Cell, Vol. 93, 693–704, May 29, 1998, Copyright ©1998 by Cell Press

Cholesterol and Bile Acid Metabolism Are Impaired in Mice Lacking the Nuclear Oxysterol Receptor LXRα

Daniel J. Peet,*†# Stephen D. Turley,‡ Wenzhen Ma,*† Bethany A. Janowski,† Jean-Marc A. Lobaccaro,*† Robert E. Hammer,*§ and David J. Mangelsdorf*†‖
*Howard Hughes Medical Institute
†Department of Pharmacology
‡Department of Internal Medicine
§Department of Biochemistry
University of Texas Southwestern Medical Center at Dallas
Dallas, Texas 75235-9050

Summary

We demonstrate that mice lacking the oxysterol receptor, LXRα, lose their ability to respond normally to dietary cholesterol and are unable to tolerate any amount of cholesterol in excess of that which they synthesize de novo. When fed diets containing cholesterol, LXRα (−/−) mice fail to induce transcription of the gene encoding cholesterol 7α-hydroxylase (Cyp7a), the rate-limiting enzyme in bile acid synthesis. This defect is associated with a rapid accumulation of large amounts of cholesterol in the liver that eventually leads to impaired hepatic function. The regulation of several other crucial lipid metabolizing genes is also altered in LXRα (−/−) mice. These results demonstrate the existence of a physiologically significant feed-forward regulatory pathway for sterol metabolism and establish the role of LXRα as the major sensor of dietary cholesterol.

Introduction

Nuclear receptors are ligand-activated transcription factors that govern aspects of every major developmental and metabolic pathway (reviewed in Kastner et al., 1995; Mangelsdorf et al., 1995). The LXRs were first identified as orphan members of the nuclear receptor superfamily whose ligands and functions are unknown (Willy and Mangelsdorf, 1998). Recently, the LXRs were shown to be activated by a specific class of naturally occurring, oxidized derivatives of cholesterol, including 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol, and 24, 25(*S*)-epoxycholesterol (Janowski et al., 1996; Lehmann et al., 1997). These oxysterols are concentrated in tissues where cholesterol metabolism and LXR expression are high, such as liver, brain, and placenta (Lavy et al., 1977; Spencer et al., 1985; Lütjohann et al., 1996). LXRs function as heterodimers with the retinoid X receptors (RXRs), and thus, the RXR/LXR complex can be activated by both RXR ligands (i.e., rexinoids) and oxysterols (Teboul et al., 1995; Willy et al., 1995; Janowski et al., 1996). Two LXR proteins (α and β) are known to exist in mammals. The expression of LXRα is restricted, with highest levels in the liver (hence, the name **l**iver **X r**eceptor) and lower but significant levels in kidney, intestine, spleen, and adrenals (Apfel et al., 1994; Willy et al., 1995). LXRβ expression is more widespread and has been found in nearly every tissue examined (Shinar et al., 1994; Song et al., 1994).

The pattern of expression of LXRs and their oxysterol ligands first suggested that these receptors may have a role in cholesterol metabolism. Cholesterol has two essential metabolic fates in mammals: conversion into steroid hormones or bile acids. Since steroid hormone synthesis is known to be governed by the orphan nuclear receptor, steroidogenic factor-1 (SF-1) (Parker and Schimmer, 1997), we have previously suggested that one plausible candidate pathway for regulation by LXRs is bile acid synthesis (Janowski et al., 1996). Consistent with this notion, we and others proposed that one likely target of LXR action is cholesterol 7α-hydroxylase (Cyp7a), the rate-limiting enzyme in the classical bile acid synthesis pathway (Janowski et al., 1996; Lehmann et al., 1997). Indeed, subsequent experiments have shown that the *Cyp7a* promoter contains a functional LXR response element that can be activated by RXR/LXR heterodimers in an oxysterol- and retinoid-dependent manner (Lehmann et al., 1997; this work; and D. J. M. and B. A. J., unpublished data). The formation of bile acids is one of two major pathways for the catabolism and excretion of cholesterol in mammals (Russell and Setchell, 1992). Perturbations in this pathway may lead to a variety of disorders, including cholesterol gallstones, atherosclerosis, and some lipid storage diseases (Akiyoshi et al., 1986; Turley and Dietschy, 1988; Carey and Duane, 1994). Together, these observations have raised the interesting possibility that LXRs may function as important transcriptional control points in bile acid metabolism.

To address further the physiological role of LXRs and their potential to regulate cholesterol catabolism, we characterized the phenotype of mice harboring targeted null mutations in each of the LXR genes. In this paper, we report that mice lacking the *Lxr*α gene lose the capacity to regulate the catabolism of dietary cholesterol in the liver. The consequence of this loss is a rapid accumulation of hepatic cholesteryl esters that eventually leads to liver failure. As predicted, LXRα null mice aberrantly regulate *Cyp7a* gene expression and have a substantially altered bile acid metabolism. In addition, several crucial regulatory proteins and enzymes involved in sterol and fatty acid synthesis are abnormally expressed, suggesting LXRα has a wide ranging effect on hepatic lipid metabolism. Taken together, our results show that LXRα functions as an essential regulatory component of cholesterol homeostasis and that no other mechanisms exist to compensate for its loss.

Results

Targeted Disruption of the *Lxr*α Gene

Figure 1A shows the strategy used to knock out the *Lxr*α gene. The mouse *Lxr*α gene includes nine exons

‖To whom correspondence should be addressed.
#Present address: Department of Biochemistry, University of Adelaide, Adelaide SA 5005, Australia.

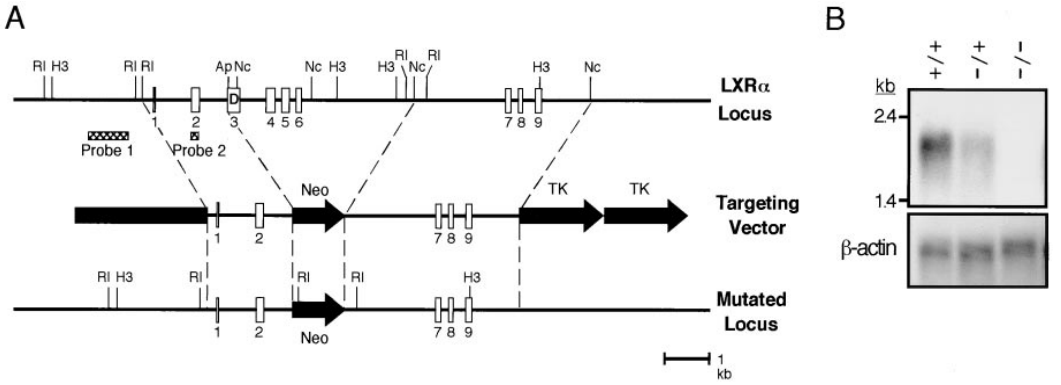

Figure 1. Targeted Disruption of the Mouse *Lxrα* Gene

(A) Targeting strategy for LXRα. A partial map of the genomic locus surrounding the *Lxrα* locus is shown. The numbered open boxes represent exons, with exon 3 containing the DNA-binding domain (designated D). Homologous recombination resulted in the replacement of exons 3–6 with the *neo* resistance gene. Restriction enzyme sites are indicated: RI, EcoRI; H3, HindIII; Ap, ApaI; Nc, NcoI. The *neo* and herpes simplex thymidine kinase (TK) genes are indicated with arrows.

(B) Northern blot of liver mRNA. Liver mRNA was hybridized using the full-length mouse *Lxrα* cDNA probe.

that encompass the translated portion of the gene. Exons 1 and 2 encode the amino-terminal portion of the receptor (A/B regions), exon 3 encodes all of the DNA-binding domain (C region), and exons 4–9 encode the hinge and ligand-binding domains (D and E regions). A targeting construct was prepared by inserting a PGK-neo cassette between an ApaI site at the beginning of exon 3 and an NcoI site in the intron between exons 6 and 7. Homologous recombination of this construct in SM-1 ES cells replaced exons 3–6 encoding the DNA-binding and ligand-binding domains of LXRα (amino acids 86–327). Two ES cell clones were chosen for generation of chimeric, heterozygous (LXRα (+/−)) and homozygous (LXRα (−/−)) mice. Mutant mice were viable and fertile, and they externally appeared normal when reared under normal laboratory conditions on standard rodent chow. Under these conditions, heterozygous and homozygous animals lived normal lifespans and bred with predicted Mendelian distributions. Analysis of liver mRNA from these mice revealed that heterozygotes retain significant levels of LXRα transcripts (∼50% of wild type), while no wild-type transcripts are detected in homozygous mice (Figure 1B). In addition, no LXRα protein was detected in homozygotes by immunoblot analysis using antibodies specific for the hinge region of LXRα (data not shown). We conclude that this disruption results in mice that are null for LXRα.

