Increased Production of Apolipoprotein B-containing Lipoproteins in the Absence of Hyperlipidemia in Transgenic Mice Expressing Cholesterol 7α-Hydroxylase*

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The finding that expression of a cholesterol 7α-hydroxylase (CYP7A1) transgene in cultured rat hepatoma cells caused a coordinate increase in lipogenesis and secretion of apoB-containing lipoproteins led to the hypothesis that hepatic production of apoB-containing lipoproteins may be linked to the expression of CYP7A1 (Wang, S.-L., Du, E., Martin, T. D., and Davis, R. A. (1997) J. Biol. Chem. 272, 19351–19358). To examine this hypothesis in vivo, a transgene encoding CYP7A1 driven by the constitutive liver-specific enhancer of the human apoE gene was expressed in C56BL/6 mice. The expression of CYP7A1 mRNA (20-fold), protein (~10-fold), and enzyme activity (5-fold) was markedly increased in transgenic mice compared with non-transgenic littermates. The bile acid pool of CYP7A1 transgenic mice was doubled mainly due to increased hydrophobic dihydroxy bile acids. In CYP7A1 transgenic mice, livers contained ~3-fold more sterol response element-binding protein-2 mRNA. Hepatic expression of mRNAs encoding lipogenic enzymes (i.e. fatty-acid synthase, acetyl-CoA carboxylase, stearoyl-CoA desaturase, squalene synthase, farnesylpyrophosphate synthase, 3-hydroxy-3-methylglutaryl-CoA reductase, and low density lipoprotein receptor) as well as microsomal triglyceride transfer protein were elevated ~3–5-fold in transgenic mice. CYP7A1 transgenic mice also displayed a >2-fold increase in hepatic production and secretion of triglyceride-rich apoB-containing lipoproteins. Despite the increased hepatic secretion of apoB-containing lipoproteins in CYP7A1 mice, plasma levels of triglycerides and cholesterol were not significantly increased. These data suggest that the 5-fold increased expression of the low density lipoprotein receptor displayed by the livers of CYP7A1 transgenic mice was sufficient to compensate for the 2-fold increase production of apoB-containing lipoproteins. These findings emphasize the important homeostatic role that CYP7A1 plays in balancing the anabolic lipoprotein assembly/secretion pathway with the cholesterol catabolic bile acid synthetic pathway.

Hepatic lipoprotein secretion requires apoB having a size that is sufficiently large to allow the formation of a lipoprotein particle containing a neutral lipid core, the availability of lipids (i.e. phospholipids, triglycerides, cholesterol, and cholesterol esters), and the intraluminal chaperone/lipid transfer protein microsomal triglyceride transfer protein (MTP)1 (reviewed in Refs. 1–6). The assembly of apoB-containing lipoproteins is abrogated when these essential requirements are not satisfied, resulting in rapid degradation of apoB within the hepatocyte (7). The most characterized pathway responsible for the rapid, co-translational degradation of incompletely translocated apoB is via a ubiquitin-dependent proteasome process (8–11). Several additional pathways that may contribute to the intracellular degradation of apoB have been described (12–15).

Cholesterol 7α-hydroxylase (CYP7A1) is a liver-specific enzyme that regulates the production of bile acids from cholesterol (16–18). Previous studies using cultured rat hepatoma cells showed that stable expression of CYP7A1 increases the cellular content of mature sterol response element-binding protein-1 (SREBP1) as well as mRNAs encoding essentially all the lipogenic enzymes required for very low density lipoprotein (VLDL) lipid production and assembly and secretion of apoB100-containing lipoproteins (19). These findings led us to hypothesize that CYP7A1 expression may indirectly regulate the assembly and secretion of VLDL via increasing the expression of SREBP, the expression of lipogenic enzymes and the expression of MTP (4, 11, 19, 20).

