Mice Expressing the Human \textit{CYP7A1} Gene in the Mouse CYP7A1 Knock-out Background Lack Induction of CYP7A1 Expression by Cholesterol Feeding and Have Increased Hypercholesterolemia When Fed a High Fat Diet*

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Cholesterol 7\(\alpha\)-hydroxylase (CYP7A1) catalyzes the rate-limiting step in the pathway responsible for the formation of the majority of bile acids. Transcription of the gene is regulated by the size of the bile acid pool and dietary and hormonal factors. The farnesoid X receptor and the liver X receptor (LXR) are responsible for regulation by bile acids and cholesterol, respectively. To study the effects of dietary cholesterol and fat upon expression of the human \textit{CYP7A1} gene, mice were generated by crossing transgenic mice carrying the human \textit{CYP7A1} gene with mice that were homozygous knockouts (\textit{cYP7A1}\(^{-/-}\)). The mice (m\textit{CYP7A1}\(^{-/-}\)/\textit{hCYP7A1}) expressed the human gene at much higher levels than did the transgenics bred in the wild-type background. A diet containing 1\% cholic acid reduced the expression of the human gene in \textit{mCYP7A1}\(^{-/-}\)/\textit{hCYP7A1} mice to undetectable levels. Cholestyramine (5\%) increased the level of expression of the human gene and the mouse gene. Thus, farnesoid X receptor-mediated regulation was preserved. A diet containing 2\% cholesterol increased expression of the mouse gene in wild-type mice, but it did not affect expression of the human gene in \textit{mCYP7A1}\(^{-/-}\)/\textit{hCYP7A1} mice. None of the diets altered the serum cholesterol or triglyceride levels in these mice; 1\% cholic acid caused a redistribution of cholesterol from the high density lipoprotein to the low density lipoprotein density in the humanized mice but not in wild-type mice. A diet containing 30\% saturated fat and 2\% cholesterol caused a decrease in \textit{CYP7A1} levels in \textit{mCYP7A1}\(^{-/-}\)/\textit{hCYP7A1} mice. The serum cholesterol levels rose in all mice fed this diet. The increase was greater in the \textit{mCYP7A1}\(^{-/-}\)/\textit{hCYP7A1} mice. Together, these data suggest that the lack of an LXR element in the region from \(-56\) to \(-49\) of the human \textit{CYP7A1} promoter may account for some of the differences in response to diets between humans and rodents.

Cholesterol is a lipid molecule that plays unique and specific roles in cellular membrane function and embryonic development. It also serves as a precursor for the synthesis of steroid hormones and bile acids. Accordingly, a sophisticated system has evolved to ensure its availability to cells. The liver plays a central role in coordinating many of the components of this process. The accumulation of excess cholesterol in blood vessels leads to atherosclerosis and its accumulation in the bile leads to gallstones. Humans, in contrast to rodents, are highly susceptible to these conditions.

Hepatic cholesterol homeostasis is controlled by the rate of cholesterol entry to the liver from dietary and endogenous lipoproteins, as well as by the rate of synthesis of new cholesterol relative to the rate of hepatic lipoprotein secretion, the rate of cholesterol degradation to bile acids, and the rate of excretion of the intact molecule into the bile. These various processes are modulated by the levels of the enzymes catalyzing the rate-limiting steps of each process. An important determinant of the level of these proteins is the rate of transcription of their genes, which is controlled, in turn, by a number of transcription factors.

Over the last few years, a large body of knowledge has been gathered regarding the regulation of these genes. A limited number of transcription factor families interact to control a network of reactions. The sterol response element binding protein family of transcription factors controls the level of enzymes in the cholesterol and fatty acid biosynthetic pathways, as well as the rate of lipoprotein uptake by the liver and nonhepatic tissues (1). Members of the peroxisome proliferation activated receptor family of nuclear hormone receptors control fatty acid metabolism in liver and fat cells (2, 3), as well as in other tissues, and thus may contribute to regulation of cholesterol metabolism as well as bile acid synthesis. Recently, it was shown (4) that two members of the nuclear hormone receptor family, the farnesoid X receptor (FXR)\(^1\) and the liver X receptor (LXR), regulate several steps in bile acid synthesis and uptake, as well as the secretion of cholesterol and other sterols by cells. Thus, subtle differences in the level of expression or the regulation of the expression of a large number of genes may be responsible for differences in responsiveness to diets or drugs in different species or even among individual organisms in a particular species.