Hepatomegaly and Cholesterol Accumulation in LXRα Null Mice

Previously, we and others have shown that LXRs are activated by a specific group of monooxidized metabolites of cholesterol, including 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol, and 24,25(*S*)-epoxycholesterol (Janowski et al., 1996; Lehmann et al., 1997). The fact that these metabolites are naturally occurring and are elevated in tissues where cholesterol metabolism is high (e.g., the liver) led to the prediction that LXRs may have a role in the regulation of cholesterol metabolism. Since wild-type murine species have an extraordinary capacity to ingest large quantities of cholesterol without deleterious effects (Breslow, 1996), one hypothesis we wished to explore was whether LXRα null mice had a defect in their ability to respond to dietary cholesterol. To test this idea, 2–3 month old, gender-matched LXRα wild-type and null mice were characterized after being reared on diets containing different cholesterol concentrations for 0, 7, 30, and 90 days. Analysis of LXRα (−/−) animals fed a standard laboratory rodent chow indicated these mice appeared identical to their wild-type littermates as determined by morphological and histological studies (see below). In retrospect, the lack of a gross phenotype on this diet in the mutant mice is perhaps not surprising, since standard rodent chow contains relatively little cholesterol (≤0.02% w/w). On this diet, the cholesterol requirement of these animals is achieved primarily through de novo biosynthesis (Osono et al., 1995).

We next tested the effect of a diet rich in cholesterol (chow supplemented with 2% cholesterol). On a per diem basis, the intake of cholesterol on this diet is approximately 20–30 times above the amount an average animal synthesizes to maintain homeostasis when its dietary needs are not being met (Osono et al., 1995). In contrast to wild-type animals, which can sustain this cholesterol-rich diet indefinitely without deleterious effects, there were dramatic morphological, histological, and chemical changes in the livers of LXRα null mice fed the same diet (Figures 2 and 3). Within 7 days of beginning the 2% cholesterol diet and chronically worsening over a 90-day period, there was a prominent color and size change in the LXRα (−/−) versus wild-type livers (Figures 2A and 3A). Histological examination of these livers demonstrated the presence of a time-dependent increase in the number and size of intracellular vacuoles, characteristic of lipid deposits (Figure 2B). Oil red O staining of liver sections verified the deposition of increasing quantities of lipid by 7 and 30 days (Figure 2C). After 90 days, the morphology of the LXRα (−/−) livers had been substantially altered, and there was distinct evidence of increased hematopoietic foci, macrophage infiltration, and progressive hepatocellular degeneration (Figures 2A–2C). Chemical analysis of these

livers showed that, consistent with the distinct color change, all of the accumulated lipid is in the form of cholesterol (Figure 3B, see also Figure 5A below), 75% of which is esterified (data not shown). Remarkably, the LXRα null animals exhibited a 15- to 20-fold increase in hepatic cholesterol within just 7 days of feeding the cholesterol-rich diet (Figure 3B). Both liver mass and cholesterol concentration continued to increase with time, and by 90 days the LXRα (−/−) livers had doubled in mass (Figures 3A and 3B) and were in the advanced stages of failure (see below). Consistent with the known ability of wild-type animals to adapt quickly to cholesterol-rich diets, the livers of LXRα (+/+) animals maintained essentially normal appearance and function. In these animals, there were no significant changes in liver histology or size, and only nominal increases in hepatic cholesterol were detected. Interestingly, there was an increase in hepatic triglyceride levels in wild type, but not LXRα null mice (Figure 3C). The elevated hepatic triglyceride level seen in the wild-type mice explains the slight pigment change in the livers of these animals (Figure 2A), even though their cholesterol levels remained low.

To confirm the dietary dependence of the defect seen in LXRα (−/−) mice, a second group of wild-type and homozygous animals was analyzed after 7 and 22 days on an intermediate cholesterol diet (chow supplemented with 0.2% cholesterol). On this diet, the average animal consumes an amount of cholesterol per day that is approximately twice what it needs to maintain normal homeostasis. As shown in Figure 3D, LXRα (−/−) mice accumulated hepatic cholesterol to levels 3- and 10-fold higher than wild-type mice when fed a 0.2% cholesterol diet for 7 and 22 days, respectively. A comparison of Figures 3B and 3D shows that the defect in LXRα (−/−) mice causes a near linear accumulation of hepatic cholesterol, regardless of whether the diet contains 0.2% or 2% cholesterol. These results demonstrate that, in the absence of LXRα, mice are unable to tolerate any dietary cholesterol in excess of what they can synthesize de novo.

Similar dietary studies were performed using heterozygous mice, which express approximately half of the levels of LXRα (Figure 1B). These mice displayed an intermediate phenotype, accumulating hepatic cholesterol concentrations to levels between those observed for LXRα (+/+) and (−/−) mice (Figure 3E). These results indicate that expression levels of LXRα are crucial for a complete response.

Significantly, there were no changes in the body weights of either LXRα (−/−) or (+/+) mice, irrespective of the type or length of the diet. However, there was a noticeable decrease in the amount of white adipose tissue in LXRα (−/−) mice after 90 days on the 2% cholesterol diet, which likely compensated for the net increase in liver mass (data not shown). Morphological and histological examinations of several other tissues where LXRα is expressed, including kidney, small intestine, spleen, and adrenal, are ongoing, but preliminary results have revealed no obvious abnormalities in these tissues thus far (data not shown). To date, no gender-specific phenotypic differences have been observed in LXRα knockout mice, although female mice tend to have higher basal and diet-induced cholesterol levels.

Analysis of the plasma from the animals used in the above dietary studies confirms and extends the conclusion that LXRα null animals have a crucial defect in hepatic metabolism of dietary cholesterol (Table 1). Plasma cholesterol levels rose significantly (>200%) over the course of the 90-day, high-cholesterol diet in LXRα (−/−) mice, while wild-type mice characteristically showed no change. There was also a dramatic increase in the levels of alanine and aspartate amino transferases (26- and 7-fold, respectively, within 30 days) in the serum of the LXRα (−/−), but not wild-type animals. These enzymes are predominantly expressed in the liver, and the large increase in their levels in serum is indicative of hepatocyte injury and necrosis and is consistent with the results of the histological studies. By 90 days, the LXRα (−/−) livers deteriorated to the point where they have lost much of their normal cell structure (Figure 2C), and this change was reflected by the observed decrease (between 30 and 90 days) in the levels of amino transferases still present in the serum of these animals (Table 1). Commensurate with impaired liver function, LXRα null mice exhibited a time-dependent decrease in serum glucose to half the normal level of their wild-type littermates when fed the high-cholesterol diet. Although several other parameters in the plasma, including triglycerides, total protein, albumin, creatinine, and electrolytes appeared normal (Table 1 and data not shown), these results demonstrate that significant liver damage has occurred by 90 days and suggest that extension of this diet will ultimately result in complete liver failure.

A characteristic of murine species is their ability to regulate intermediate and low-density lipoprotein (IDL/LDL) cholesterol in serum tightly even under prolonged conditions of elevated cholesterol intake. Since the data from Table 1 revealed an increase in plasma cholesterol in the LXRα null mice, a plasma lipoprotein profile was generated from wild-type and homozygous mice fed standard or 2% cholesterol chow diets for 30 days. As predicted, wild-type animals were resistant to large increases in dietary cholesterol and maintained very low circulating levels of IDL/LDL cholesterol (Figures 4A and 4B). However, LXRα (−/−) animals produced an ~5-fold increase in IDL/LDL cholesterol and only minimal changes in HDL when compared to LXRα (+/+) animals fed the 2% cholesterol–supplemented diet (Figure 4B).

Cholesterol and Bile Acid Metabolism Defects in LXRα Null Mice

The phenotype described above provides convincing evidence that one of the roles of LXRα is to regulate dietary cholesterol metabolism in the liver. Since the primary pathway for catabolic elimination of cholesterol is through bile acid metabolism, we next examined the response of several important components in this pathway. An 8-day dietary study similar to the one described above was performed on 3-month-old male mice, comparing wild-type and LXRα (−/−) animals fed standard or 2% cholesterol–supplemented chow. The animals in this group were analyzed for rates of fecal bile acid excretion, and bile acid pool size and composition (Figure 5). Similar to the results obtained above, LXRα (−/−) animals manifest a 16-fold increase in hepatic cholesterol when fed a 2% cholesterol chow diet (Figure 5A).

