determined by the method of Bradford (32). BACAT (3) and BACTE (10) activities were measured as previously described.

Antibody production and affinity purification

Based on the mouse BACAT amino acid sequence (33), a peptide with the sequence NH_2-CAAQEHSWKEIQKFLK-COOH

(with a cysteine added at the NH_2-terminal end for coupling of the peptide) was synthesized and used to raise antisera in rabbits (Sigma Genosys, Suffolk, UK). The reactivity of this antibody with BACAT was verified by Western blotting using recombinant His-tagged human BACAT protein expressed and purified from *Escherichia coli*. An antibody was also generated in rabbits against a peptide corresponding to the sequence NH_2-CNPSMIPIE-KAKGPI-COOH of the PTE-Ib (34) by Sigma Genosys. Both antibodies were purified from rabbit sera using peptide affinity column chromatography before Western blotting.

Western blot analysis

Western blot analysis was performed in principal as described previously (35) on 5–38 μg of protein separated by electrophoresis using 10% sodium dodecylsulfate polyacrylamide gels followed by electrotransfer to nitrocellulose membranes. The membranes were blocked in 50 mM Tris-Base, 0.15 M NaCl, and 0.5% Tween 20 (TST) containing 1% BSA, incubated for 1 h with the BACAT antibody (diluted 1:300 in TST buffer containing 0.1% BSA), washed 3 × 10 min in TST-0.1%BSA, and then incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (diluted 1:10,000 in TST-0.1% BSA). After washing the membranes 3 × 5 min with TST buffer, the signal was detected using ECL (Amersham Life Science, Arlington Heights, IL). The filters were exposed to X-ray film, and quantitation was carried out using Image Master VDS Software (Amersham Pharmacia Biotech, Uppsala, Sweden). The membrane was stripped according to standard procedures (Bio-Rad Laboratories) and reprobed with the PTE-Ib antibody as described above.

Northern blot analysis

Total RNA was prepared from mouse livers using the QuickPrep^R total RNA extraction kit (Amersham Pharmacia Biotech, Uppsala, Sweden), and Northern blot analysis was carried out as described previously (35). The filters were probed with a ^{32}P-labeled full-length cDNA probe for mouse BACAT (33) and a ^{32}P-labeled cDNA probe for β-actin. The blots were exposed to X-ray film at −70°C, and signals were quantified using the Image Master VDS Software.

FXR coactivator recruitment assay

The ligand binding domain of human FXR (amino acids 222–472 based on GenBank accession number U68233) was expressed in *E. coli* as an NH_2-terminal His-tagged protein using the pET28a vector (Novagen), and the protein was purified by affinity chromatography. The activity of recombinant human FXR was determined in an FXR coactivator recruitment assay. Eu^{3+}-coupled anti-His antibody (anti-His-Eu^{3+}) and allophycocyanin-coupled streptavidin were obtained from Perkin-Elmer Life Sciences. An N-terminally biotinylated peptide (NH_2-CPSSHSSLTERHKIL-HRLLQEGSPS-COOH) (Innovagen AB) derived from steroid receptor coactivator 1 was used as coactivator. A 15 μl reaction volume contained 20 mM Tris (pH 7.5), 0.125% CHAPS, 2 mM dithiothreitol, 0.05% BSA, 0.11 μg/ml anti-His-Eu^{3+}, 2.3 μg/ml allophycocyanin-coupled streptavidin, 60 nM human FXR-ligand binding domain, 120 nM biotinylated steroid receptor coactivator 1 peptide, and the appropriate ligand. Antagonist activity was measured in the presence of 80 μM CDCA in the reaction mixture. After the addition of all reagents, plates were incubated for 1.5 h at room temperature and time resolved fluorescence was measured in a Victor2 (Perkin-Elmer Life Sciences). Excitation was at 340 nm, and fluorescence was measured at 615 and 665 nm. Specific signals were calculated by dividing the 665 nm signal by the 615 nm signal and multiplying the fraction by 10,000.

Statistical analysis

Statistical differences between groups were tested by Student's *t*-test. All results are considered significant at $P < 0.05$.

RESULTS

PPARα activation represses peroxisomal BACAT activity and protein level in mouse liver

To test the possibility that PPARα could regulate the final reaction in bile salt biosynthesis, the bile acid amidation step, we measured BACAT activity in liver homogenates from untreated and WY-14,643-treated wild-type and PPARα-null mice. The specific activity of taurine conjugation with CA-CoA was decreased to ∼20% in the WY-14,643-treated wild-type mouse liver homogenates compared with untreated wild-type mice ($P < 0.05$) (**Fig. 1**). Similar results were obtained for taurine conjugation with CDCA-CoA (data not shown). In untreated PPARα-null mice, the BACAT activity was significantly higher than that in untreated wild-type mice ($P < 0.05$) but was unaffected by treatment with WY-14,643 ($P = 0.23$) (Fig. 1).

In a follow-up study using freshly prepared liver homogenates, the PPARα-dependent downregulation of BACAT specific activity was confirmed (data not shown). In wild-type mice, the liver weights increased after WY-14,643 treatment from 0.74 ± 0.04 g to 1.96 ± 0.26 g, and a slight increase was seen in PPARα-null mice, from 0.99 ± 0.01 g to 1.12 ± 0.24 g. In untreated wild-type mice, the total liver activity of taurochenodeoxycholate formation was 3.02 μmol/min/liver compared with a total activity of 1.20 μmol/min/liver for the WY-14,643-treated mice. Thus, in spite of the hepatomegaly (∼2.5-fold) caused by PPARα activation, the total capacity to amidate CDCA was decreased by 60%. The corresponding figures for total taurocholate formation were 3.01 μmol/min/liver in untreated mice and 1.96 μmol/min/liver for the WY-14,643-treated mice.