The coordinate induction of VLDL assembly and secretion observed in rat hepatoma cells expressing a CYP7A1 transgene was reminiscent of the coordinate changes in the biosynthesis of VLDL lipids and secretion of apoB observed in primary hepatocytes obtained from carbohydrate-induced (21) and fasted (22–24) rats. Subsequent studies of livers from fasted and refed carbohydrate-induced mice showed that changes in the hepatic content of SREBPs could account for the observed changes in fat and sterol metabolism (25). Fasted mice displayed reduced levels of SREBP1c and SREBP2, whereas livers from carbohydrate-refed mice displayed mainly an increase in SREBP1c (SREBP2 returned to normal levels) (25). SREBP1c preferentially increases the transcription of mRNAs involved in fatty acid biosynthesis (26–28). In contrast, SREBP2 appears to increase preferentially the transcription of mRNAs involved in cholesterol metabolism (26). Thus, the relative bal-

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† The abbreviations used are: MTP, microsomal triglyceride transfer protein; SREBP, sterol response element-binding protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HPLC, high pressure liquid chromatography.
Induction of VLDL Production by CYP7A1

23305

MATERIALS AND METHODS

Transgenic Vector—Transgenic mice expressing the rat CYP7A1 cDNA were generated at the Gladstone Institute of Cardiovascular Disease. The transgene (pLV.7 plasmid) was constructed using a DNA construct containing the hepatic control region of the human apolipoprotein E3 gene and a gift from John M. Taylor (29). The rat CYP7A1 cDNA (1.8 kilobase pairs), which contained all the coding region and 58 base pairs of 3′-untranslated region with EcoRI sites on both ends, was ligated into the pLIV plasmid at the MfeI site (29). When expressed in cultured cells, this vector produced a single CYP7A1 mRNA (2.4 kilobases) species that is distinct from the endogenous mouse CYP7A1 mRNA multiple bands around 4.2 kilobases. After establishing the correct orientation of the cDNA, the transgenic vector was isolated from bacterial sequence using SacI and SpeI restriction enzymes. The 7.6-kilobase pair transgenic construct was gel-purified using a Qiagen gel extraction kit (QIAGEN Inc.). The construct was microinjected into single cell embryos of strain C57BL/6J SJ and implanted into pseudopregnant female mice.

CYP7A1 Transgenic Mice—Tails of pups were clipped; DNA was obtained with a QIAGEN DNAeasy kit, and polymerase chain reaction was performed using the appropriate vector-specific primers. Pups (f0) showing genomic integration of the desired transgenic sequences were bred with C57BL/6J mice, and male progeny from these two lines of CYP7A1 transgenic mice were selected for the present study. Female progeny from these two lines of CYP7A1 transgenic mice were used as a positive control. Male and female progeny from these two lines of CYP7A1 transgenic mice were assayed as previously described (30).

De Novo Synthesized [35S]Methionine-labeled ApoB—Mice were injected with 1 μCi of [35S]methionine/mouse via the tail vein after an overnight (15 h) fast. Blood was collected every hour post-injection for 4 h. Plasma proteins were isolated from the plasma and analyzed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Schleicher & Schuell). The nonspecific binding sites of the membranes were blocked using 10% defatted dried milk, followed by addition of the indicated primary antibody. The relative amount of primary antibody bound to the proteins was detected with the species-specific horseradish peroxidase-conjugated IgG. After washing, blots were developed using the ECL detection kit (Amersham Pharmacia Biotech) on high performance liquid chromatography (Hyperfilm ECL, Amersham Pharmacia Biotech). Polyclonal antibodies were used against apoB, MTP, CYP7A1, and protein-disulfide isomerase.

Bile Acid Pool Analysis—The bile acid pool size was determined by HPLC (34). Upon death under anesthesia, the abdomen was opened, and the gallbladder, liver, and small intestine were excised and homogenized in 5 mL of 100% ethanol. [24-3H]Taurocholate was added to the homogenate as a recovery standard. The homogenate was centrifuged at 1700 rpm for 20 min. The supernatant was concentrated under a nitrogen stream and resuspended in distilled water. This was run through a PrepSep C18 column and eluted with 100% methanol. The sample was concentrated to a 1000 μL volume, and 10 μL of bile acid sample was loaded onto a Beckman UltraspHERE C18, HPLC column under isocratic elution using 67% methanol and water containing 0.1% KH2PO4, pH 5.4. The flow rate was 0.75 mL/min, and absorbance was read at 205 nm. The bile acid pool size was calculated using known standards and the [24-3H]taurocholate recovery standard.