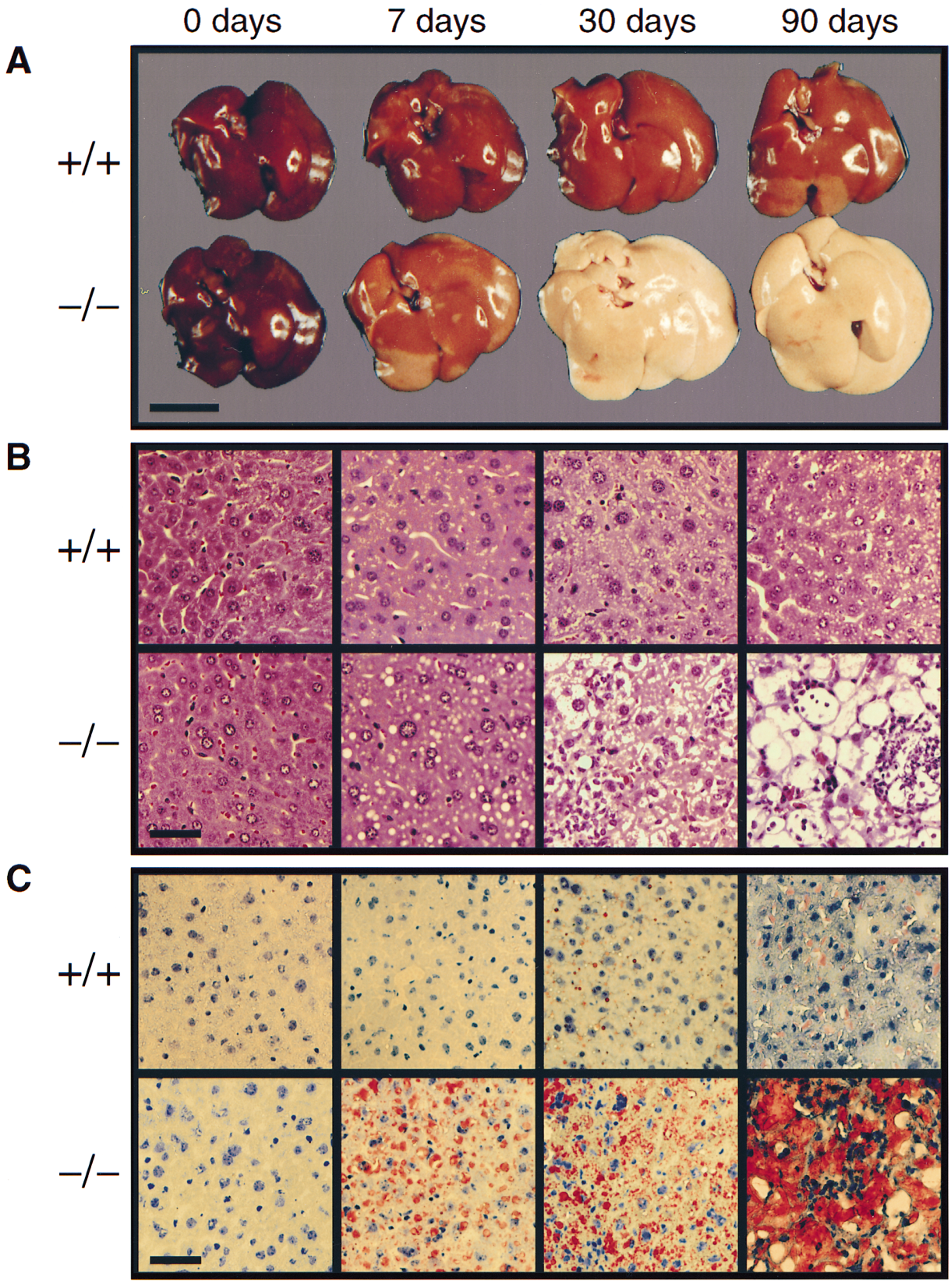

Figure 2. Morphology and Histology of Livers from LXRα Knockout versus Wild-Type Mice on High-Cholesterol Diets

(A) Gross morphology of livers from female LXRα null (−/−) and wild-type (+/+) mice fed chow supplemented with 2% cholesterol for 0, 7, 30, or 90 days. The development of fatty livers in the LXRα (−/−) mice is evident after 7 days on the high-cholesterol diet. Bar = 10 μm. Liver sections from (A) were prepared for histology and stained with hematoxylin and eosin (B) or oil red O (C). The unstained vacuoles visible in the hematoxylin- and eosin-stained sections of the LXRα (−/−) mice on the high-cholesterol diet stain positive (red color) for lipids with oil red O. Bars = 10 μm.

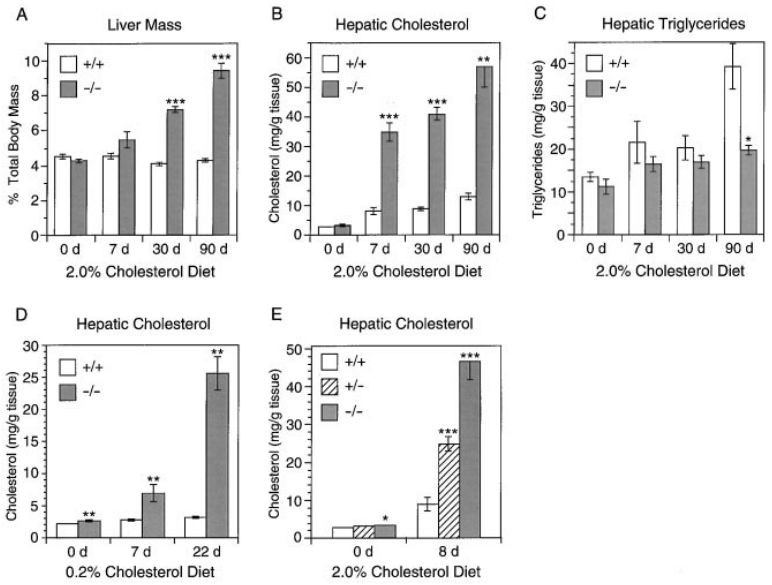

Figure 3. Liver Chemistries from LXRα Knockout versus Wild-Type Mice on High or Intermediate Cholesterol Diets

(A) Liver mass relative to total body mass of LXRα (−/−) and (+/+) female mice fed chow supplemented with 2% cholesterol for 0, 7, 30, or 90 days. Measurements of hepatic cholesterol (B) and triglycerides (C) quantitated enzymatically from extracts of the livers in (A). The animals used in this study are the same as in Figure 2. (D) Hepatic cholesterol concentrations of LXRα (−/−) and (+/+) male mice fed chow supplemented with 0.2% cholesterol for 0, 7, or 22 days. (E) Hepatic cholesterol concentrations of LXRα (+/+), (+/−), and (−/−) female mice fed chow supplemented with 2.0% cholesterol for 0 or 7 days. All values are expressed as mean ± SEM, n = 5. Significant differences compared to wild-type mice on the same diet: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Within 8 days, the concentration of hepatic cholesterol rose in LXRα null mice from its normal value of 2.46 ± 0.05 mg/g to 40.09 ± 0.82 mg/g tissue, whereas hepatic cholesterol in wild-type mice increased nominally from 2.21 ± 0.03 mg/g to 5.03 ± 0.19 mg/g tissue. Interestingly, even on the low-cholesterol diet there was a statistically significant difference ($P < 0.001$) between the hepatic cholesterol concentration in LXRα (+/+) and (−/−) mice (2.21 ± 0.03 and 2.46 ± 0.05 mg/g, respectively; Figure 5A, inset). These phenotypes were independent of gender (compare Figures 3B [female] to 5A [males]).

The ability of wild-type animals to maintain a relatively low hepatic cholesterol concentration when cholesterol intake is high is largely due to the compensatory increase in bile acid synthesis (Figures 5B and 5C). The 123% increase in fecal bile acid excretion (Figure 5B) and 36% increase in bile acid pool size (Figure 5C) were substantial and expected, based on previous studies showing that bile acid metabolism is responsible for metabolic clearance of dietary cholesterol (Carey and Duane, 1994; S. D. T., M. Schwarz, D. K. Spady, and J. M. Dietschy, unpublished data). In marked contrast, the bile acid pool size of the LXRα (−/−) mice was significantly lower than wild-type mice and did not change when the animals were fed the cholesterol-rich diet (Figure 5C). In addition, bile acid excretion in the LXRα (−/−) mice increased less than half the amount it did in wild-type animals (Figure 5B). Since ordinarily bile acid excretion reflects bile acid synthesis, these results imply that LXRα null mice have a decreased ability to upregulate bile acid synthesis.