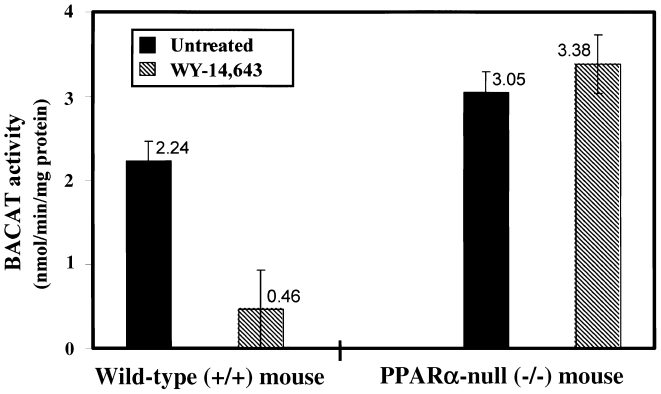

Fig. 1. Peroxisome proliferator-activated receptor α (PPARα)-dependent downregulation of bile acid-CoA:amino acid *N*-acyltransferase (BACAT) activity in mouse liver homogenates. Liver homogenates were prepared from untreated and WY-14,643-treated wild-type (+/+) and PPARα-null (−/−) mice, and taurocholate formation was measured as described in Materials and Methods. The data shown are means ± SD (n = 4).

The liver homogenates were fractionated into postnuclear supernatants, which were further fractionated into mitochondrial, light-mitochondrial" (enriched in peroxisomes), microsomal, and cytosolic fractions, and each of these fractions was characterized by the measurement of marker enzyme activities, BACAT and BACTE activities. Treatment with WY-14,643 resulted in a decrease in the specific activity of taurochenodeoxycholate formation in all particulate fractions, whereas the specific activity remained essentially unchanged in the cytosolic fraction (**Fig. 2A**). In contrast, catalase activity was increased in the light-mitochondrial fraction (as a consequence of peroxisome proliferation) and in the cytosolic fraction (Fig. 2B). Approximately 70% of the total BACAT activity was recovered in the particulate fraction (peroxisomal activity), and ~30% was recovered in the cytosolic fraction. Treatment of mice with WY-14,643 changed the distribution, so that 20% was recovered in the particulate fraction and 80% was recovered in the cytosolic fraction. Previous subcellular fractionation studies have shown that the BACAT activity is also detected in the peroxisomal fraction of rat and human liver (3, 36). As peroxisomes are present in all particulate fractions, the repression of BACAT activity in these fractions by WY-14,643 treatment is apparently attributable to repression of the peroxisomal BACAT expression. The light-mitochondrial fractions, being enriched in peroxisomes, were therefore fractionated on 15–45% (w/v) Optiprep density gradients to obtain purified peroxisomes. BACAT activity and protein concentration were measured in all gradient fractions, which showed that the BACAT activity was localized to the peroxisomal fractions. The specific BACAT activity decreased by more than 90% in the peroxisomal fraction upon WY-14,643 treatment (**Fig. 3A**), whereas catalase activity was only decreased by ~50% (Fig. 3B). The difference in the enrichment of catalase activity from light-mitochondrial fractions to purified peroxisomes from control and WY-14,643 treated mice (Figs. 2B, 3B) is attributable to a very large increase in the amount of peroxisomes in the light-mitochondrial fraction after treatment with WY-14,643 compared with the amount of peroxisomes in the light-mitochondrial fraction from untreated mice. To test whether the decrease in BACAT activity in the peroxisomal fractions was attributable to a decrease in BACAT protein, the fractions were analyzed by Western blotting using a peptide antibody that recognizes recombinant BACAT (Fig. 3C). The BACAT protein was detected as an ~46 kDa protein in the light-mitochondrial, cytosolic, and peroxisomal fractions. In agreement with the activity data, a 10-fold decrease in BACAT protein was observed in the peroxisomal fraction after treatment with WY-14,643 (Fig. 3C), whereas cytosolic liver BACAT protein remained unchanged by the treatment. As a control, the membrane was stripped and hybridized with a peptide antibody directed against PTE-Ib, the expression of which has previously been shown to be upregulated by clofibrate treatment (34). In contrast to BACAT, the expression of PTE-Ib was clearly increased in the light-mitochondrial, cytosolic, and peroxisomal fractions in response to WY-14,643 treatment (Fig. 3C). Thus, changes in BACAT protein levels recognized by the BACAT antibody correlated well with changes in the specific activities of BACAT in the subcellular fractions.

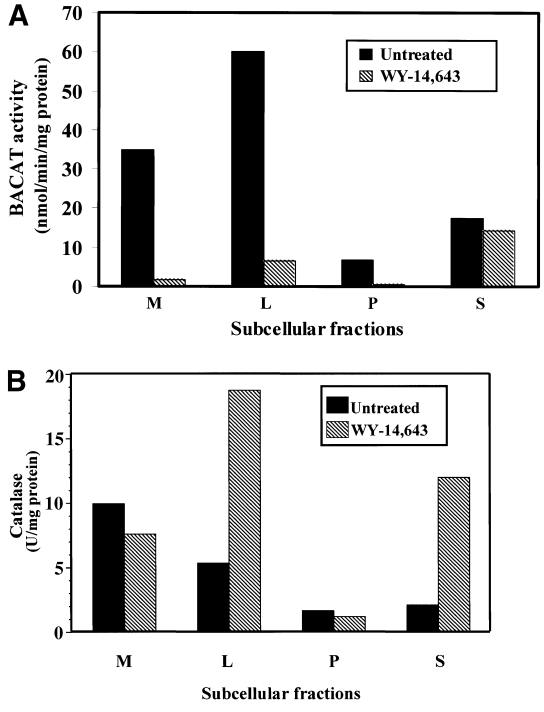

Fig. 2. Changes in activities of BACAT and catalase in subcellular fractions after treatment with WY-14,643. Liver postnuclear fractions prepared from homogenates of eight pooled livers from untreated mice (black bars) and three pooled livers from WY-14,643-treated mice (hatched bars) were fractionated and analyzed as described in Materials and Methods. The data shown are duplicate measurements of taurochenodeoxycholate formation (A) and catalase activity (B) in subcellular fractions. M, mitochondrial fraction; L, light-mitochondrial fraction; P, microsomal fraction; S, cytosolic fraction.

Regulation of hepatic mouse BACAT mRNA

The PPARα involvement in the regulation of BACAT mRNA expression was examined by Northern blot analysis. Using a full-length mouse BACAT cDNA probe, two mRNAs of 2.2 and 4 kb were detected, and quantitation of the major transcript of 2.2 kb showed a significant reduction after treatment of wild-type mice with WY-14,643 ($P < 0.05$) (**Fig. 4A**). In untreated PPARα-null mice, BACAT mRNA levels were lower than those in wild-type mice ($P < 0.05$), and the mRNA level remained unchanged after WY-14,643 treatment ($P = 0.5$).