Statistical Analysis—Results are given as means ± S.D. Statistical significance was determined by Student’s t test using double-tailed p values. Values of p < 0.05 were considered to be significant.

RESULTS

We constructed a transgenic vector that we anticipated would provide stable and high level expression of CYP7A1 by the liver. A construct containing the coding region of rat CYP7A1 and the liver-specific enhancer region of the human CYP7A1 RNase Protection Assay—In vitro transcribed radiolabeled antisense and sense strand RNAs were synthesized according to the manufacturer’s protocol using T7 RNA polymerase (MaxiScript, Ambion Inc.). The resulting transcripts were gel-purified using a 5% denaturing acrylamide gel. The transcriptions were excised and eluted from the gel matrix and subsequently ethanol-precipitated. Approximately 30,000 cpm of the in vitro transcript was used to hybridize to 20 μg of total RNA. The RNase protection assay was performed using a HybSpeed kit (Ambion Inc.) with RNase T1 and RNase A following the manufacturer’s specifications. The protected RNA fragments were run through a 5% denaturing acrylamide gel and visualized by PhosphorImager analysis (Molecular Dynamics, Inc.).
apoE gene promoter region (29) was found to express high levels of CYP7A1 mRNA when transiently transfected into HepG2 cells (data not shown). This transgene was subsequently injected into blastocysts, which were implanted into pseudo-pregnant female mice (29). Approximately 30% of the newborn mice were shown to contain the rat CYP7A1 transgene, as determined by polymerase chain reaction analysis of genomic DNA obtained from the tail.

First generation transgenic mice were bred with C57BL/6J mice. Progeny from each mating were examined for the presence of rat CYP7A1 mRNA (derived from the transgene) in liver. Two litters expressed rat CYP7A1 at levels similar to those reported in Fig. 1. There was no significant difference in the expression of rat CYP7A1 mRNA between male and female mice. These transgenic mice also displayed increases in the hepatic expression of the LDL receptor and SREBP2 mRNAs that were similar to those displayed by fifth generation transgenic mice with a genetic background that was estimated to be >90% C57BL/6J (see Fig. 3). Thus, the phenotype described below for CYP7A1 transgenic mice is not likely the result of an epigenetic event caused by integration of the transgene.

**CYP7A1 Transgenic Mice Express CYP7A1 mRNA, Protein, and Enzyme Activity at Constitutively High Levels—**

Rat CYP7A1 mRNA (derived from the transgene) was clearly evident in the livers of CYP7A1 transgenic mice, whereas it was undetected in the livers of non-transgenic mice (Fig. 1A). The mRNA expression level of the rat CYP7A1 transgene was estimated to be >50-fold greater than that of the endogenous mouse CYP7A1. The expression of the transgene mRNA appeared to be liver-specific (i.e. using the RNase protection assay, we could not detect rat CYP7A1 mRNA in brain, lungs, heart, kidneys and skeletal muscle (data not shown)). Western blots of microsomes obtained from the livers of three transgenic mice displayed >9-fold more immunoreactivity toward an immunofluorescence-purified antibody raised against rat CYP7A1 compared with microsomes from non-transgenic mice (Fig. 1B). The increased expression of CYP7A1 mRNA and protein in transgenic mice resulted in an ~6-fold increase in CYP7A1 enzyme activity of hepatic microsomes (Fig. 1C).

**Transgenic Expression of CYP7A1 Increases the Endogenous Bile Acid Pool Due Mainly to Increased Taurochenodeoxycholate—**

The bile acid pool was ~2-fold greater in CYP7A1 transgenic mice compared with their non-transgenic littermates (Fig. 2A). It is interesting to note the stark difference in biliary bile acid composition between the CYP7A1 transgenic mice and non-transgenic mice. In the CYP7A1 transgenic mice, the relative content of the more hydrophobic (dihydroxy) biliary bile acids, taurochenodeoxycholic acid (+10-fold) and taurohyodeoxycholic acid (+5-fold), was increased, whereas the relative content of the more hydrophilic (trihydroxy) bile acids, taurocholic acid (~90%) and taumuricholic acid (~50%), was significantly decreased (Fig. 2B).