An analysis of bile acid pool composition revealed a striking difference in this parameter as well. Over 95% of the murine biliary bile acid pool is composed of cholic and muricholic acids (Akiyoshi et al., 1986). The ratio of cholic acid to muricholic acid is tightly regulated and has a significant effect on the animal's ability to absorb cholesterol in the intestine; the higher the ratio, the more cholesterol that is potentially capable of being absorbed (Cohen et al., 1977; Uchida et al., 1980). As shown in Figure 5D, wild-type animals respond to increased dietary cholesterol by substantially lowering this ratio (from 1.34 ± 0.08 to 0.88 ± 0.05). In contrast, LXRα (−/−) mice have a significantly higher basal ratio (2.03 ± 0.23), which is completely unresponsive to increased cholesterol (2.21 ± 0.57). The above results are consistent with the notion that LXRα (−/−) mice lack a cholesterol-sensing mechanism that governs the clearance of excess cholesterol through bile acid synthesis.

Regulation of *Cyp7a* by LXRα

The rate-limiting step in the classical bile acid synthesis pathway utilizes the liver-specific enzyme cholesterol 7α-hydroxylase (Cyp7a), which converts cholesterol into 7α-hydroxycholesterol (Russell and Setchell, 1992). In

Table 1. Plasma Analysis of Wild Type and LXRα (−/−) after 2% Cholesterol Diets[a]

Test	Genotype	0 Days	7 Days	30 Days	90 Days
Cholesterol	+/+	84	82	105	82
(mg/dL, ± <3.5%)	−/−	72	127	162	145
Alanine transaminase	+/+	17	19	13	15
(U/L, ± <5.5%)	−/−	12	98	314	188
Aspartate transaminase	+/+	39	44	41	45
(U/L, ± 7%)	−/−	44	160	303	197
Glucose	+/+	231	247	200	219
(m/dL, ± <3%)	−/−	178	195	109	121
Triglycerides	+/+	52	38	39	43
(mg/dL, ± <5%)	−/−	46	35	29	31
Total protein	+/+	4.7	ND	4.4	4.4
(g/dL, ± <4%)	−/−	4.6	4.7	5.4	4.7
Albumin	+/+	1.7	1.5	1.5	1.5
(g/dL, ± <3%)	−/−	1.6	1.6	1.6	1.4

[a] All measurements performed on plasma pooled from groups of five female mice.

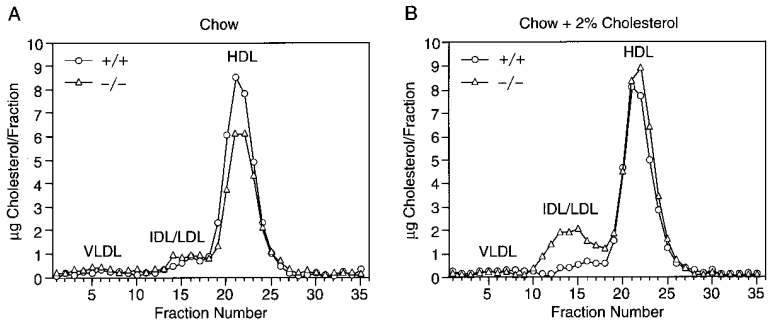

Figure 4. Plasma Lipoprotein Profiles of LXRα Knockout versus Wild-Type Mice

Plasma lipoprotein profiles of LXRα (−/−) and (+/+) female mice fed chow alone (A) or supplemented with 2% cholesterol (B) for 30 days. Fasting plasma samples from 5 mice were pooled, fractionated by FPLC, and total cholesterol in each fraction was quantitated as described in the Experimental Procedures. Total plasma cholesterol concentrations are presented in Table 1. The animals used in this study are the same as in Figure 2.

murine species, this enzyme is transcriptionally regulated by dietary cholesterol (Rudling, 1992), and we and others have shown that the *Cyp7a* promoter contains a functional LXR response element (i.e., LXRE) (Lehmann et al., 1997; see also below). In addition to Cyp7a, a second enzyme called oxysterol 7α-hydroxylase (Cyp7b) exists as part of an alternative bile acid synthesis pathway, which is not transcriptionally regulated by cholesterol (Schwarz et al., 1997). Therefore, it was of interest to characterize the response of these genes in the LXRα (−/−) mice. As expected, *Cyp7a* mRNA was up-regulated (5.9-fold) in wild-type mice fed a high-cholesterol diet for 7 days (Figure 6A). However, there was no cholesterol-induced up-regulation of Cyp7a in LXRα (−/−) mice, consistent with the notion that this gene is a direct target of LXRα action. In contrast to Cyp7a, there were no differences in expression of Cyp7b in either LXR (+/+) or (−/−) mice (Figure 6A).

In addition to normally expressing LXRα, the liver also expresses the closely related receptor, LXRβ (Figure 6B). Since both of these receptors have similar ligand specificities (Janowski et al., 1996; Lehmann et al., 1997), we wished to address why LXRβ cannot rescue the bile acid phenotype in LXRα (−/−) mice. As shown in Figure 6B, the expression of the *Lxr*α and β genes is not regulated by dietary cholesterol, indicating that the LXRs are not likely to be autoregulated in the liver. More importantly, there was no compensatory increase in the level of hepatic LXRβ in LXRα (−/−) mice. Furthermore, cotransfection studies with LXRα and β expression plasmids demonstrate that the LXRE found in the *Cyp7a* promoter is a substantially stronger response element for LXRα than it is for LXRβ (Figure 6C). These results corroborate the previous findings of Lehmann and coworkers, showing that LXRα has a substantially higher affinity than LXRβ for binding to the *Cyp7a* LXRE (Lehmann et al., 1997). Taken together with the striking phenotype of LXRα (−/−) mice, we conclude that LXRα and β can differentially regulate gene expression and, thus, may have nonoverlapping roles.

Impaired Expression of Hepatic Genes Involved in Multiple Lipid Pathways

In addition to the genes involved in bile acid metabolism, the expression of 18 other genes involved in cholesterol and fatty acid metabolism were investigated by Northern blot analysis (Figure 7). End product repression of cholesterol biosynthesis is a well-studied pathway that is controlled primarily by the transcription factor SREBP-2 (Brown and Goldstein, 1997). As expected, the liver mRNA levels of SREBP-2, as well as the cholesterogenic enzymes, hydroxymethyl glutaryl-coenzyme A (HMG-CoA) synthase and reductase, farnesyl diphosphate (FPP) synthase, and squalene synthase were down-regulated in response to cholesterol in both LXRα (+/+) and (−/−) animals (Figure 7). Surprisingly, however, there was a

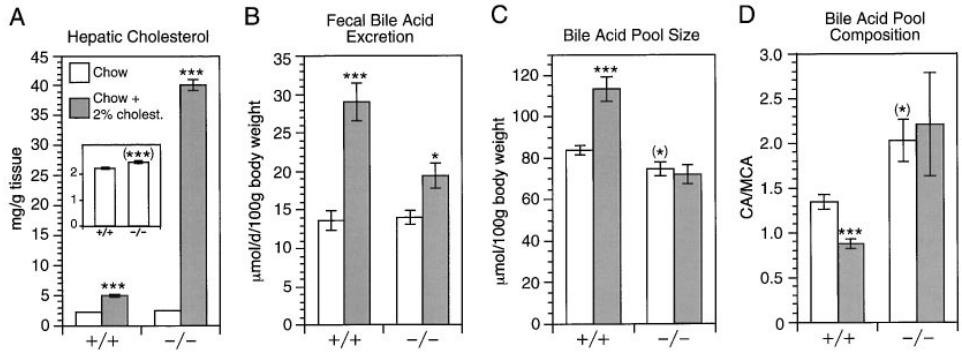

Figure 5. Bile Acid Pool Size and Excretion in LXRα Knockout versus Wild-Type Mice

(A) Hepatic cholesterol concentrations from LXRα (−/−) and (+/+) male mice fed chow alone or supplemented with 2% cholesterol for 8 days. Inset, enlargement of hepatic cholesterol concentrations of LXRα (−/−) and (+/+) mice on a chow-only diet showing a statistically significant difference (2.21 ± 0.033 versus 2.46 ± 0.050 mg/g liver, $P < 0.001$). (B) Fecal bile acid excretion was determined from extraction and analysis of stool samples collected from the mice in (A) over the final 72 hr period of the 8 day diet. (C) Bile acid pool size from gallbladder, surrounding liver, and small intestine taken from mice in (A). (D) Bile acid composition of pool in (C). CA, cholic acid; MCA, muricholic acid. All values are expressed as mean ± SEM, n = 10. Significant differences compared to same genotype on chow diet: *, $P < 0.05$; ***, $P < 0.001$. Significant differences compared to wild-type mice on chow diet: (*), $P < 0.05$; (***), $P < 0.001$.