Cholestyramine treatment sequesters bile acids in the intestine and results in a compensatory upregulation of bile acid biosynthesis (37, 38). Therefore, we analyzed the gene expression of the cytosolic BACAT after cholestyramine treatment. The results showed that hepatic BACAT mRNA levels increased by 2- to 3-fold after treatment with cholestyramine in both wild-type mice ($P < 0.05$) and PPARα-null mice ($P < 0.05$) (Fig. 4B).

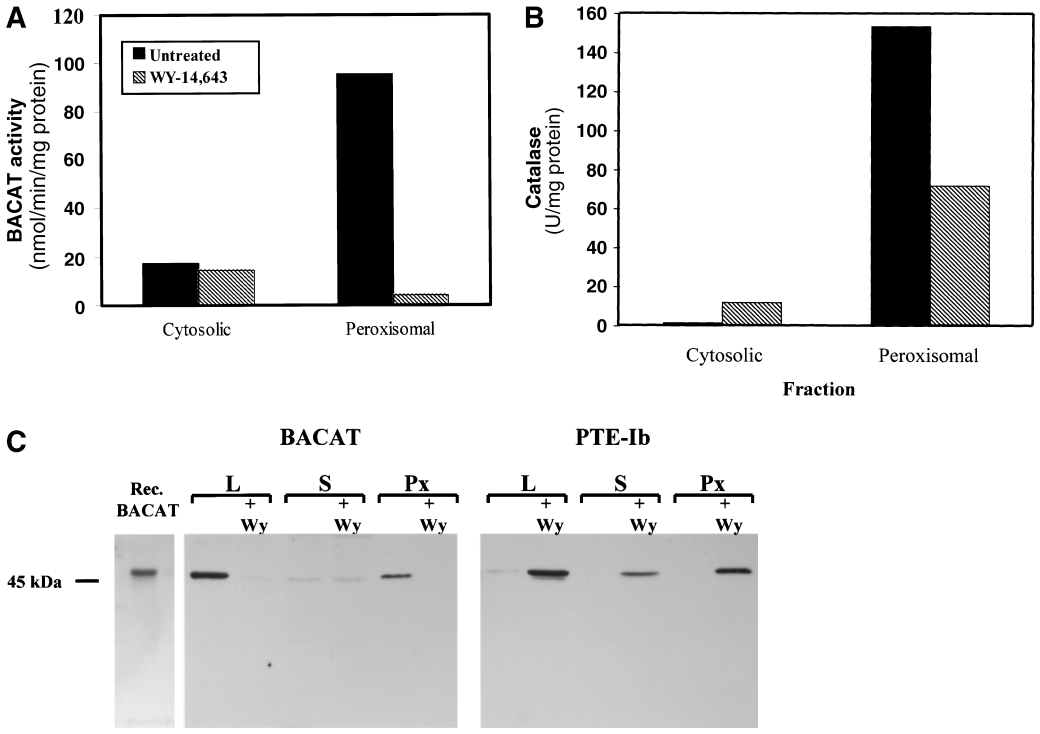

Fig. 3. Downregulation of peroxisomal, but not cytosolic, BACAT activity and protein levels after treatment with WY-14,643. Purified peroxisomes were isolated by density gradient centrifugation of light-mitochondrial fractions prepared from homogenates of pooled livers from untreated (black bars) and WY-14,643-treated (hatched bars) mice. A: Formation of taurochenodeoxycholate was measured in vitro by radio-HPLC analysis as described in Materials and Methods. B: Catalase activity in cytosolic and purified peroxisomal fractions. C: Western blot analysis of recombinantly expressed (Rec.) BACAT protein and subcellular fractions using a BACAT primary antibody as described in Materials and Methods. Left panel: Western blot analysis of recombinant His-tagged BACAT protein expressed in *E. coli*, purified using nickel-affinity chromatography. Middle panel: Five micrograms of peroxisomal protein (Px) and 38 μg of protein from the cytosolic (S) and light-mitochondrial (L) fractions were used for Western blot analysis. Right panel: The blot shown in the middle panel was stripped and reprobed with an antibody raised against peroxisomal acyl-CoA thioesterase Ib (PTE-Ib). Wy, WY-14,643.

BACTE activity is regulated by WY-14,643 treatment

We have previously shown that the specific activity of BACTE was increased 3.5 times in liver homogenates by WY-14,643 treatment of wild-type mice, but the activity remained unchanged by treatment of the PPARα-null mice (10). To further investigate the effect of WY-14,643 treatment on BACTE activity in various subcellular compartments, the same subcellular liver fractions used for the BACAT activity measurements were used for the BACTE activity measurements. The specific activity of BACTE was found to be increased by WY-14,643 treatment in the cytosolic, light-mitochondrial, and microsomal fractions. However, no increase was evident in the mitochondrial fraction (**Fig. 5**). In the cytosolic fraction, the chenodeoxycholoyl-CoA thioesterase activity was 3 times higher (Fig. 5) and the choloyl-CoA thioesterase activity was increased 4.6 times (data not shown) in WY-14,643-treated compared with untreated wild-type mice. In the light-mitochondrial fraction, the corresponding increases were 2- and 1.7-fold, respectively. As alluded to above, WY-14,643 treatment of mice results in hepatomegaly and increases total liver protein. Total protein in the light-mitochondrial and cytosolic fractions were 3-fold higher compared with the fractions from livers of untreated mice, leading to an ∼10-fold increase in the total liver activity of cytosolic BACTE and an ∼6-fold increase the total activity in the light-mitochondrial fraction. The increase in the light-mitochondrial fraction was mainly attributable to a 5- to 6-fold increase of the BACTE activity in the peroxisomal fraction. Thus, although PPARα activation downregulates the expression of peroxisomal BACAT protein (leaving the expression of the cytosolic BACAT essentially unchanged), BACTE activity is increased in both peroxisomes and cytosol, which together may promote decreased bile acid amidation and increased formation of unconjugated bile acids in the liver.

