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Published in:
The Journal of Biological Chemistry

DOI:
10.1074/jbc.M206522200

Publication date:
2002

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 37, Issue of September 13, pp. 33870–33877, 2002

Printed in U.S.A.

Increased Hepatobiliary and Fecal Cholesterol Excretion upon Activation of the Liver X Receptor Is Independent of ABCA1*

Received for publication, July 1, 2002, and in revised form, July 5, 2002
Published, JBC Papers in Press, July 8, 2002, DOI 10.1074/jbc.M206522200

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The ATP-binding cassette transporter ABCA1 is essential for high density lipoprotein (HDL) formation and considered rate-controlling for reverse cholesterol transport. Expression of the *Abca1* gene is under control of the liver X receptor (LXR). We have evaluated effects of LXR activation by the synthetic agonist T0901317 on hepatic and intestinal cholesterol metabolism in C57BL/6J and DBA/1 wild-type mice and in ABCA1-deficient DBA/1 mice. In wild-type mice, T0901317 increased expression of *Abca1* in liver and intestine, which was associated with a ~60% rise in HDL. Biliary cholesterol excretion rose 2.7-fold upon treatment, and fecal neutral sterol output was increased by 150–300%. Plasma cholesterol levels also increased in treated *Abca1*$^{-/-}$ mice (+120%), but exclusively in very low density lipoprotein-sized fractions. Despite the absence of HDL, hepatobiliary cholesterol output was stimulated upon LXR activation in *Abca1*$^{-/-}$ mice, leading to a 250% increase in the biliary cholesterol/phospholipid ratio. Most importantly, fecal neutral sterol loss was induced to a similar extent (+300%) by the LXR agonist in DBA/1 wild-type and *Abca1*$^{-/-}$ mice. Expression of *Abcg5* and *Abcg8*, recently implicated in biliary excretion of cholesterol and its intestinal absorption, was induced in T0901317-treated mice. Thus, activation of LXR in mice leads to enhanced hepatobiliary cholesterol secretion and fecal neutral sterol loss independent of (ABCA1-mediated) elevation of HDL and the presence of ABCA1 in liver and intestine.

Reverse cholesterol transport (RCT)[1] or centripetal cholesterol flux is a key process in maintenance of whole body cholesterol homeostasis (1–6). RCT involves efflux of excess cholesterol from peripheral cells toward nascent high density lipoprotein (HDL) and its transport to the liver, followed by hepatic uptake mediated by scavenger receptor class B type I (SR-BI), biliary secretion in the form of cholesterol or bile salt, and finally disposal into feces. HDL-mediated RCT is generally assumed to underlie the well known epidemiological relationship between high HDL cholesterol levels and low risk for development of atherosclerosis.

Efflux of cholesterol from peripheral cells, including macrophages in the vessel wall, is now known to be mediated in part by the ATP-binding cassette transporter ABCA1 (7–10). *Abca1* mRNA is widely distributed throughout the body, with high expression levels in macrophages, hepatocytes, and enterocytes (11, 12). This distribution pattern has recently been confirmed for the ABCA1 protein (13). The role of ABCA1 in hepatocytes is currently unknown, but may involve formation of pre-β-HDL particles (14). In the intestine, ABCA1 has been suggested to be involved in cholesterol efflux from enterocytes into the lumen, thereby regulating the efficiency of intestinal cholesterol absorption (15, 16).

HDL is considered a major source for bile-destined cholesterol and phospholipid (17, 18). Yet, we have recently demonstrated that, despite the absence of HDL, hepatobiliary cholesterol flux and fecal sterol excretion are not affected in *Abca1* knockout mice (19). Our results thus questioned whether ABCA1 has indeed an important role in control of mass cholesterol transport from the periphery to the liver and suggest that its major peripheral function is removal of excess cholesterol from macrophages. Haghpassand *et al.* (20) showed convincingly that efflux from macrophages constitutes only a small fraction of HDL cholesterol.

Several genes involved in control of cholesterol metabolism, including *Abca1*, are transcriptionally regulated by the liver X receptor (LXR) (21–24). Two LXR isoforms have been identified, LXRα (NR1H3) and LXRβ (NR1H2) (25, 26). Upon stimulation by oxysterols, activated LXR forms a heterodimer with the retinoid X receptor (RXR, NR2B1), binds to DNA, and influences gene expression. It has been proposed that a high dietary cholesterol intake (via subsequent formation of oxysterols) activates LXR, which, in turn, induces expression of genes involved in cholesterol disposal (27, 28). Because of its prominent position in controlling cholesterol homeostasis, pharmacological activation of LXR is considered a promising approach to raise HDL, to improve RCT, and thereby to prevent the development of atherosclerosis. Treatment of rodents with LXR (or retinoid X receptor) agonists indeed results in elevation of plasma HDL levels (29, 30) and reduced intestinal cholesterol absorption (23).