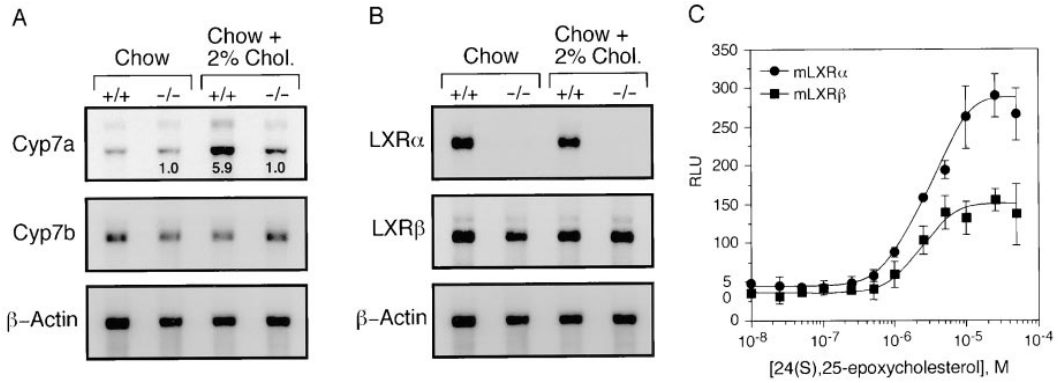

Figure 6. Transcriptional Regulation of Sterol 7α-Hydroxylases by LXRs

Northern blots of liver mRNA isolated from LXRα (−/−) and (+/+) male mice fed chow alone or chow supplemented with 2% cholesterol for 7 days, and probed for (A) cholesterol 7α-hydroxylase (Cyp7a) and oxysterol 7α-hydroxylase (Cyp7b), or (B) for LXRα and LXRβ. mRNA was isolated from five mice in each group and pooled prior to Northern blotting. Quantitation of bands relative to β-actin controls was performed as described in the Experimental Procedures. Fold changes in *Cyp7a* mRNA are compared to (+/+) mice on the chow diet and are representative of two or more independent experiments. Similar results were observed with female mice on the same diet. (C) LXRα and β differentially regulate transcription through the *Cyp7a* LXRE. Mouse Hepa1-6 cells were cotransfected with the luciferase reporter plasmid TK-CYP7A-LXREx3-LUC together with the expression plasmids for mLXRα or mLXRβ, and incubated with various concentrations of 24(*S*),25-epoxycholesterol. RLU, relative light units.

significant increase in the expression of these genes in the LXRα (−/−), but not wild-type, animals fed a low-cholesterol diet. At present, the consequence of the altered up-regulation of these genes is not fully understood and will require further study; however, the lack of a severe phenotype under low dietary cholesterol conditions suggests this misregulation may not be deleterious. Furthermore, no differences were seen in the regulation of SREBP cleavage activating protein (SCAP), sterol carrier protein, LDL receptor, HDL receptor (SR-B1), or apolipoprotein gene expression in the LXRα null mice (Figure 7).

In contrast to the up-regulation of genes involved in cholesterol biosynthesis, three genes involved in fatty acid metabolism, SREBP-1, stearoyl CoA desaturase-1, and fatty acid synthase, were markedly down-regulated in LXRα (−/−) mice on the low-cholesterol diet (Figure 7). Again, the consequence of the altered expression of these genes on this diet does not appear to be deleterious, but further study will be required to determine if there are other metabolic defects in these mice under other dietary conditions (e.g., low or high fatty acid–supplemented diets). The expression of several other genes, including the three peroxisome proliferator activated receptors (PPARs) and acetyl CoA carboxylase were unchanged in the mutant versus wild-type mice.

Discussion

Model of the Regulation of Cholesterol Homeostasis

In this paper, the in vivo role of LXRα was investigated by generating knockout mice. The results from this investigation support a model in which LXRα plays a crucial role in regulating the catabolism of dietary cholesterol in the liver (Figure 8). It is well established that cholesterol homeostasis is maintained in normal animals through two regulated pathways: de novo synthesis and catabolism. Biosynthesis is governed by the accumulation of sterols in the liver, which feed back and inhibit further synthesis through a well-documented pathway involving the transcription factors SREBP-1 and SREBP-2 (Brown and Goldstein, 1997). When cholesterol is included in the diet at levels surpassing the biosynthetic rate, a feed-forward pathway is activated that increases liver cholesterol catabolism and bile acid mobilization (Russell and Setchell, 1992). The ability to greatly upregulate this feed-forward pathway explains why mice fed excessive amounts of cholesterol are able to maintain relatively normal lipid physiology. However, the mechanism by which this pathway is regulated has remained unknown. Our results demonstrate that LXRα is a key component of this regulation. By binding to the oxysterols that exist in liver when there is a surplus of cholesterol (Janowski et al., 1996; Lehmann et al., 1997), we propose that LXRα acts as a cholesterol sensor and upregulates expression of various components of cholesterol catabolism. In keeping with this model, we have shown that the absence of LXRα results in a block in the catabolic pathway, which in turn leads to the rapid accumulation of hepatic cholesterol. As the sensor, LXRα must be exquisitely responsive, since even a slight increase in dietary cholesterol results in a pronounced accumulation of hepatic sterols in LXRα (−/−) animals (Figure 3D). Furthermore, we have shown that a major mechanism by which the presence of LXRα keeps the level of dietary cholesterol in check is by positively regulating transcription of the rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase (Cyp7a). This regulatory paradigm is the antithesis of the feed-back control mechanism that transcriptionally represses the rate-limiting enzyme in cholesterol synthesis (Brown and Goldstein, 1997) and provides an elegant symmetry to the regulation of cholesterol homeostasis (Figure 8).

LXRα Regulation of Bile Acid Metabolism

In support of the notion that a primary target of LXRα regulation is bile acid metabolism, we have demonstrated that LXRα knockout animals have marked differences in bile acid excretion, pool size, and composition.

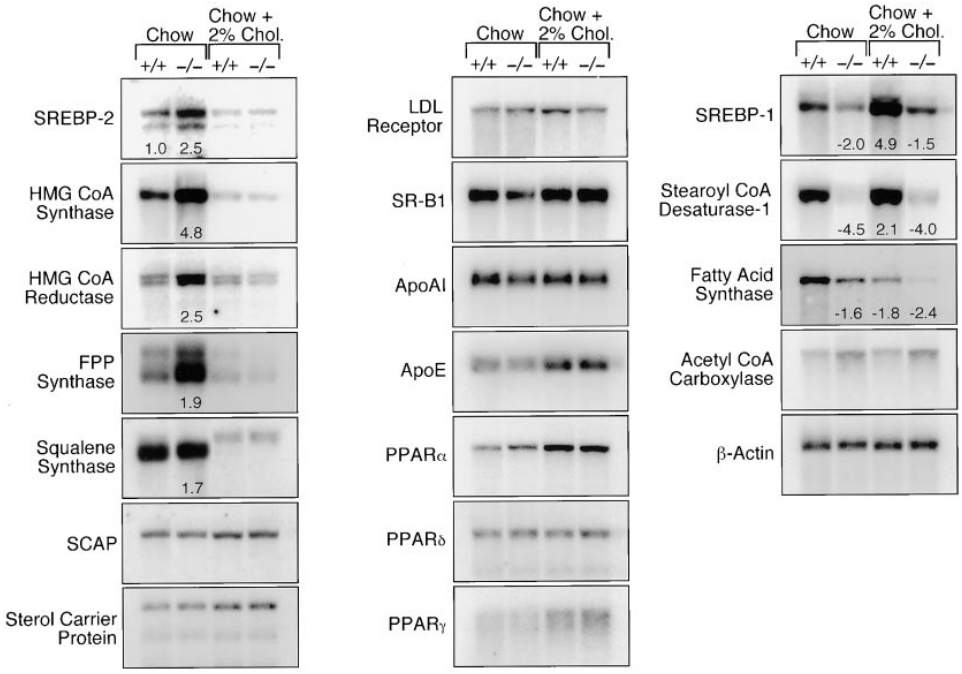

Figure 7. Northern Blot Analysis of Various Lipid Regulating Genes in LXRα Knockout versus Wild-Type Mice

Northern blots of liver mRNA isolated from LXRα (−/−) and (+/+) male mice fed chow alone or supplemented with 2% cholesterol for 7 days. The source of mRNA was the same as in Figure 6. Quantitation of bands relative to β-actin controls was performed as described in the Experimental Procedures. Fold changes in mRNA are compared to (+/+) mice on the chow diet and are representative of two or more independent experiments. Similar results were observed with female mice on the same diet.