Bile acid-CoA thioesters are weak antagonists of FXR

Nonesterified fatty acids act as agonists for the PPARα (39, 40), whereas the corresponding CoA thioesters act as antagonists (41). Both bile acids and bile salts have been shown to be agonists for FXR (17–21). Therefore, we tested the possibility that bile acid-CoA thioesters may act as antagonists for FXR, using a FXR coactivator recruit-

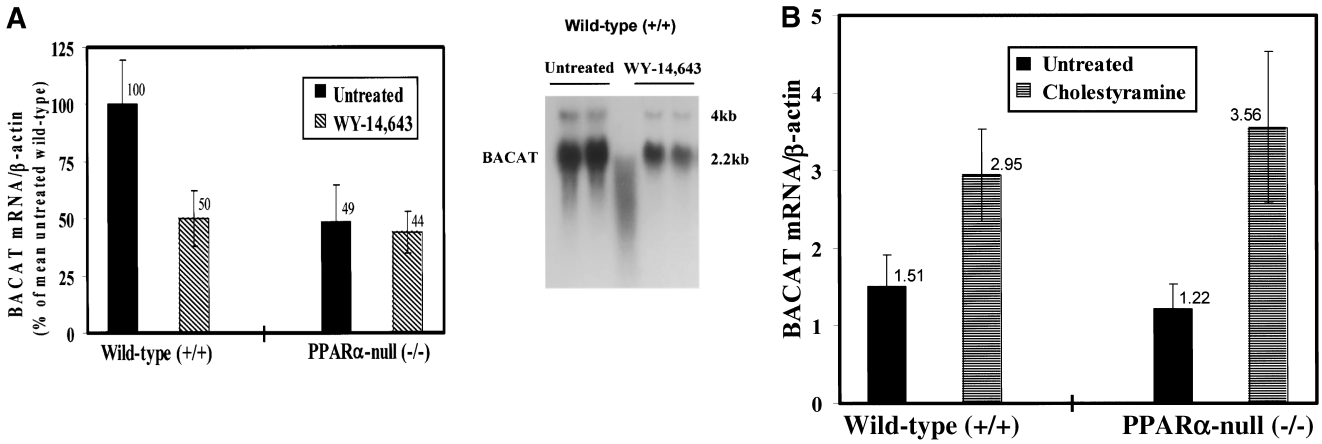

Fig. 4. Hepatic BACAT mRNA levels after treatment of mice with WY-14,643 and cholestyramine. A: Northern blot analysis was carried out on 20 μg of liver total RNA from untreated and WY-14,643-treated wild-type and PPARα-null mice using ^{32}P-labeled mouse BACAT cDNA probe as described. The signal for the 2.2 kb transcript of mouse BACAT was normalized to the signal for β-actin in each sample, and the mean value for the group of untreated wild-type mice was set to 100%. The data shown are means ± SD (n = 7). A typical Northern blot of untreated and WY-14,643-treated wild-type mice is shown to the right. B: Northern blot analysis was carried out on 20 μg of liver total RNA from groups of four untreated (black bars) and five cholestyramine-treated (hatched bars) wild-type (+/+) and PPARα-null (−/−) mice using ^{32}P-labeled mouse BACAT cDNA probe as described. The signal for BACAT was normalized to β-actin in each sample. The data shown are means ± SD (n = 4–5).

ment assay. CDCA-CoA, CA-CoA, and LCA-CoA were all found to antagonize the CDCA-induced FXR/steroid receptor coactivator 1 complex by direct binding to FXR (**Fig. 6**). The rank order of potency was CDCA-CoA > LCA-CoA > CA-CoA. In this experimental system, free CDCA acted as an agonist, with an EC_{50} value of ~50 μM, whereas the corresponding CoA thioester (CDCA-CoA) was the most potent antagonist, with an IC_{50} of ~100 μM. LCA-CoA acted as a weak antagonist (IC_{50} ~ 200 μM), whereas CA-CoA was not titrated to a high enough concentration to allow an estimation of IC_{50} value. None of the tested bile acid-CoA thioesters could induce the recruitment of the coactivator peptide of steroid receptor coactivator 1 to FXR, suggesting that they are not agonists for the FXR. Free CoA had no effect on FXR activation in this assay.

DISCUSSION

This study shows that treatment with WY-14,643 downregulated the total liver bile acid amidation activity in mice, whereas BACTE activity was upregulated, both effects being PPARα dependent. The most intriguing finding was that the decreased liver bile acid amidation activity was attributable to a selective decrease in peroxisomal BACAT, whereas the activity remained essentially unchanged in the cytosolic fraction. This conclusion is based on the following findings: subcellular fractionation experiments showed that both activities are present in peroxisomes and cytosol of mouse liver, similar to the distribution in rat and human liver (3, 11, 36). Characterization of the subcellular fractions demonstrated that both BACAT activity and protein were decreased more than 10-fold in the peroxisomes by WY-14,643 treatment, whereas activity and protein remained essentially unchanged in the cytosolic fractions. The explanation for the apparent discrepancy between changes in BACAT mRNA levels and activity is not yet clear. However, using Green Fluorescent Protein localization experiments, we recently showed that the "classic" BACAT is mainly localized in the cytosol (42). Therefore, the existence of a second, yet unidentified, BACAT is predicted (at least in the mouse) that is mainly peroxisomal. Further support for this idea was recently presented by Gonzalez and colleagues (43), who showed that the BACAT gene is under strong regulation of the hepatocyte nuclear factor 4α (HNF-4α). They demonstrated that in spite of essentially undetectable levels of BACAT expression in HNF-4α-null mice, most of the bile acids in gallbladder bile were conjugated, with an increase in glycine conjugation.

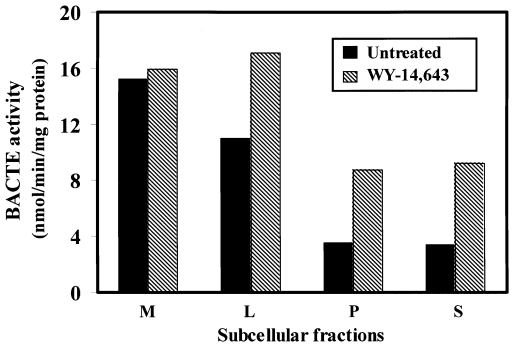

Fig. 5. Changes in the specific activity of bile acid-CoA thioesterase (BACTE) activities in subcellular fractions after treatment of mice with WY-14,643. Chenodeoxycholoyl-CoA thioesterase activity was measured in the mitochondrial (M), light-mitochondrial (L), microsomal (P), and cytosolic (S) fractions obtained after subcellular fractionation of pooled mouse liver homogenates as described in Fig. 2 and in Materials and Methods.