The rate of cholesterol degradation to bile acids is controlled in part by the enzyme cholesterol 7\(\alpha\)-hydroxylase (CYP7A1), which catalyzes the initial step in the pathway of bile acid synthesis that is responsible for more than 50\% of bile acid synthesis.

\(^{1}\) The abbreviations used are: FXR, farnesoid X receptor; LXR, liver X receptor; CYP7A1, cholesterol 7\(\alpha\)-hydroxylase; LDL, low density lipoprotein; HDL, high density lipoprotein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
were studied.

In these cases, the increase in bile acid synthesis induced by cholesterol feeding leads to a decrease in CYP7A1 levels (12, 13).

To establish an in vivo model system for studying the regulation of the human gene, we generated transgenic mice that expressed the human CYP7A1 gene under the control of its own promoter (18). Human CYP7A1 mRNA levels were low in these mice. These mice were crossed with CYP7A1+/− mice. The mice lacking the mouse gene and expressing the human gene are referred to as mCYP7A1+/−/hCYP7A1 mice. The phenotypes of these mice, as well as some aspects of the regulation of the human CYP7A1 gene in these mice when fed a variety of diets, were studied.

MATERIALS AND METHODS

**Animals**—FVB, 129SvJ, and B6,129-CYP7A1−/− (referred to hereafter as CYP7A1−/−) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and bred in our facility. Preparation of the FVB mice containing the human CYP7A1 transgene has been described previously (18). Mice were crossbred, and the offspring were screened for the presence of the human gene and for the absence of the mouse gene using polymerase chain reaction and primers specific for each species. A line of mice that had three copies of the human transgene and the highest level of expression was chosen for further studies (18).

**Diets**—Mice were fed Prolab Isopro RMH 3000 diet (PMI Nutrition International, Brentwood, MO) containing 5% fat. In some experiments, either 1% cholic acid, 2% cholesterol, or 5% cholestyramine was added to ground Prolab diet and made into pellets. The “recuse” diet was ground chow with 1% cholic acid and water, supplemented with Kritter Vites (Mardale Laboratories, Glendale Heights, IL) as described by Schwarz et al. (19). In another set of experiments, mice were fed a chow diet containing 20% of calories from coconut oil, such that 25% of the calories were from fat and 1.25% from cholesterol.

**Lipid Analysis**—Cholesterol and triacylglycerol levels in the serum samples were assayed using Sigma diagnostic kits 352-20 and 336-10. **Lipoprotein Profiles**—Serum lipoproteins were separated by fast performance liquid chromatography using two Superose 6 HR 40/30 Columns in series (Amersham Biosciences) as described by Plump et al. (20). Serum (30–100 μl) was injected, the flow rate was 0.5 ml/min, and 1-ml fractions were collected. A buffer containing 0.15 x NaCl, 1 mm EDTA, and 0.02% azide, pH 7.4, was used. The cholesterol and triglyceride content of each fraction was determined using the Sigma diagnostic kits modified to bring the samples into the linear range. Low density lipoprotein (LDL) and high density lipoprotein (HDL) were isolated from plasma and used as standards.

**Cell Culture and Northern Analysis**—HepG2 cells were cultured in minimum essential medium (Invitrogen) with 10% fetal calf serum as described previously (21). RNA isolation and Northern analysis was carried out as described previously (22).

**Quantification of the Level of mRNA of hCYP7A1 in the CYP7A1−/− Mice**—Real time PCR was used to quantify the levels of CYP7A1 mRNA. Primers and a probe that was specific for the human or mouse CYP7A1 were designed using the Applied Biosciences (Foster City, CA) software, and assays were run on an Applied Biosciences ABI 7700 SDS, using their standard conditions. Primers corresponded to nucleotides +1019 to +1040 and +1061 to +1105 of the human gene and nucleotides +971 to +995 and +1037 to +1061 of the mouse gene. DNA sequences from +1042 to +1171 of the mouse gene and from +1003 to +1032 of the human gene were used as the species-specific probes.