Of particular interest are the differences in bile acid pool composition, since this parameter not only reflects cholesterol catabolism, but also has an effect on cholesterol absorption from the diet (Cohen et al., 1977; Uchida et al., 1980). The substantial increase in the ratio of cholic acid to muricholic acid seen in the LXRα (−/−) mice suggests that these animals may potentially absorb more dietary cholesterol. Thus, in addition to regulating cholesterol catabolism and excretion, LXRα may also govern cholesterol uptake. It is also of interest that LXRα (−/−) mice maintain a statistically higher basal concentration of hepatic cholesterol, even when fed a low (0.02%) cholesterol diet (Figures 3B and 5A, inset).

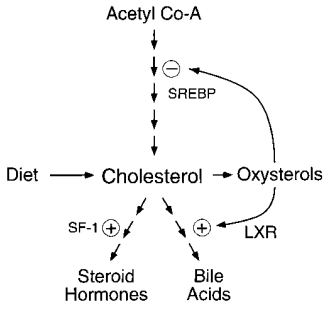

Figure 8. Model for the Regulation of Cholesterol Homeostasis

Cholesterol metabolism is governed by two mechanisms: feed-back repression of biosynthesis through the inhibition of the transcription factor SREBP and feed-forward induction of catabolism by ligand-dependent activation of the oxysterol receptor LXRα. See text for details.

Significantly, although the hepatic cholesterol level is higher than normal, it does not increase further as long as these animals maintain their dietary cholesterol intake below the maximum amount they are able to synthesize. However, once the dietary intake exceeds the biosynthetic rate, hepatic cholesterol accumulation increases in an almost linear fashion (Figures 3B and 3D). Taken together, these phenotypes demonstrate that the up-regulation of Cyp7a enzyme activity is crucial for the metabolic clearance of dietary cholesterol, but additional LXRα targets may also exist. Consistent with this notion, in ongoing experiments we have shown that even when all Cyp7a activity is inhibited in wild-type animals by feeding a high-cholesterol diet artificially supplemented with cholic acid (Jelinek et al., 1990; Shefer et al., 1991, Schwarz et al., 1997), these animals exhibit a similar but not identical phenotype to that seen in LXRα null mice fed the same diet without cholic acid.

LXRα Regulation of Sterol and Fatty Acid Metabolism

The transcriptional regulation of several genes involved in both sterol and fatty acid synthesis were also shown to be altered in LXRα (−/−) mice (Figure 7). Under conditions of low dietary cholesterol, the levels of all the major cholesterogenic enzyme mRNAs were increased, including that for the rate-limiting enzyme HMG CoA reductase. Concordant with these increases, mRNA levels of SREBP-2, a known transcriptional regulator of these genes, were also elevated. Although previous studies have shown that a clear relationship exists between the

up-regulation of these genes and cholesterol synthesis in normal animals (Brown and Goldstein, 1997), a number of the products of these genes are also known to be posttranslationally regulated. In ongoing experiments with LXRα null animals, we have noted that the increased expression of these enzymes does not result in the predicted increase in hepatic cholesterol synthesis. Although preliminary, these results suggest the existence of a separate regulatory pathway for these enzymes that uncouples them from cholesterol biosynthesis. Future studies will be directed at understanding the consequence of this aberrant up-regulation, including whether or not an alternate lipid biosynthetic pathway is involved, perhaps generating an LXRα ligand. On this point, we note that one of the highest affinity ligands for LXRα is the liver-specific sterol 24(*S*),25-epoxycholesterol (Figure 6; B. A. J., S. A. Jones, S. A. Kliewer, and D. J. M., unpublished data), which is believed to be synthesized directly from squalene in a shunt pathway that bypasses the last several steps in the synthesis of cholesterol (Spencer, 1994).

In contrast to cholesterogenic genes, mRNA for genes involved in fatty acid synthesis were down-regulated in LXRα knockout mice. The most dramatic decrease was seen with stearoyl CoA desaturase-1, the enzyme required for synthesis of Δ9-unsaturated fatty acids that are essential constituents of all membrane phospholipids and triglycerides. These enzymes are known to be transcriptionally regulated by SREBP-1 (also called ADD1), which was originally characterized for its effects on adipocyte differentiation (Tontonoz et al., 1993) and is believed to have more of a role in fatty acid metabolism. In LXRα (−/−) mice, the levels of both the mRNA (Figure 7) and active nuclear form (data not shown) of SREBP-1 are down-regulated, implying that LXRα may govern synthesis of essential fatty acid components by directly controlling SREBP-1 expression. This phenotype is consistent with that of transgenic mice expressing a constitutively active form of SREBP-1, in which fatty acid synthesizing enzymes are dramatically elevated, resulting in a fatty liver phenotype (Shimano et al., 1996). Dietary studies varying the fat intake of these animals are under way to understand further the role of LXRα in this pathway.

The loss of LXRα leads to one other noteworthy phenotype: an increase in plasma LDL cholesterol. This phenotype is rather unique, since it is well known that mice are highly resistant to any changes in circulating LDL unless mutations are introduced that alter expression of apolipoproteins or the LDL receptor (Breslow, 1996). We observed no statistically relevant LXRα-dependent changes in the mRNA levels of apolipoproteins A and E (Figure 7), apolipoprotein B (data not shown), LDL receptor, or HDL receptor, yet there was a significant increase in LDL cholesterol plasma levels after feeding a cholesterol-rich diet. These findings suggest the LXRα null mouse may be a useful animal model for the further study of cholesterol homeostatic diseases such as atherosclerosis.

A Family of Oxysterol Receptors

In addition to bile acid synthesis, cholesterol also serves as a precursor in steroid hormone synthesis. As might be predicted, these two metabolic pathways have similar intermediary enzymatic steps that involve hydroxylation of the side chain and ring structures of cholesterol. Likewise, it is perhaps not surprising to learn that both pathways are transcriptionally regulated by nuclear receptors (Figure 8). Similar to LXRα's regulation of bile acid metabolism, the orphan nuclear receptor steroidogenic factor-1 (SF-1) has been shown to regulate several steps in the steroidogenic pathway, including expression of the rate-limiting cholesterol side chain cleavage enzyme, Cyp11a (Parker and Schimmer, 1997). The knockout of the gene encoding SF-1 results in complete adrenal and gonadal agenesis, confirming the role of this nuclear receptor in steroidogenesis (Luo et al., 1994). Moreover, SF-1 has also recently been shown to be activated by oxysterols (Lala et al., 1997). Interestingly, the SF-1 activators exhibit a different rank order of specificity than LXR ligands (Lala et al., 1997). These results suggest that, like the receptors for steroids, retinoids, and prostanoids, there exists a family of receptors for oxysterols whose function is to regulate important metabolic pathways. At least one other oxysterol receptor, LXRβ, is already known to exist, and the elucidation of its biological role is a future goal.

Conclusions

In conclusion, the characterization of the LXRα knockout mouse provides unequivocal evidence that LXRα subserves a key role in cholesterol homeostasis. The finding that LXRα is a crucial checkpoint in this process may have broad implications for its potential use as a target in human disease. For example, genetic screening for LXRα mutations may prove useful in identifying patient cohorts with inherited defects in cholesterol metabolism (especially of dietary origin). Likewise, the availability of the LXRα knockout mouse as an animal model and the future development of nonsteroidal LXRα agonists should provide useful tools for exploring the utility of LXRα as a pharmaceutical target.

Experimental Procedures

Construction of the LXRα Targeting Vector

Mouse LXRα genomic clones were obtained by screening a 129Sv λ genomic library using the 1.4 kb full-length cDNA of human LXRα (Willy et al., 1995). The targeting vector was constructed using a neo-TK template plasmid containing a neomycin and two tandem herpes simplex virus thymidine kinase cassettes (Rosahl et al., 1993). This vector was designed to replace exons 3–6 by homologous recombination, which contain the complete DNA-binding domain and majority of the ligand-binding domain of LXRα (amino acids 86–327). A short arm was generated by blunt end inserting the 1.9 kb EcoRI-ApaI fragment containing exons 1 and 2 into the unique BamHI site 5′ of the neo cassette, and the longer arm by blunt end inserting the 4.0 kb NcoI fragment containing exons 7–9 into the unique XhoI site located between the neo and TK cassettes (Figure 1). The targeting vector was linearized at a unique SalI site prior to electroporation.