**Transgenic Expression of CYP7A1 Increases the Hepatic Expression of Lipogenic Genes—**

Compared with the livers of non-transgenic littersmates, the livers of CYP7A1 transgenic mice displayed significantly greater levels of mRNAs encoding enzymes involved in fatty acid synthesis (acetetyl-CoA carboxylase, 4.3-fold increase; and fatty-acid synthase, 5.8-fold increase) and cholesterol metabolism (3-hydroxy-3-methylglutaryl-CoA reductase, 5.9-fold increase; farnesyl-diphosphate synthase, 3.9-fold increase; squalene synthase, 4.9-fold increase; and the LDL receptor, 5.2-fold increase) (Fig. 3). Although the level of SREBP1 mRNA was similar in both groups of mice, in CYP7A1 transgenic mice, SREBP2 mRNA levels were 3-fold greater than in non-transgenic littersmates (Fig. 3).

The livers of CYP7A1 transgenic mice also displayed increased expression of mRNAs encoding MTP (3-fold increase) and stearoyl-CoA desaturase (4.8-fold increase), two gene products thought to be required for the assembly and secretion of apoB-containing lipoproteins (35, 36) (Fig. 3). Consistent with previous studies indicating that hepatic apoB mRNA expression is resistant to changes in expression (24), the livers of both groups of mice showed similar levels of apoB mRNA (Fig. 3). It is interesting to note that the changes in the expression of mRNAs displayed by CYP7A1 transgenic mice are similar to those observed in SREBP2 transgenic mice (26). Increased expression of hepatic LDL receptors was also observed in hamsters expressing CYP7A1 via an adenovirus transgene (37).

In CYP7A1 transgenic mice, the expression of endogenous (mouse) CYP7A1 was reduced to undetectable levels (Fig. 3), a
result expected from the doubling of the endogenous bile acid pool size (Fig. 2). The alternative (acidic) bile acid biosynthetic pathway is controlled by oxysterol 7α-hydroxylase (CYP7B1) mRNA (38, 39). The livers of CYP7A1 transgenic mice expressed a 4.8-fold reduction in the expression of CYP7B1 compared with the livers of their non-transgenic siblings (Fig. 3). Feeding mice bile acids has been reported to cause a modest reduction in the hepatic expression of CYP7B1 mRNA (39).

Transgenic Expression of CYP7A1 Increases MTP Protein Levels—To further investigate MTP expression in CYP7A1 transgenic mice, we performed Western blot analysis of hepatic microsomes from transgenic and non-transgenic mice at mid-light. MTP protein levels were increased; 1.7-fold (*, p = 0.025) in CYP7A1 transgenic mice compared with non-transgenic littermates when normalized to protein-disulfide isomerase (data not shown).

Transgenic Expression of CYP7A1 Increases the Assembly and Secretion of Triglyceride-rich Lipoproteins—We quantitated the relative rate of accumulation of triglycerides and [35S]methionine-labeled apoB in the blood of mice treated with Triton WR-1339. Triton WR-1339 prevents the metabolism and removal of lipoproteins from plasma (40). The rate of accumulation of VLDL in plasma following intravenous injection of Triton WR-1339 therefore provides a means to estimate their production rates. Following Triton WR-1339 administration, there were significantly greater amounts of triglyceride that accumulated in the plasma of CYP7A1 transgenic mice compared with non-transgenic littermates (Fig. 4). Furthermore, least-squares analysis of the linear rate of accumulation of plasma triglycerides showed that CYP7A1 transgenic mice displayed a slope that was 2.2-fold greater than that obtained with non-transgenic mice.

The rates of accumulation of [35S]methionine-labeled apoB100 (1.65-fold, *, p = 0.025) and apoB48 (2.4%, *, p = 0.05) were also increased in CYP7A1 transgenic mice compared with non-transgenic littermates (Fig. 5). These combined data indicate that the hepatic assembly and secretion of apoB-containing lipoproteins are increased in CYP7A1 transgenic mice.