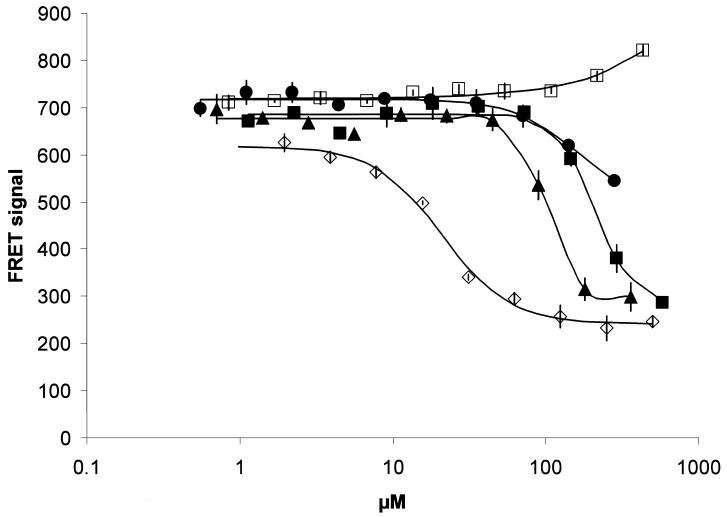

Fig. 6. Bile acid-CoA thioesters are antagonists of the farnesoid X receptor (FXR) in vitro. The binding of bile acid-CoA thioesters to FXR was examined in a coactivator recruitment assay as described in Materials and Methods. Chenodeoxycholic acid (CDCA) was an agonist with an EC_{50} value of ~50 μM. All three bile acid-CoA thioesters antagonized CDCA-mediated FXR activation with the rank order of potency CDCA-CoA (closed triangles) > lithocholic acid-CoA (LCA-CoA) (closed squares) > cholic acid-CoA (CA-CoA) (closed circles). For CDCA-CoA and LCA-CoA, the IC_{50} values were determined to be ~100 μM and ~200 μM, respectively. CA-CoA was not titrated to sufficiently high concentrations to determine the IC_{50} value. CoA (open squares) alone did not inhibit CDCA-mediated FXR activation. LCA (open diamonds) was used as a control. FRET, fluorescence resonance energy transfer.

In contrast to BACAT, BACTE activity was upregulated in both cytosol and peroxisomes by WY-14,643 treatment. The regulation of the enzyme activity was essentially the same for both bile acid-CoA substrates in vitro. The assay conditions used in this study to measure BACAT activity allow us to conclude that the observed decrease in BACAT activity would not be attributable to competition for substrate by BACTE in vitro. However, in vivo, it is likely that BACTE and BACAT compete for the common bile acid-CoA substrate. BACAT apparently plays different roles in cytosol and peroxisomes, as illustrated in **Fig. 7**. Recently, a human BACS was identified that is localized in the endoplasmic reticulum and mainly activates bile acids (CDCA, DCA, and LCA) to the corresponding CoA thioesters, whereas the human peroxisomal very long-chain acyl-CoA synthetase mainly activates 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) to THCA-CoA (5). It is likely that the peroxisomal THCA-CoA synthetase initiates peroxisomal β-oxidative side chain cleavage of THCA-CoA and 3α,7α-dihydroxy-5β-cholestanoyl-CoA to form de novo synthesized bile acid-CoAs, which has been shown to occur mainly in peroxisomes (44). CA-CoA and CDCA-CoA formed by peroxisomal β-oxidation are the substrates for peroxisomal BACAT before excretion into bile (36). Therefore, the very strong downregulation of peroxisomal BACAT in combination with an upregulation of BACTE likely influences the rate of amidation of de novo synthesized bile acids so that newly formed CA-CoA and CDCA-CoA thioesters from peroxisomal β-oxidation are efficiently converted to unconjugated bile acids at the expense of bile salt formation by PPARα activation, as illustrated in Fig. 7. A compensatory increase of amidation by the microsomal BACS and cytosolic BACAT of de novo synthesized bile acids is less likely. BACS is the rate-limiting enzyme in the microsomal-cytosolic pathway. Consequently, CoA activation of both deconjugated bile acids returning from the gut and newly formed bile acids from the peroxisomes probably exceeds its capacity, resulting in the accumulation of unconjugated bile acids in liver tissue.

To the best of our knowledge, our finding that peroxisomal BACAT is downregulated by WY-14,643 treatment of mice is the first report on the downregulation of a per-

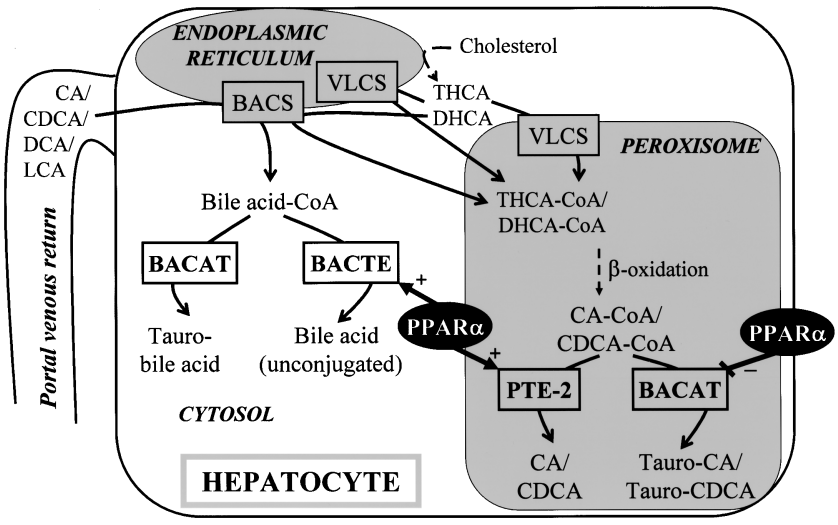

Fig. 7. Model for bile acid amidation pathways in mouse liver. The primary bile acid-CoA thioesters produced in the peroxisomes are either amidated by the peroxisomal BACAT or thiolytically cleaved by the peroxisomal acyl-CoA thioesterase 2 (PTE-2). Peroxisomal BACAT expression is downregulated, whereas PTE-2 is upregulated via PPARα. Unconjugated bile acids recycled back to the liver in the enterohepatic circulation are reactivated by the microsomal bile acid-CoA-synthetase (BACS) before amidation by the cytosolic BACAT or thiolytically cleaved by BACTE. In cytosol, BACAT is not regulated, whereas the activity of BACTE is highly upregulated via PPARα. DCA, deoxycholic acid; DHCA, 3α,7α-dihydroxy-5β-cholestanoic acid; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; VLCS, very long-chain acyl-CoA synthetase.