A standard curve was prepared for the human and mouse genes by amplifying the sequences from +705 to +1250 and from +666 to +1210, respectively. The DNA fragments were purified, and a standard curve was prepared by carrying out real time polymerase chain reaction with known amounts of cDNA and the appropriate human or mouse probes. This technique measures the absolute amount of RNA present. In all samples, GAPDH mRNA levels were measured to provide an internal standard.

**Statistics**—Data were analyzed using Statview (SAS Institute, Cary, NC). Analysis of variance or group t-tests were carried out as appropriate.

**RESULTS**

**Survival of CYP7A1−/− and hCYP7A1/CYP7A1−/− Mice**—In the original characterization of homozygous knock-out mice for the CYP7A1 gene, it was reported (23) that most of the homozygotes died at or shortly after birth, unless they and their mothers were fed a diet supplemented with bile acids and fat soluble vitamins. A subsequent preliminary report (24) suggested that this was not the case, and the mice could live through the neonatal period without dietary supplementation. To resolve this issue, and to learn whether the human gene could rescue the knock-out phenotype if it indeed was lethal, two sets of animals were bred at the same time under identical conditions and without dietary supplementation for either the mothers or the pups. Three female CYP7A1−/− mice that were mated with CYP7A1+/− male mice became pregnant. The litters contained three, three, and four viable pups. There were no surviving pups after 14, 13, and 10 days, respectively. In contrast, five female mice homozygous for CYP7A1−/− but bearing at least one copy of the human CYP7A1 gene, when mated with male CYP7A1−/− mice that had at least one copy of the human CYP7A1 gene, had litters of three to eight pups (average 4.5 pups), and all pups survived the neonatal period and grew to adulthood.

The weight of these pups at 1 month of age was the same as the weight of normal mice of the FVB and 129SvJ strains of similar age and was greater than the weight of CYP7A1−/− mice that lack the human gene that were raised on the rescue diet (Fig. 1). The CYP7A1−/− mice on the rescue diet gained more weight than the mCYP7A1−/−/hCYP7A1 mice, or the control mice after the first month, and by 3 months of age, the CYP7A1−/− female mice weighed the same as the FVB and mCYP7A1−/−/hCYP7A1 female mice, whereas the CYP7A1−/− male mice were still somewhat smaller than the comparison male mice (Fig. 1).

**Northern Analysis of CYP7A1 mRNA in the Transgenic Mice**—Livers were removed from the various strains of mice, mRNA was prepared, and Northern analysis was carried out (Fig. 2). The levels of human CYP7A1 mRNA were considerably higher in mCYP7A1−/−/hCYP7A1 mice than in HepG2 cells but were variable, and the degree of variability was greater than one would expect because the mice constituted a mixture of heterozygotes and homozygotes for the hCYP7A1 gene. The mRNA was the same size as that of HepG2 cells, and two bands were present, as is usually seen with the human CYP7A1 (25). The ratio of the two sizes of mRNA was similar in the liver of CYP7A1−/− mice and in HepG2 cells. No human CYP7A1 mRNA was detected in the CYP7A1−/− mice or in any other tissue of the mCYP7A1−/−/hCYP7A1.

**Quantification of the Level of mRNA of Human CYP7A1 in the CYP7A1−/− Mice**—Real time polymerase chain reaction...
FIG. 1. Weight of mice of different genotypes. FVB, 129SvJ, and mCYP7A1+/-/hCYP7A1 mice were placed on a chow diet. mCYP7A1−/− mice were placed on a chow diet supplemented with 1% cholic acid and water containing multivitamins. The mice were weighed at 1 and 3 months of age. Data are presented as mean ± S.E. Female FVB, n = 10; male 129SvJ, n = 18; female CYP7A1−/−, n = 21; female mCYP7A1−/−/hCYP7A1, n = 10; male FVB, n = 6; male 129SvJ, n = 5; male CYP7A1−/−, n = 18; male mCYP7A1−/−/hCYP7A1, n = 28. *, p < 0.001 compared with the other three strains.