Despite Increased Hepatic Production of apoB100-containing Lipoproteins, CYP7A1 Mice Display No Increase in Plasma or Hepatic Lipids—In contrast to the significant increased production of hepatic apoB-containing lipoproteins displayed by
CYP7A1 transgenic mice, lipoproteins did not accumulate in plasma (Fig. 6, A–D). Although the concentration of triglyceride was slightly increased in the plasma of CYP7A1 mice, this 12% increase was not statistically significant. Furthermore, the plasma levels of cholesterol in non-high-density lipoprotein lipoproteins were significantly decreased in CYP7A1 transgenic mice (~50%; p < 0.01) (Fig. 6A). We also examined the cholesterol content of lipoproteins that were fractionated by fast protein liquid chromatography. The results confirm the previous results showing that in the plasma of CYP7A1 transgenic mice, the cholesterol content of the apoB-containing lipoproteins (intermediate density lipoprotein and LDL; fractions 21–36) contained significantly less cholesterol (Fig. 6C). Plasma from CYP7A1 transgenic mice also contained significantly less cholesterol in the high density lipoprotein fractions (fractions 36–51) (Fig. 6C). Although the plasma of CYP7A1 transgenic mice contained less cholesterol compared with the plasma obtained from non-transgenic littermates, the plasma content of both apoB100 and apoB48 was similar (Fig. 6D). Thus, despite the significant 2-fold increase in hepatic production of apoB-containing lipoproteins in CYP7A1 mice, lipoproteins did not accumulate in plasma.

**DISCUSSION**

Our results show that hepatic “overexpression” of a CYP7A1 transgene in mice leads to a 2-fold increase in the production of apoB100-containing lipoproteins. Further analysis indicated that the increased hepatic lipoprotein assembly/secrection displayed by CYP7A1 mice occurs in response to an induction of lipogenic biosynthetic enzymes whose transcription is increased by SREBP. The associated induction by SREBP2 of hepatic expression of LDL receptor mRNA was sufficient to prevent the accumulation in plasma of apoB-containing lipoproteins despite the increased lipoprotein production displayed CYP7A1 transgenic mice. The apparent coordinate linkage of the cholesterol/bile acid catabolic pathway with the anabolic lipoprotein assembly pathway contributes to the maintenance of cholesterol and lipoprotein homeostasis in C57BL/6 mice.

The CYP7A1 transgene provided constitutive high level expression of CYP7A1 mRNA, protein, and enzyme activity in the livers of recipient mice (Fig. 1). Since transgenic mice showed normal fecundity, pregnancy, litter size, sex distribution, weight gain, fecal consistency, general health, and longevity (data not shown), the artificially increased expression of CYP7A1 did not impair essential physiological functions. It is interesting to note that the size of the bile acid pool of CYP7A1 transgenic mice was increased only ~2-fold (Fig. 2), whereas CYP7A1 enzyme activity increased ~6-fold (Fig. 1C). Thus, the expansion of the bile acid pool size was disproportionately less than expected. In other studies of mice fed a diet containing 0.2% cholate, the bile acid pool was also increased only 2-fold (34). These combined data suggest that an as yet to be defined process may limit expansion of the bile acid pool of mice beyond 2-fold.

The finding that the expression of CYP7B was decreased ~70% (Fig. 3) suggests that bile acid production by the alternative bile acid synthetic pathway (38, 41) was diminished in CYP7A1 transgenic mice. Thus, in CYP7A1 transgenic mice, the alternative bile acid synthetic pathway contributes less to the bile acid pool, whereas the CYP7A1-dependent pathway contributes more. It has been generally noted that dihydroxy bile acids (e.g. chenodeoxycholic acid) are the preferential products of the alternative (oxysterol-derived) bile acid synthetic pathway (17). It is therefore somewhat surprising that the bile acid pool of CYP7A1 transgenic mice contains relatively more hydrophobic dihydroxy bile acids and less hydrophilic trihydroxy bile acids (e.g. increased taurochenodeoxycholic acid and less taurocholic and tauro-β-muricholic acids) (Fig. 2B). The marked decrease in cholic acid may be explained by bile acid repression of CYP8B1, which diverts de novo synthesized intermediates from forming chenodeoxycholic acid so that they produce cholic acid (42, 43). Thus, the doubling of the bile acid pool in CYP7A1 transgenic mice may have repressed 12α-hydroxylation, causing a compensatory increase in taurochenedoxycholic acid.