oxisomal enzyme via PPARα. The mechanism by which the expression of the peroxisomal BACAT is downregulated is not clear, but it may involve competition by PPARα and some other transcription factor for binding to the same response element. Such a mechanism has been demonstrated to downregulate several other genes. Several extraperoxisomal enzymes involved in bile acid synthesis are also regulated via PPARα. Cholesterol 7α-hydroxylase gene expression and activity are reduced in humans and mice by treatment with fibrates and WY-14,643, leading to reduced bile acid excretion (23, 45–47). PPARα has also been shown to upregulate the expression of sterol 12α-hydroxylase and thereby regulate bile acid composition (24). The human apical sodium-dependent bile salt transporter gene, which is mainly expressed in cholangiocytes, kidney, ileum, and cecum, was recently shown to be positively regulated via PPARα in cholangiocytes, which may contribute to the decreased bile salt excretion during PPARα activation (48). The physiological consequences of decreased bile acid amidation are not yet clear. However, our present finding that PPARα apparently mediates decreased bile acid amidation in response to WY-14,643 treatment is in agreement with an overall decrease in bile acid synthesis and excretion. Together with our finding of increased expression of BACTE in cytosol and peroxisomes, it is likely that PPARα will promote decreased amidation of de novo synthesized bile acids, and possibly also of recycled bile acids, leading to an increase in free bile acids in mouse liver. Whether PPARα activation leads to the accumulation of free bile acids in liver could be assessed by measuring hepatic levels of bile acids and bile salts. Detection of biliary bile acids may not reflect intrahepatic levels of unconjugated bile acids because of impaired secretion into the bile canaliculi. In five patients with biliary atresia, the accumulation of unconjugated bile acids (from 2.9% to 4.6% of total bile acids/salts) was observed in liver tissue, whereas only trace amounts were detected in their sera (9). Similar studies should be performed on patients with other liver disorders to address the possibility that unconjugated hydrophobic bile acids may be pathogenic to the liver parenchyma.

After submission of this article, a paper was published online showing that clofibrate treatment of rats induced BACAT mRNA and cytosolic BACAT protein and activity by ~3-fold (49). The reason for the different regulation in rat and mouse is not yet clear, but it could be related to the fact that rats lack a gallbladder and therefore may show different regulation of expression of BACAT. Interestingly, BACAT expression is upregulated via the FXR in rat but not in mouse (22), demonstrating a further species difference between rat and mouse in the regulation of the BACAT gene.

Bile acid feeding reduces the expression of Cyp7A1 and bile acid synthesis. Conversely, treatment with cholestyramine, a nonabsorbable drug that increases fecal loss of bile acids, upregulates Cyp7A1 expression and bile acid synthesis. These effects are mediated via FXR, for which free bile acids and bile salts are more or less potent activators, respectively (17, 19, 21). In an attempt to test the impact of increased synthesis of bile acids on BACAT expression, we treated mice with cholestyramine. A 2- to 3-fold increase in BACAT mRNA levels was found as a result of cholestyramine treatment in both wild-type and PPARα-null mice. In contrast, treatment of mice with CDCA had no effect on BACAT mRNA expression (data not shown), in accordance with recent findings by others (22, 43). These data on cholesterylamine and bile acid treatment suggest that bile acids do not regulate BACAT gene expression via FXR but possibly via some other mechanism in mice.

In view of previous findings that CoA thioesters of fatty acids may act as antagonists for PPARα (41) whereas free fatty acids are ligands for PPARα (39, 40), we tested the possibility that bile acid-CoA thioesters act as antagonists for FXR. By using an FXR coactivator recruitment assay, bile acid-CoA thioesters were shown to antagonize CDCA-mediated activation of the receptor. Interestingly, CDCA-CoA was the most potent antagonist, followed by LCA-CoA, whereas CA-CoA was found to be a very weak (if at all) antagonist. The physiological relevance of our findings that CoA thioesters of bile acids may act as antagonists for FXR is not yet clear. However, in light of the PPARα-mediated downregulation of the bile acid amidation activity and upregulation of BACTE activity, which together may change the cellular contents of free and amidated bile acids as well as levels of bile acid-CoAs, PPARα activation may mediate cross-talk to FXR signaling. Complex cross-talk between PPARα and FXR is further substantiated by recent findings showing that bile acids can antagonize the action of PPARα (50) and that bile acids can induce the expression of the human PPARα gene via FXR (51).

In conclusion, this study has shown that PPARα activation in mice decreased the peroxisomal capacity to amidate bile acids and upregulated both peroxisomal and cytosolic BACTE activity. These effects are likely to decrease bile acid amidation in vivo, probably mediating metabolic cross-talk between PPARα and FXR. Under certain conditions, PPARα activation may cause an accumulation of toxic unconjugated bile acids in liver tissue and induce or exacerbate liver disease.

The authors thank Rigmor Sletta for skillful technical assistance and Frank J. Gonzalez and Jeffrey Peters for the PPARα-null mice. Large-scale production and purification of the recombinant human FXR-ligand binding domain was carried out by the AstraZeneca Biotech Lab in Södertälje, Sweden. The authors thank James O'Byrne for the Western blot analysis of recombinant BACAT protein. This work was supported by grants from the Sigurd K. Thoresen Foundation, Heje's Foundation, Valborg Aschehoug's Foundation, EWS Stiftelsen, the Swedish Research Council, and the Swedish Heart and Lung Foundation.