was used to quantify the levels of hCYP7A1 mRNA. In all samples, primers and a probe for GAPDH mRNA were used as an internal control for the amount of mRNA added. In the first study, the effect of the presence of the mouse CYP7A1 gene on the level of expression of the human CYP7A1 gene was examined. HepG2 cells were used as a reference standard. In mice that were homozygous for the normal mouse CYP7A1 gene, the human CYP7A1 mRNA was barely detectable (Fig. 3). In heterozygous knock-out mice (CYP7A1−/−), the transgene mRNA was more readily detectable. In homozygous knock-out mice (CYP7A1−/−), the level of human mRNA was even higher. Overall expression of the human CYP7A1 gene in the CYP7A1−/− mice was 9-fold greater than it was in the CYP7A1+/- mice. The highest level of expression in the mouse heterozygotes, however, was lower than the lowest level observed in mice that were null for the mouse gene. Thus, the presence of the mouse gene suppresses expression of the human gene.

The absolute level of CYP7A1 mRNA in normal mice was compared with that in the mCYP7A1−/−/hCYP7A1 mice, using known amounts of mouse and human CYP7A1 mRNA as standards. In female mice lacking the endogenous CYP7A1 gene, (mCYP7A1−/−/hCYP7A1 female mice), the human CYP7A1 mRNA level was about one-sixth that exhibited by normal FVB mice of comparable age on the same diet (Fig. 4). In male mice, this difference was less pronounced, with male mCYP7A1−/−/hCYP7A1 having about one-third the level of CYP7A1 mRNA of normal male mice. Interestingly, the level of CYP7A1 in normal female mice was twice that of the normal male mouse, but the gender difference was not noticeable in the mCYP7A1−/−/hCYP7A1 mice.

Effect of Cholestyramine, Cholic Acid, and Cholesterol on CYP7A1 mRNA Levels in Normal and mCYP7A1−/−/hCYP7A1 Mice—Transcriptional regulation of the CYP7A1 gene in response to increases and decreases in the size of the bile acid pool was studied. For the feeding experiments, groups of normal mice were set up containing approximately equal numbers of FVB and 129SvJ mice, to account for the mixed genetic background of the mCYP7A1−/−/hCYP7A1 mice, although there were no noticeable differences in CYP7A1 mRNA levels in the two strains.

Addition of 1% cholic acid to the diet of normal mice resulted in a reduction of 90% the level of mouse CYP7A1 mRNA (Fig. 5, A and C), regardless of gender. Additionally, feeding of bile acids to the mCYP7A1−/−/hCYP7A1 mice resulted in an even greater reduction of the level of human CYP7A1 mRNA (Fig. 5, B and D). Upon cholic acid feeding, the level of human mRNA was less than 1% that of the mouse mRNA. In contrast, the mouse mRNA level after cholic acid feeding was about half the
CYP7A1<sup>−/−</sup> Mice Lack Induction of CYP7A1 Expression

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Mice fed a high fat, high cholesterol diet were compared before and after the feeding experiments (data not shown).

When examined by fast performance liquid chromatography, the lipoprotein profile as assessed by the distribution of cholesterol and triglyceride was the same in animals fed the control diet as in those fed the 5% cholestyramine or 2% cholesterol diets. However, in the mCYP7A1<sup>−/−</sup>/hCYP7A1 mice fed cholic acid, there was a redistribution of cholesterol from the HDL to the LDL density region. This was observed in the four male and four female mice studied (Fig. 7).

Effect of a High Fat, High Cholesterol Diet on Serum Lipids and CYP7A1 mRNA Levels in Normal and mCYP7A1<sup>−/−</sup>/hCYP7A1 Mice—To learn whether the human and mouse CYP7A1 genes in the mCYP7A1<sup>−/−</sup>/hCYP7A1 mouse may differ in their response to a diet that may be atherogenic in primates but not in mice, mice were fed a high fat (25% total calories, 20% calories saturated fat), 1.5% cholesterol diet without cholic acid supplementation. Serum cholesterol levels rose in all of the mice (Fig. 8). Cholesterol levels in mCYP7A1<sup>−/−</sup>/hCYP7A1 mice were considerably more elevated than in normal mice (Fig. 8). Data from the two control strains were pooled in this experiment because there were no differences in response between the two strains. The same effect was seen in both male and female mice (Fig. 8).