The recent discovery demonstrating the importance of bile acid structure in activating the ligand-dependent farnesoid X receptor transcription factor, which regulates the expression of CYP7A1, the ileal bile acid-binding protein (44–46) and the canalicular bile acid export protein (47), emphasizes the complex interrelationships between bile acid pool composition, gene expression, and physiology of bile acids. CYP7A1 transgenic mice may provide an experimental model to explore the mechanisms that determine the composition of the bile acid pool.

A major impetus for undertaking these studies was to examine the influence that CYP7A1 has on hepatic production of apoB-containing lipoproteins. The results of these studies show for the first time that augmented expression of CYP7A1 via transgenic constitutive expression in mice increases the production of apoB-containing lipoproteins by increasing the hepatic expression of mRNAs whose transcription is increased by mature SREBP (Fig. 3). These mRNAs include lipogenic enzymes regulating the synthesis of fatty acids (e.g. acetyl-CoA carboxylase (48), fatty-acid synthase (49), and steraryl-CoA desaturase (36)) and cholesterol (e.g. 3-hydroxy-3-methylglutaryl-CoA reductase (50), farnesyl-diphosphate synthase (51), and squalene synthase (52)). There was also increased hepatic expression of MTP and SREBP2 mRNAs in CYP7A1 transgenic mice, whereas the expression of apoB mRNA was similar in both groups (Fig. 3).

These changes in the expression of lipogenic enzymes were associated with increased production of apoB-containing lipoproteins (Figs. 4 and 5). In the mouse, apoB100 is exclusively

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**Fig. 4. Transgenic expression of CYP7A1 increases the hepatic production and secretion of triglycerides.** Triton WR-1339 was injected into the tail veins of mice. At the indicated times, blood was obtained from the retro-orbital sinus. The content of triglycerides was determined by an enzyme assay. Each point represents the mean ± S.D. of four mice/group. *, significant difference (p < 0.01) between non-transgenic (open bars) and CYP7A1 transgenic (hatched bars) mice. The slope of the rate of increase in plasma triglycerides was 2.2-fold greater in CYP7A1 mice than in non-transgenic littermates.
Thus, the concordant 2-fold increase in the accumulation of both triglycerides (Fig. 4) and \[^{35}S\]methionine-labeled apoB100 (Fig. 5) displayed by CYP7A1 transgenic mice treated with Triton WR-1339 suggests that hepatic VLDL assembly and secretion were coordinately increased by CYP7A1. Lipogenesis is a major determinant of how much apoB enters the VLDL assembly/secretion pathway and how much is degraded by the alternative ubiquitin-dependent proteasome pathway (reviewed in Ref. 4). The increased production of apoB100-containing lipoproteins without a change in apoB mRNA expression displayed by CYP7A1 transgenic mice is consistent with this proposal.

The increased production of apoB-containing lipoproteins displayed by CYP7A1 transgenic mice is similar to the pheno-
type displayed by rat hepatoma cells (McA-RH7777) that express a CYP7A1 transgene (19). There is one potentially important difference. In the livers of CYP7A1 transgenic mice, SREBP2 mRNA was selectively increased (Fig. 3), whereas in rat hepatoma cells (McA-RH7777) expressing a CYP7A1 transgene, SREBP1 was selectively increased (19). Our findings showing that CYP7A1 transgenic mice displayed increased expression of SREBP2 mRNA are consistent with those showing that SREBP2 is induced in the livers of hamsters treated with a regimen that decreases cellular cholesterol levels (i.e. a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor and a bile acid-binding resin) (56). The basis for why CYP7A1 expression in rat hepatoma cells (McA-RH7777) increases the expression of SREBP1, but not that of SREBP2 (19), may be related to cellular differences in oxysterol metabolism. Recent studies showed that treating rat hepatoma cells (McA-RH7777) with a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor selectively increased SREBP1c due to an oxysterol-mediated activation of liver X receptor (28).