REFERENCES

1. Chiang, J. Y. L. 1998. Regulation of bile acid synthesis. *Front. Biosci.* **3:** d176–d193.
2. Vessey, D. A., J. Whitney, and J. L. Gollan. 1983. The role of conju-

gation reactions in enhancing biliary secretion of bile acids. *Biochem. J.* **214:** 923–927.
3. Solaas, K., A. Ulvestad, O. Søreide, and B. F. Kase. 2000. Subcellular organization of bile acid amidation in human liver: a key issue in regulating the biosynthesis of bile salts. *J. Lipid Res.* **41:** 1154–1162.
4. Kase, B. F., I. Björkhem, and J. I. Pedersen. 1983. Formation of cholic acid from 3α,7α,12α-trihydroxy-5β-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* **24:** 1560–1567.
5. Mihalik, S. J., S. J. Steinberg, Z. Pei, J. Park, D. G. Kim, A. K. Heinzer, G. Dacremont, R. J. A. Wanders, D. A. Cuebas, K. D. Smith, and P. A. Watkins. 2002. Participation of two members of the very long-chain acyl-CoA synthetase family in bile acid synthesis and recycling. *J. Biol. Chem.* **277:** 24771–24779.
6. Wheeler, J. B., D. R. Shaw, and S. Barnes. 1997. Purification and characterization of a rat liver bile acid coenzyme A ligase from rat liver microsomes. *Arch. Biochem. Biophys.* **348:** 15–24.
7. Matoba, N., M. Une, and T. Hoshita. 1986. Identification of unconjugated bile acids in human bile. *J. Lipid Res.* **27:** 1154–1162.
8. Goto, J., H. Miura, M. Inada, T. Nambara, T. Nagakura, and H. Suzuki. 1988. Determination of bile acids in liver tissue by gas chromatography-mass spectrometry with negative ion chemical ionization detection. *J. Chromatogr.* **452:** 119–129.
9. Abukawa, D., M. Nakagawa, K. Iinuma, M. Nio, R. Ohi, and J. Goto. 1998. Hepatic and serum bile acid composition in patients with biliary atresia: a microanalysis using gas chromatography-mass spectrometry with negative ion chemical ionization detection. *Tohoku J. Exp. Med.* **185:** 227–237.
10. Hunt, M. C., K. Solaas, B. F. Kase, and S. E. H. Alexson. 2002. Characterization of an acyl-CoA thioesterase that functions as a major regulator of peroxisomal lipid metabolism. *J. Biol. Chem.* **277:** 1128–1138.
11. Solaas, K., R. J. Sletta, O. Søreide, and B. F. Kase. 2000. Presence of choloyl- and chenodeoxycholoyl-coenzyme A thioesterase activity in human liver. *Scand. J. Clin. Lab. Invest.* **60:** 91–102.
12. Martinez-Diez, M. C., M. A. Serrano, M. J. Monte, and J. J. Marin. 2000. Comparison of the effects of bile acids on cell viability and DNA synthesis by rat hepatocytes in primary culture. *Biochim. Biophys. Acta.* **1500:** 154–160.
13. Gaull, G. E., and C. E. Wright. 1987. Taurine conjugation of bile acids protects human cells in culture. *Adv. Exp. Med. Biol.* **217:** 61–67.
14. Benedetti, A., D. Alvaro, C. Bassotti, A. Gigliozzi, G. Ferretti, T. La Rosa, A. Di Sario, L. Baiocchi, and A. M. Jezequel. 1997. Cytotoxicity of bile salts against biliary epithelium: a study in isolated bile ductule fragments and isolated perfused rat liver. *Hepatology.* **26:** 9–21.
15. Rolo, A. P., C. M. Palmeira, and K. B. Wallace. 2002. Interactions of combined bile acids on hepatocyte viability: cytoprotection or synergism. *Toxicol. Lett.* **126:** 197–203.
16. Camponella, L., G. Favero, D. Mastrofini, and M. Tomassetti. 1996. Toxicity order of cholanic acids using an immobilised cell biosensor. *J. Pharm. Biomed. Anal.* **14:** 1007–1013.
17. Parks, D. J., S. G. Blanchard, R. K. Bledsoe, G. Chandra, T. G. Consler, S. A. Kliewer, J. B. Stimmel, T. M. Wilson, A. M. Zavacki, D. D. Moore, and J. M. Lehmann. 1999. Bile acids: natural ligands for an orphan nuclear receptor. *Science.* **284:** 1365–1368.
18. Chiang, J. Y., R. Kimmel, C. Weinberger, and D. Stroup. 2000. Farnesoid X receptor responds to bile acids and represses cholesterol 7α-hydroxylase gene (CYP7A1) transcription. *J. Biol. Chem.* **275:** 10918–10924.
19. Wang, H., J. Chen, K. Hollister, L. C. Sowers, and B. M. Forman. 1999. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell.* **3:** 543–553.
20. Sinal, C. J., M. Tohkin, M. Miyata, J. M. Ward, G. Lambert, and F. J. Gonzalez. 2000. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell.* **102:** 731–744.
21. Makishima, M., A. Y. Okamoto, J. J. Repa, H. Tu, R. M. Learned, A. Luk, M. V. Hull, K. D. Lustig, D. J. Mangelsdorf, and B. Shan. 1999. Identification of a nuclear receptor for bile acids. *Science.* **284:** 1362–1365.
22. Pircher, P. C., J. L. Kitto, M. L. Petrowski, R. K. Tangirala, E. D. Bischoff, I. G. Schulman, and S. K. Westin. 2003. Farnesoid X receptor regulates bile acid-amino acid conjugation. *J. Biol. Chem.* **278:** 27703–27711.
23. Post, S. M., H. Duez, P. P. Gervois, B. Staels, F. Kuipers, and H. M. Princen. 2001. Fibrates suppress bile acid synthesis via peroxisome proliferator-activated receptor-alpha-mediated downregulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase expression. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1840–1845.
24. Hunt, M. C., Y-Z. Yang, G. Eggertsen, C. M. Carneheim, M. Gåfvels, C. Einarsson, and S. E. H. Alexson. 2000. The peroxisome proliferator-activated receptor alpha (PPARα) regulates bile acid biosynthesis. *J. Biol. Chem.* **275:** 28947–28953.
25. Shah, P. P., and E. Staple. 1968. Synthesis of coenzyme A esters of some bile acids. *Steroids.* **12:** 571–575.
26. Lee, S. S., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, P. M. Fernandez-Salguero, H. Westphal, and F. J. Gonzalez. 1995. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15:** 3012–3022.
27. Prydz, K., B. F. Kase, I. Björkhem, and J. I. Pedersen. 1986. Formation of chenodeoxycholic acid from 3alpha,7alpha-dihydroxy-5beta-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* **27:** 622–628.
28. Slinde, E., and T. Flatmark. 1973. Determination of sedimentation coefficients of subcellular particles of rat liver homogenates. A theoretical and experimental approach. *Anal. Biochem.* **56:** 324–340.
29. Beaufay, H., A. Amar-Costesec, E. Feytmans, D. Thines-Sempoux, M. Wibo, M. Robbi, and J. Berthet. 1974. Analytical study of microsomes and isolated subcellular membranes from rat liver. 3. Subfractionation of the microsomal fraction by isopycnic and differential centrifugation in density gradients. *J. Cell Biol.* **61:** 188–200.
30. Baudhuin, P., H. Beaufay, Y. Rahman-Li, O. Z. Sellinger, R. Wattiaux, P. Jacques, and C. De Duve. 1964. Tissue fractionation studies. 17. Intracellular distribution of monoamine oxidase, aspartate aminotransferase, alanine aminotransferase, D-amino acid oxidase and catalase in rat-liver tissue. *Biochem. J.* **92:** 179–184.
31. Wanders, R. J. A., M. Kos, B. Roest, A. J. Meijer, G. Schrakamp, H. S. Heymans, W. H. Tegelaers, H. van den Bosch, R. B. Schutgens, and J. M. Tager. 1984. Activity of peroxisomal enzymes and intracellular distribution of catalase in Zellweger syndrome. *Biochem. Biophys. Res. Commun.* **123:** 1054–1061.
32. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
33. Falany, C. N., H. Fortinberry, E. H. Leiter, and S. Barnes. 1997. Cloning, expression, and chromosomal localization of mouse liver bile acid CoA:amino acid N-acyltransferase. *J. Lipid Res.* **38:** 1139–1148.
34. Hunt, M. C., S. Nousiainen, M. Huttunen, K. Orii, L. T. Svensson, and S. E. H. Alexson. 1999. Peroxisome proliferator-induced long chain acyl-CoA thioesterases comprise a highly conserved novel multi-gene family involved in lipid metabolism. *J. Biol. Chem.* **274:** 34317–34326.
35. Hunt, M. C., P. J. G. Linquist, J. M. Peters, F. J. Gonzalez, U. Diczfalusy, and S. E. H. Alexson. 2000. Involvement of the peroxisome proliferator-activated receptor alpha in regulating long-chain acyl-CoA thioesterases. *J. Lipid Res.* **41:** 814–823.
36. Kase, B. F., and I. Björkhem. 1989. Peroxisomal bile acid-CoA: amino-acid N-acyltransferase in rat liver. *J. Biol. Chem.* **264:** 9220–9223.
37. Beher, W. T., M. E. Beher, and B. Rao. 1966. Bile acid and cholesterol metabolism in the mouse as affected by cholestyramine. *Proc. Soc. Exp. Biol. Med.* **122:** 881–884.
38. Garbutt, J. T., and T. J. Kenney. 1972. Effects of cholestyramine on bile acid metabolism in normal man. *J. Clin. Invest.* **51:** 2781–2789.
39. Kliewer, S. A., S. S. Sundseth, S. A. Jones, P. J. Brown, G. B. Wisely, C. Koble, P. Devchand, W. Wahli, T. M. Willson, J. M. Lenhard, and J. M. Lehmann. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc. Natl. Acad. Sci. USA.* **94:** 4318–4323.
40. Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. USA.* **94:** 4312–4317.
41. Elholm, M., I. Dam, C. Jørgensen, A. M. Krogsdam, D. Holst, I. Kratchmarova, M. Göttlicher, J. Å. Gustafsson, R. Berge, T. Flatmark, J. Knudsen, S. Mandrup, and K. Kristiansen. 2001. Acyl-CoA esters antagonize the effects of ligands on peroxisome prolifera-