The lipoprotein distribution profiles also changed in all mice. In the control strains, the percentage of cholesterol in the HDL fraction fell significantly, although the absolute value did not change. The amount of cholesterol in the LDL and VLDL fractions increased. In the mCYP7A1<sup>−/−</sup>/hCYP7A1 mice, a similar pattern of change was seen, with the VLDL levels rising proportionately more than in the control strains (data not shown).

CYP7A1 mRNA levels were also determined at the end of the 2-week feeding period. In control mice, the CYP7A1 mRNA levels decreased by about 80%, despite the absence of bile acids and the presence of cholesterol in the diet (Fig. 9). In mCYP7A1<sup>−/−</sup>/hCYP7A1 mice, the decrease in CYP7A1mRNA was even greater. There was no detectable CYP7A1 mRNA in either male or female mCYP7A1<sup>−/−</sup>/hCYP7A1 mice after 2 weeks on the diet (Fig. 9).
Mice Lack Induction of CYP7A1 Expression

**FIG. 5. Effect of diet on CYP7A1 levels in normal and mCYP7A1−/−/hCYP7A1 mice.** The same experiment as described in Fig. 4 was carried out, except that in addition to the chow diet, groups of mice were fed 1% cholic acid for 1 week, 5% cholestyramine for 1 week, or 2% cholesterol for 2 weeks mixed in the chow. Units are the same as in Fig. 4. Data are presented as mean ± S.E. A, normal female mice. Chow, n = 19. B, mCYP7A1−/−/hCYP7A1 female mice. Chow, n = 10; male mice and only 12% of the mouse mRNA level in female mice were first bred to become heterozygous and then homozygous knock-outs of the mouse CYP7A1 gene, the level of mRNA thus suppressing expression of the human gene. Indeed, as the presence in the rat CYP7A1 gene, a binding site for LXR is absent from the homologous segment of the promoter region of the human gene (−56 to −49) (15). To determine whether this nucleotide sequence divergence might affect bile acid and cholesterol physiology, transgenic mice were generated containing the entire human gene and adjacent regulatory regions (18).

The level of the human CYP7A1 mRNA was much lower than the level of the endogenous mouse CYP7A1 mRNA. Because the size of the endogenous pool of bile acids in the mouse is severalfold greater per gram of liver than the size of this pool in man, it is possible that the mouse gene is intrinsically expressed at a higher level, creating a larger bile acid pool and thus suppressing expression of the human gene. Indeed, as the mice were first bred to become heterozygous and then homozygous knock-outs of the mouse CYP7A1 gene, the level of mRNA for human CYP7A1 rose concomitant with a decrease of the level of the mouse mRNA. In the transgenic mice, the human mRNA level was about 30% of the level of the mouse mRNA in male mice and only 12% of the mouse mRNA level in female mice. However, the levels of human CYP7A1 mRNA in the

**FIG. 6. Serum cholesterol levels in normal mice and mCYP7A1−/−/hCYP7A1 mice.** Blood was drawn from male and female mice of the FVB, 129 SvJ, and mCYP7A1−/−/hCYP7A1 mice fed a chow diet. Serum cholesterol was determined. Data are presented as mean ± S.E. Male FVB, n = 10; male SvJ, n = 11; male mCYP7A1−/−/hCYP7A1, n = 23; female FVB, n = 11; female SvJ, n = 11; female mCYP7A1−/−/hCYP7A1, n = 20. Female mice of the FVB and the SvJ strains had lower cholesterol than the male mice, p < 0.01 and 0.03, respectively. The level in female mCYP7A1−/−/hCYP7A1 mice was lower than levels in female mice of the FVP and SvJ strains. *, p < 0.001 and 0.05, respectively.