The additional finding that MTP mRNA was increased in the livers of SREBP2 transgenic mice (26) is consistent with the proposal that the increase in SREBP2 mRNA in the livers of CYP7A1 transgenic mice may mediate the increased MTP mRNA expression. Several studies support the concept that, in mice, MTP expression may be rate-limiting for the production of apoB-containing lipoproteins (57–60). The increased hepatic expression of MTP mRNA together with the increased expression of lipogenic enzymes contributes to the increased production of apoB-containing lipoproteins displayed by CYP7A1 transgenic mice.

Despite the increased production of apoB-containing lipoproteins displayed by CYP7A1 transgenic mice treated with Triton WR-1339, the plasma from these mice showed no evidence of increased lipoproteins (Fig. 6). These data suggest that the capacity for metabolizing and removing apoB-containing lipoproteins from the plasma of CYP7A1 transgenic mice exceeded the 2-fold increase in their production. These metabolic processes include lipoprotein lipase- and hepatic lipase-mediated lipolysis, followed by removal from plasma of the resulting remnant particles by the LDL receptor (61–66).

SREBP2 is a more potent inducer of the LDL receptor than is SREBP1 (26, 28). LPL expression can be also activated by SREBP2 (67). Thus, the selective increased expression of SREBP2 mRNA in the livers of CYP7A1 transgenic mice is likely to be responsible for the 5.2-fold increased expression of LDL receptor mRNA (Fig. 3). Increased hepatic expression of LDL receptors may also account for the significant reduction of intermediate density lipoprotein and LDL cholesterol in the plasma of CYP7A1 transgenic mice (Fig. 6). Our findings are consistent with those showing that the livers of hamsters expressing a CYP7A1 adenovirus transgene display increased expression of the LDL receptor, increased rates of hepatic LDL clearance, and reduced plasma LDL cholesterol (37).

In humans, some moderate forms of hypertriglyceridemia are associated with both increased hepatic production of triglyceride-rich lipoproteins (68–70) and impaired metabolism and removal from plasma of apoB100-containing lipoproteins (71). In some hypertriglyceridemic patients, the production of hepatic triglyceride-rich lipoproteins was found to vary in parallel with rates of bile acid synthesis (68–70, 72). Reduced absorption of bile acids displayed by type IV hypertriglyceridemic patients may be responsible for increased bile acid synthesis (73). The linkage between the bile acid synthetic pathway and hypertriglyceridemia becomes more apparent when type IV patients are treated with cholestyramine, which induces both CYP7A1 expression and the hepatic production of VLDL triglycerides (43). Conversely, feeding hypertriglyceridemic patients chenodeoxycholic acid, which represses CYP7A1, reduces plasma triglyceride levels (75).

The recent finding that genetic loss of the ileal bile acid receptor results in a familial form of type IV hyperlipidemia (72) provides further evidence suggesting that stimulation of the bile acid synthetic pathway is involved. Our findings demonstrating that CYP7A1 transgenic mice display a 2-fold increased production of apoB100/triglyceride lipoproteins, but no accumulation of triglyceride in plasma, suggest that stimulation of the bile acid synthetic pathway is not by itself sufficient to cause hypertriglyceridemia.

Our data suggest that SREBP-mediated gene expression links the anabolic VLDL production pathway to the cholesterol/bile acid catabolic pathway through changes in hepatic cholesterol levels and metabolism (19). The recent discovery that the nuclear hormone liver X receptor and farnesoid X receptor are activated by oxysterols (76, 77) and bile acids (44–46), respectively, provides an additional connection between CYP7A1 and VLDL production. Many of the intermediates that are formed during the conversion of cholesterol to bile acids are oxysterols that activate the liver X receptor (76, 77). Moreover, the finding that the liver X receptor activates both CYP7A1 (78) and SREBP1c (28, 79) expression provides an additional mechanism linking the bile acid biosynthetic pathway to the anabolic VLDL production pathway. Our studies of CYP7A1 transgenic mice provide support linking cholesterol/oxysterol metabolism to the hepatic expression of genes controlling lipoprotein production and metabolism.

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