tor-activated receptor alpha conformation, DNA binding, and interaction with co-factors. *J. Biol. Chem.* **276:** 21410–21416.
42. O'Byrne, J., M. C. Hunt, D. K. Rai, M. Saeki, and S. E. H. Alexson. 2003. The human bile acid-CoA:amino acid N-acyltransferase functions in the conjugation of fatty acids to glycine. *J. Biol. Chem.* **278:** 34237–34244.
43. Inoue, Y., A. M. Yu, J. Inoue, and F. J. Gonzalez. 2004. Hepatocyte nuclear factor 4alpha is a central regulator of bile acid conjugation. *J. Biol. Chem.* **279:** 2480–2489.
44. Kase, B. F., J. I. Pedersen, B. Strandvik, and I. Björkhem. 1985. In vivo and in vitro studies on formation of bile acids in patients with Zellweger syndrome. Evidence that peroxisomes are of importance in the normal biosynthesis of both cholic and chenodeoxycholic acid. *J. Clin. Invest.* **76:** 2393–2402.
45. Kesäniemi, A., and S. M. Grundy. 1984. Influence of gemfibrozil and clofibrate on metabolism of cholesterol and plasma triglycerides in man. *J. Am. Med. Assoc.* **251:** 2241–2246.
46. Bertolotti, M., M. Concari, P. Loria, N. Abate, A. Pinetti, M. E. Guicciardi, and N. Carulli. 1995. Effects of different phenotypes of hyperlipoproteinemia and of treatment with fibric acid derivative on the rates of cholesterol 7α-hydroxylation in humans. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1064–1069.
47. Ståhlberg, D., E. Reihnér, M. Rudling, L. Berglund, K. Einarsson, and B. Angelin. 1995. Influence of bezafibrate on hepatic cholesterol metabolism in gallstone patients: reduced activity of cholesterol 7α-hydroxylase. *Hepatology.* **21:** 1025–1030.
48. Jung, D., M. Fried, and G. A. Kullak-Ublick. 2002. Human apical sodium-dependent bile salt transporter gene (SLC10A2) is regulated by the peroxisome proliferator-activated receptor alpha. *J. Biol. Chem.* **277:** 30559–30566.
49. He, D., S. Barnes, and C. N. Falany. 2003. Rat liver bile acid CoA: amino acid N-acyltransferase: expression, characterization, and peroxisomal localization. *J. Lipid Res.* **44:** 2242–2249.
50. Sinal, C. J., M. Yoon, and F. J. Gonzalez. 2001. Antagonism of the actions of peroxisome proliferator-activated receptor-alpha by bile acids. *J. Biol. Chem.* **276:** 47154–47162.
51. Pineda Torra, I., T. Claudel, C. Duval, V. Kosykh, J. C. Fruchart, and B. Staels. 2003. Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. *Mol. Endocrinol.* **17:** 259–272.