**DISCUSSION**

The nucleotide sequence divergence between the human and the mouse CYP7A1 gene in the segment from −56 to −49 of the promoter region results in significant differences in the regulation of this gene in the two species. This divergence may explain, at least in part, the basis for the differences in susceptibility to hypercholesterolemia between humans and rodents. Recent work in a number of laboratories (8, 10, 27–30) has defined an intricate and coordinated scheme for the regulation of bile acid synthesis and uptake and for cholesterol transport. Central to the regulatory process are two nuclear hormone receptors, FXR and LXR, that control the transcription of a number of genes coding for the proteins that mediate several of the synthetic and transport processes essential for maintaining cellular and whole body cholesterol homeostasis. In contrast to its presence in the rat CYP7A1 gene, a binding site for LXR is absent from the homologous segment of the promoter region of the human gene (−56 to −49) (15). To determine whether this nucleotide sequence divergence might affect bile acid and cholesterol physiology, transgenic mice were generated containing the entire human gene and adjacent regulatory regions (18).
livers of knock-out mice were severalfold higher than the level of CYP7A1 mRNA in HepG2 cells but still lower than the mouse mRNA levels. The mice studied were a combination of heterozygotes and homozygotes, and this may account for some of the variability observed in the levels of hCYP7A1 mRNA. A more likely explanation for the differences between the human and mouse CYP7A1 gene expression, however, is that endogenous sterols, such as 27-OH-cholesterol (31), induce expression of the mouse CYP7A1 gene through the LXR element to a greater degree than hepatocyte nuclear factor-1 stimulates the human gene, thus allowing greater expression of the mouse gene despite the size of its bile acid pool.

The differences in the amounts of CYP7A1 mRNA in male and female mice is consistent with previous reports (32, 33) concluding that gender-related differences in the bile acid pool size are caused by differences in CYP7A1 expression and not by differences in the alternate pathway of bile acid synthesis, because the effect of gender was not observed in CYP7A1-/- mice (23). In the mCYP7A1-/-/hCYP7A1 mice, there were no differences between male mice and female mice. This result is consistent with a report (34) showing no differences in bile acid synthesis between male and female humans. Whether this is caused directly by the difference in the DNA sequence of the LXR/hepatocyte nuclear factor-1 binding site, by another species-related difference in the promoter or enhancer DNA sequences, or by consequences of the LXR sequence difference on other parameters of cholesterol metabolism remains unknown. Nevertheless, this finding supports using these mice as tools to understand human bile acid and cholesterol metabolism.

Although the level of expression of the human gene was lower than that of the mouse gene, it was sufficient to rescue the knock-out mice from the almost complete perinatal mortality observed in the knock-outs unless the newborns and their mothers are maintained on a diet supplemented with bile acids and vitamins (23). The mCYP7A1-/-/hCYP7A1 animals, the level of human CYP7A1 mRNA was unchanged by cholesterol feeding compared with the mCYP7A1-/-/hCYP7A1 on a chow diet. A decrease in CYP7A1 mRNA with cholesterol feeding has been reported in monkeys.
CYP7A1−/− Mice Lack Induction of CYP7A1 Expression

Males

![Graph showing serum cholesterol levels in males for Chow and Western diets.](Image)

Females

![Graph showing serum cholesterol levels in females for Chow and Western diets.](Image)

FIG. 8. Effect of “Western” diet on serum cholesterol levels in normal and mCYP7A1−/−/hCYP7A1 mice. Mice were fed a diet with 25% of the calories from fat (20% saturated) with 1.5% cholesterol for 2 weeks. The serum cholesterol levels were determined before and after the diet. Data are presented as mean ± S.E. Chow normal male, n = 21; chow normal female, n = 22; chow mCYP7A1−/−/hCYP7A1 male, n = 23; high fat normal male and high fat normal female, n = 8; mCYP7A1−/−/hCYP7A1 male and female, n = 4. * p < 0.01 compared with chow; **, p < 0.05 mCYP7A1−/−/hCYP7A1 on high fat diet compared with normal mice on high fat diet.

FIG. 9. Effect of Western diet on CYP7A1 mRNA levels in normal and mCYP7A1−/−/hCYP7A1 mice. CYP7A1 levels were determined as described in the legend Fig. 3 using the livers of the mice fed the high fat diet described in Fig. 8. Data are presented as mean ± S.E. The CYP7A1 level of the mice fed the high fat diet was divided by the mean CYP7A1 level on the chow diet and multiplied by 100 to obtain percentage of the level on the control diet. Data are presented as mean ± S.E. n = 8 for normal mice, male and female (four FVB and four SVJ) and n = 4 for mCYP7A1−/−/hCYP7A1 mice, male and female. *, p < 0.05.