Generation of LXRα Null Mice

Ablation of the mouse *Lxrα* gene locus was accomplished according to previously described methods (Ishibashi et al., 1993; Rosahl et al., 1993). SM-1 mouse ES cells were grown on irradiated STO feeder cells. Approximately 5×10^7 cells were electroporated with 1 mg/ml of linearized targeting vector and subjected to selection with G418 and FIAU. Resistant clones were analyzed by Southern blotting after

HindIII digestion, using probes 1 and 2 indicated in Figure 1A. Two positive clones were expanded and injected into C57BL/6 blastocysts to produce five male chimeric mice, which transmitted the mutant gene through the germline. The mice were genotyped by Southern blotting of tail DNA after EcoRI digestion. Subsequent experimental procedures were performed on the C57BL/6–129Sv mixed strain descendants (F2 and subsequent generations) and repeated with the second clone, with the exception of the 90-day 2% cholesterol diet. The mice were maintained on a 12 hr light/12 hr dark cycle and fed ad libitum a standard, cereal-based mouse/rat diet (No. 7001, Harlan Teklad) containing 4% (w/w) fat and approximately 0.02% (w/w) cholesterol. Where indicated, this diet was supplemented with 0.2% or 2% (w/w) cholesterol (ICN). The mice were euthanized after a 5 hr fast during the early phase of the light cycle unless otherwise indicated. All mouse procedures used in this study were performed in accordance with the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center at Dallas.

Northern Blotting

Total RNA was extracted using RNA STAT-60 (Tel-Test, Inc.), and mRNA isolated using oligo(dT)-cellulose columns (Pharmacia Biotech). Equivalent amounts of mRNA from 4–6 mice were pooled, and 4–10 μg subjected to Northern blot analysis as described (Lehrman et al., 1987). Mouse cDNA probes for *Lxr*α and *Lxr*β (Lehmann et al., 1997); cholesterol 7α-hydroxylase (Ishibashi et al., 1996); oxysterol 7α-hydroxylase (Schwarz et al., 1997); *PPAR*α, *PPAR*δ, *PPAR*γ (Kliewer et al., 1994); and 15 other lipid regulatory genes (Shimano et al., 1997) were prepared as described. *Sr-B1* cDNA probe was a gift from Dr. Helen Hobbs. Probes for SCAP and sterol carrier protein were prepared by RT-PCR with mouse liver poly(A)$^+$ RNA as described previously for other probes (Shimano et al., 1996). PCR primers used were: SCAP, 5′-TTAAGCTTTGTCCCGGGCATTCCAA CTGG-3′ and 5′-TTGAATTCGACTTGGTGAGCACCAACACAT-3′; and sterol carrier protein, 5′-AAGAAGCTTGAAGAGGAAGGGGAA-3′ and 5′-AGCTTAGCTTTGTCCGGCTGAAG-3′ (Mori et al., 1991). Northerns were quantitated using a phosphorimager (Molecular Dynamics) and standardized against β-actin controls.

Histological Procedures

Liver, kidney, spleen, adrenal, small intestine, brain, testis, ovary, skin, muscle, white and brown adipose tissue, heart, and lung tissue were fixed in formalin (Accustain; Sigma), embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin by a standard procedure. Frozen tissue sections were stained with oil red O using standard procedures. Sections were examined under bright-field microscopy with an Olympus model BX50 photomicroscope.

Plasma and Tissue Chemical Analyses

Mice were anaesthetized with sodium pentobarbital and exsanguinated via the ascending vena cava. Blood was transferred to tubes containing EDTA (Microcuvette CB1000 capillary tubes; Sarstedt, Inc.), and the plasma isolated by centrifugation. Pooled plasma (from five mice) was analyzed for cholesterol, triglyceride, and lipoproteins as previously described (Ishibashi et al., 1993). Whole livers were removed, weighed, and sections taken (0.2 g) for lipid analysis. The lipids were extracted and analyzed for triglycerides and cholesterol as previously described (Bucolo and David, 1973; Yokode et al., 1990). Plasma analysis of glucose, aspartate aminotransferase, alanine aminotransferase, cholesterol, triglycerides, albumin, and total protein levels was performed using a Paramax RX automated analyzer (Dade International).

Bile Acid Analyses

In studies involving the measurement of bile acid pool size, composition, and excretion, male mice were housed individually in plastic cages containing wood shavings and were fed their respective diets ad libitum. Stools were collected from each animal over the 72 hr immediately prior to study, dried, weighed, and ground in a mechanical blender. Aliquots were taken for the measurement of total bile acid content by an enzymatic method as described (Turley et al., 1997). The daily stool output (g/day per 100 g body weight) and fecal bile acid content (μmol/g) were used to calculate the rate of bile acid excretion (μmol/day per 100 g body weight). After the mice were weighed, anesthetized, and exsanguinated, the entire small intestine and its contents, along with the bulk of the liver and the gallbladder, were combined and extracted in ethanol with [24-^{14}C] taurocholic acid (New England Nuclear) added to correct for procedural losses. The total bile acid content of the extract was quantitated by HPLC (M. Schwarz, D. W. Russell, J. M. Dietschy, and S. D. T., unpublished data). These data, together with the respective value for recovery of the internal standard, were used to calculate bile acid pool size (μmol/100 g body weight) and composition. Irrespective of genotype or diet, taurocholic acid and tauromuricholic acid accounted for more than 95% of the bile acids in the pool in all cases. No attempt was made to resolve what fraction of the muricholic acid peak corresponded to β, α, and ω forms of this bile acid.

Cell Culture and Cotransfection Assays

Transient transfections in Hepa1-6 mouse liver cells were performed as described (Willy et al., 1995). Cells were maintained at 5% CO_2 in DMEM supplemented with 10% fetal bovine serum. Receptor expression plasmids encoding mouse LXRα or LXRβ in CMX vectors were cotransfected with a luciferase reporter plasmid (TK-CYP7A-LXREx3-LUC) containing three tandem copies of the DR-4 sequence (gcttTGGTCActcaAGTTCAagtta) from the rat *Cyp7a* gene (Chiang and Stroup, 1994). 24(*S*),25-epoxycholesterol was dissolved in ethanol and added to cells after transfection in medium containing 5% cabosil-treated newborn calf serum. All transfections were normalized to a β-galactosidase internal standard as a control. Data are presented as mean relative light units (RLUs) from triplicate assays ± SEM.

Statistical Analyses

Values are expressed as mean ± standard error of the mean (SEM). The significance of differences between mean values were evaluated using the unpaired Student's *t*-test. Sample groups showing heterogeneity of variance were appropriately transformed before analysis.

Acknowledgments

We thank Drs. Patricia Willy for antibodies, and Yasushi Kisanuki, Joyce Repa, Margrit Schwarz, and Mala Mahendroo for animal studies; Dr. E. J. Corey for 24(*S*), 25-epoxycholesterol; Drs. David Russell, Michael Brown, Joseph Goldstein, Helen Hobbs for Northern probes, technical resources, and critical review; Drs. John Dietschy, Jay Horton, Hitoshi Shimano, and James Richardson for critical comments. We thank Dr. David Spady for providing methodology for bile acid pool size analysis, and Dr. Iichiro Shimomura and Bobby Korn for mouse SCAP cDNA. We thank Jeffrey Graven, Brian Jefferson, Elizabeth Moore, Stephen Osterman, Scott Clark, and Heather Lawrence for expert technical assistance. D. J. M. is an Associate Investigator, and D. J. P. and J.-M. L. are Associates of the Howard Hughes Medical Institute (HHMI). This work was funded by HHMI and grants from the Robert A. Welch Foundation, US Public Health Service (HL09610), and Moss Heart Fund.

Received March 20, 1998; revised April 27, 1998.

References

Akiyoshi, T., Uchida, K., Takase, H., Nomura, Y., and Takeuchi, N. (1986). Cholesterol gallstones in alloxan-diabetic mice. J. Lipid Res. *27*, 915–924.

Apfel, R., Benbrook, D., Lernhardt, E., Ortiz, M.A., Salbert, G., and Pfahl, M. (1994). A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. Mol. Cell. Biol. *14*, 7025–7035.

Breslow, J.L. (1996). Mouse models of atherosclerosis. Science *272*, 685–688.

Brown, M.S., and Goldstein, J.L. (1997). The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell *89*, 331–340.

Bucolo, G., and David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. *19*, 476–482.

Carey, M.C., and Duane, W.C. (1994). Enterohepatic circulation. In The Liver: Biology and Pathobiology, I.M. Arias, J.L. Boyer, N. Fausto, W.B. Jakoby, D. Schachter, and D.A. Shafritz, eds. (New York: Raven Press Ltd.), pp. 719–767.