between CYP7A1 activity and apolipoprotein B-containing lipoprotein production. In the mCYP7A1−/−/hCYP7A1 mice, there is virtually no CYP7A1 mRNA when these mice are fed cholic acid and thus presumably a very low level of bile acid synthesis. This is in contrast to the normal mouse, which continues to express CYP7A1 mRNA at about 8–10% of the normal level, which leads to continued bile acid production even in the case of maximal expansion of the bile acid pool. Thus, the observations reported here, that there is an increase in VLDL/LDL and a decrease in HDL, lend support to that hypothesis (37) and are consistent with the finding of Pullinger et al. (38) that in two individuals who lack CYP7A1, there is statin-resistant hypercholesterolemia.

The lack of an increase in serum cholesterol with cholesterol feeding alone is of interest. The response of serum cholesterol to cholesterol feeding is highly variable and relatively small compared with the response to the total fat and saturated fat content of the diet (39). In one careful study (40), cholesterol feeding had an effect on serum cholesterol when fed with saturated fat but not with unsaturated fat. When mice were fed a diet high in saturated fat (20%) and cholesterol (1.5%), the level of CYP7A1 fell significantly in the control mice. This was unexpected because the diet contained cholesterol, which should have stimulated CYP7A1, and lacked bile acids, which would have suppressed it. Thus the basis for the down-regulation is not immediately apparent, but the effects of saturated fat feeding on the FXR-LXR system are worthy of future studies. The high fat diet did not decrease CYP7A1 levels to nearly as great an extent as did bile acid feeding in the normal mice, and the residual level should have been enough to maintain a bile acid pool size of the same or a greater magnitude as in normal humans on a per-gram-of-liver basis. This is consistent with the finding that in the normal mice, the rise in serum cholesterol was modest and resulted in a level that is the normal range for humans. In contrast, the effect of the “Western diet” on the mCYP7A1−/−/hCYP7A1 mice was more dramatic. Expression of CYP7A1 was virtually eliminated. In mCYP7A1−/−/hCYP7A1 mice, serum cholesterol, which started at about the same level as in normal mice, more than doubled, reaching potentially atherogenic levels of over 300 mg/dl.

There is, however, no direct relationship between CYP7A1 levels and serum cholesterol levels. Mice with either high or very low levels of CYP7A1 can have normal serum cholesterol levels. Alternatively, some mice with elevated serum chole-
terol levels still have significantly higher CYP7A1 levels than mice with virtually no CYP7A1. This is unlikely to be caused by changes in the alternate pathway of bile acid synthesis, because this does not seem to be regulated by either cholesterol or bile acids. Thus, the interplay among the factors that regulate several mechanisms of cholesterol homeostasis is critical in determining lipoprotein phenotype.

It has been suggested that cholic acid formation is primarily the result of synthesis through the classical pathway, whereas chenodeoxycholate in humans or the muricholates in rodents are formed by the alternate pathway of bile acid biosynthesis. The finding that the CYP7A1 knock-out mice had a reduced ratio of choles to muricholate (20% cholic acid) is consistent with this notion (23). The mice described here have a percentage of choles similar to that of normal mice, 48% in FVB and 62% in mCYP7A1

Taken together, the results of the present report suggest that there are significant differences in both the level of expression and the regulation of the human and mice CYP7A1 genes. These may be attributed, at least in part, to the lack of an LXR element in the human promoter. Lack of the LXR site could lead to lower base-line expression of CYP7A1 because of lack of stimulation by endogenous oxysterols and cholesterol. The relatively smaller bile acid pool may make humans more susceptible to gallstone formation. In addition, the lack of induction during cholesterol feeding and the exaggerated response to saturated fat feeding may render humans more susceptible to diet-induced hypercholesterolemia and thus more susceptible to atherosclerosis.

While this manuscript was in review and after our preliminary report (41), a brief report (42) was published that used a construct similar to ours to generate hCYP7A1 mice in the mCYP7A1 background. These mice also lack induction of human CYP7A1 with cholesterol feeding. There were no data on the absolute CYP7A1 levels or the effects of other dietary manipulations in that article. Together, these reports, along with our previous data (14, 15), establish firmly that lack of an element in the human CYP7A1 gene is the basis of important differences in cholesterol metabolism in humans compared with rodents.

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