Chiang, J.Y.L., and Stroup, D. (1994). Identification and characterization of a putative bile acid-responsive element in cholesterol 7α-hydroxylase gene promoter. J. Biol. Chem. *269*, 17502–17507.

Cohen, B.I., Raicht, R.F., and Mosbach, E.H. (1977). Sterol metabolism studies in the rat. Effect of primary bile acids (sodium taurochenodeoxycholate and sodium taurocholate) on sterol metabolism. J. Lipid Res. *18*, 223–231.

Ishibashi, S., Brown, M.S., Goldstein, J.L., Gerrard, R.D., Hammer, R.E., and Herz, J. (1993). Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J. Clin. Invest. *92*, 883–893.

Ishibashi, S., Schwarz, M., Frykman, P.K., Herz, J., and Russell, D.W. (1996). Disruption of cholesterol 7α-hydroxylase gene in mice. I. Postnatal lethality reversed by bile acid and vitamin supplementation. J. Biol. Chem. *271*, 18017–18023.

Janowski, B.A., Willy, P.J., Rama-Devi, T., Falck, J.R., and Mangelsdorf, D.J. (1996). An oxysterol signaling pathway mediated by the nuclear receptor LXRα. Nature *383*, 728–731.

Jelinek, D.F., Andersson, S., Slaughter, C.A., and Russell, D.W. (1990). Cloning and regulation of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis. J. Biol. Chem. *265*, 8190–8197.

Kastner, P., Mark, M., and Chambon, P. (1995). Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell *83*, 859–869.

Kliewer, S.A., Forman, B.M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K., and Evans, R.M. (1994). Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc. Natl. Acad. Sci. USA *91*, 7355–7359.

Lala, D.S., Syka, P.M., Lazarchik, S.B., Mangelsdorf, D.J., Parker, K.L., and Heyman, R.A. (1997). Activation of the orphan nuclear receptor steroidogenic factor 1 by oxysterols. Proc. Natl. Acad. Sci. USA *94*, 4895–4900.

Lavy, U., Burstein, S., Gut, M., and Javitt, N.B. (1977). Bile acid synthesis in man. II. Determination of 7α-hydroxycholesterol, (22R)-22-hydroxycholesterol, and 26-hydroxycholesterol in human meconium. J. Lipid Res. *18*, 232–238.

Lehmann, J.M., Kliewer, S.A., Moore, L.B., Smith-Oliver, T.A., Oliver, B.B., Su, J.L., Sundseth, S.S., Winegar, D.A., Blanchard, D.E., Spencer, T.A., and Willson, T.M. (1997). Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J. Biol. Chem. *272*, 3137–3140.

Lehrman, M.A., Russell, D.W., Goldstein, J.L., and Brown, M.S. (1987). Alu-Alu recombination deletes splice acceptor sites and produces secreted low density lipoprotein receptor in a subject with familial hypercholesterolemia. J. Biol. Chem. *262*, 3354–3361.

Luo, X., Ikeda, Y., and Parker, K.L. (1994). A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell *77*, 481–490.

Lütjohann, D., Breuer, O., Ahlborg, G., Nennesmo, I., Sidén, Å., Diczfalusy, U., and Björkhem, I. (1996). Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. Proc. Natl. Acad. Sci. USA *93*, 9799–9804.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R.M. (1995). The nuclear receptor superfamily: the second decade. Cell *83*, 835–839.

Mori, T., Tsukamoto, T., Mori, H., Tashiro, Y., and Fujiki, Y.. (1991). Molecular cloning and deduced amino acid sequence of nonspecific lipid transfer protein (sterol carrier protein 2) of rat liver: a higher molecular mass (60kDa) protein contains the primary sequence of nonspecific lipid transfer protein as its C-terminal part. Proc. Natl. Acad. Sci. USA *88*, 4338–4342.

Osono, Y., Woollett, L.A., Herz, J., and Dietscht, J.M. (1995). Role of low density lipoprotein receptor in the flux of cholesterol through the plasma and across the tissues of the mouse. J. Clin. Invest. *95*, 1124–1132.

Parker, K.L., and Schimmer, B.P. (1997). Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr. Rev. *18*, 361–377.

Rosahl, T.W., Geppert, M., Spillane, D., Herz, J., Hammer, R.E., Malenka, R.C., and Südhof, T.C. (1993). Short-term synaptic plasticity is altered in mice lacking synapsin I. Cell *75*, 661–670.

Rudling, M. (1992). Hepatic mRNA levels for the LDL receptor and HMG-CoA reductase show coordinate regulation in vivo. J. Lipid Res. *33*, 493–501.

Russell, D.W., and Setchell, K.D.R. (1992). Bile acid biosynthesis. Biochemistry *31*, 4737–4749.

Schwarz, M., Lund, E.G., Lathe, R., Björkhem, I., and Russell, D.W. (1997). Identification and characterization of a mouse oxysterol 7α-hydroxylase cDNA. J. Biol. Chem. *272*, 23995–24001.

Shefer, S., Nguyen, L.B., Salen, G., Ness, G.C., Tint, G.S., Batta, A.K., Hauser, S., and Rani, I. (1991). Regulation of cholesterol 7α-hydroxylase by hepatic 7α-hydroxylated bile acid flux and newly synthesized cholesterol supply. J. Biol. Chem. *266*, 2693–2696.

Shimano, H., Horton, J.D., Hammer, R.E., Shimomura, I., Brown, M.S., and Goldstein, J.L. (1996). Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J. Clin. Invest. *98*, 1575–1584.

Shimano, H., Shimomura, I., Hammer, R.E., Herz, J., Goldstein, J.L., Brown, M.S., and Horton, J.D. (1997). Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the *SREBP-1* gene. J. Clin. Invest. *100*, 2115–2124.

Shinar, D.M., Endo, N., Rutledge, S.J., Vogel, R., Rodan, G.A., and Schmidt, A. (1994). *NER*, a new member of the gene family encoding the human steroid hormone nuclear receptor. Gene *147*, 273–276.

Song, C., Kokontis, J.M., Hiipakka, R.A., and Liao, S. (1994). Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. Proc. Natl. Acad. Sci. USA *91*, 10809–10813.

Spencer, T.A. (1994). The squalene dioxide pathway of steroid biosynthesis. Acc. Chem. Res. *27*, 83–90.

Spencer, T.A., Gayen, A.K., Phirwa, S., Nelson, J.A., Taylor, F.R., Kandutsch, A.A., and Erickson, S.K. (1985). 24(S),25-Epoxycholesterol: evidence consistent with a role in the regulation of hepatic carcinogenesis. J. Biol. Chem. *260*, 13391–13394.

Teboul, M., Enmark, E., Li, Q., Wikström, A.C., Pelto-Huikko, M., and Gustafsson, J.-Å. (1995). OR-1, a member of the nuclear receptor superfamily that interacts with the 9-*cis*-retinoic acid receptor. Proc. Natl. Acad. Sci. USA *92*, 2096–2100.

Tontonoz, P., Kim, J.B., Graves, R.A., and Spiegelman, B.M. (1993). ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. Mol. Cell. Biol. *13*, 4753–4759.

Turley, S.D., and Dietschy, J.M. (1988). The metabolism and excretion of cholesterol by the liver. In The Liver: Biology and Pathobiology, I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz, eds. (New York: Raven Press, Ltd.), pp. 617–641.

Turley, S.D., Spady, D.K., and Dietschy, J.M. (1997). Regulation of fecal bile acid excretion in male golden Syrian hamsters fed a cereal-based diet with and without added cholesterol. Hepatology *25*, 797–803.

Uchida, K., Nomura, Y., and Takeuchi, N. (1980). Effects of cholic acid, chenodeoxycholic acid and their related bile acids on cholesterol, phospholipid and bile acid levels in serum, liver, bile and feces of rats. J. Biochem. (Tokyo) *87*, 187–194.

Willy, P.J., and Mangelsdorf, D.J. (1998). Nuclear orphan receptors: the search for novel ligands and signaling pathways. In Hormones

and Signaling, Volume 1, B.W. O'Malley, ed. (San Diego: Academic Press), pp. 307–358.

Willy, P.J., Umesono, K., Ong, E.S., Evans, R.M., Heyman, R.A., and Mangelsdorf, D.J. (1995). LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev. *9*, 1033–1045.

Yokode, M., Hammer, R.E., Ishibashi, S., Brown, M.S., and Goldstein, J.L. (1990). Diet-induced hypercholesterolemia in mice: prevention by overexpression of LDL receptors. Science *250*, 1273–1275.