In this study, we have investigated the role of ABCA1 in LXR-controlled pathways of hepatobiliary and fecal cholesterol

* This work was supported by Grants 902-23-191 and 902-23-193 from the Netherlands Organization for Scientific Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: RCT, reverse cholesterol transport; HDL, high density lipoprotein; SR-BI, scavenger receptor class B type I; LXR, liver X receptor; FPLC, fast protein liquid chromatography; VLDL, very low density lipoprotein.

output in mice. For this purpose, wild-type and ABCA1-deficient mice (31) were treated with the synthetic LXR agonist T0901317 (23, 29). Surprisingly, both T0901317-treated *Abca1*$^{-/-}$ and wild-type mice showed similarly increased rates of hepatobiliary cholesterol output and increased fecal sterol loss independent of (ABCA1-mediated) elevation of plasma HDL levels and the (putative) role of ABCA1 in intestinal cholesterol absorption.

EXPERIMENTAL PROCEDURES

Animals—Male C57BL/6J mice (2–3 months old) were purchased from Harlan (Horst, The Netherlands). *Abca1*$^{-/-}$ mice with a DBA/1 background (6–8 months old) and age-matched DBA/1 wild-type mice were obtained from IFFA Credo (Saint-Germain-sur-L'Arbresle, France). Because of the limited supply of homozygous knockout mice, both male and female mice were used in these experiments. Animals received standard mouse chow (Hope Farms BV, Woerden, The Netherlands) and water *ad libitum*.

Experimental Methods—The synthetic LXR agonist T0901317, kindly provided by Organon BV (Oss, The Netherlands), was solubilized in Me_2SO. This solution was diluted 1:1 with chremophor and further diluted 1:9 with mannitol/water (5%). Animals received 20 μmol of T0901317/kg/day by gavage at 4 p.m. Control groups were treated with the solvent only. All animals were housed separately, and feces of individual mice were collected from days 4 to 5. At day 5, mice were anesthetized by intraperitoneal injection of Hypnorm (fentanyl/fluanisone, 1 ml/kg) and diazepam (10 mg/kg). Bile was collected for 30 min by cannulation of the gallbladder. During bile collection, body temperature was stabilized using a humidified incubator. At the end of the collection period, animals were killed by cardiac puncture. Blood was collected in EDTA-containing tubes. Livers were excised and weighed. The small intestine was rinsed with cold phosphate-buffered saline and divided into three equal parts. Parts of both the liver and intestine were snap-frozen in liquid nitrogen and stored at −80 °C for mRNA isolation and biochemical analysis. Samples for microscopic evaluation were frozen in isopentane and stored at −80 °C or fixed in paraformaldehyde for hematoxylin/eosin and oil red O staining. C57BL/6J mice used for RNA isolation and lipid analysis only were killed without prior bile collection. Tissues were immediately removed, snap-frozen in liquid nitrogen, and manipulated as described below.

Analytical Methods—Bile salts were measured enzymatically (32). Commercially available kits were used for the determination of free cholesterol (Wako, Neuss, Germany); total cholesterol, HDL cholesterol, and triglycerides (Roche Molecular Biochemicals, Mannheim, Germany); and phospholipids and free fatty acids (Wako) in plasma. Hepatic and biliary lipids were extracted according to Bligh and Dyer (33). Phospholipids in bile and liver were determined as described by Böttcher *et al.* (34). Cholesterol in bile was measured according to Gamble *et al.* (35). Hepatic cholesterol and triglyceride contents were analyzed as described above. Feces were lyophilized, weighed, and homogenized. Neutral sterols and bile salts were analyzed according to Arca *et al.* (36) and Setchell *et al.* (37), respectively. Pooled plasma samples from all animals of one group were used for lipoprotein separation by fast protein liquid chromatography (FPLC) as described previously (38).

RNA Isolation and PCR Methods—Total RNA was isolated with Trizol (Invitrogen) and quantified using Ribogreen (Molecular Probes, Inc., Eugene, OR). cDNA synthesis was done according to Bloks *et al.* (39). For C57BL/6J mice, all three intestinal samples per mouse were analyzed separately, whereas for DBA/1 and *Abca1*$^{-/-}$ mice, equal amounts of RNA from the three distinct parts of the small intestine were pooled prior to reverse transcription. Real-time quantitative PCR (40) was performed using an Applied Biosystems 7700 sequence detector according to the manufacturer's instructions. Primers were obtained from Invitrogen. Fluorogenic probes, labeled with 6-carboxyfluorescein and 6-carboxytetramethylrhodamine, were made by Eurogentec (Seraing, Belgium); all sequences are listed in Table I. All expression data were subsequently standardized for 18 S rRNA, which was analyzed in separate runs.

Statistics—Statistical analyses were performed using SPSS Version 10.0 for Windows (SPSS Inc., Chicago, IL). Treated and untreated groups were compared by Student's *t* test for large data series of biochemical parameters and by the Mann-Whitney *U* test for the remaining, as indicated. A p value <0.05 was considered statistically significant.

TABLE I

Primer sequences used in mRNA quantification by real-time reverse transcription-PCR

Quantitative real-time PCR was performed as described under "Experimental Procedures." All probes were labeled with 6-carboxyfluorescein and 6-carboxytetramethylrhodamin at the 5′- and 3′-ends, respectively.

	Accession No.	Forward	Reverse	Probe
Srebp1a	Ref. 58	GAGGCGGCTCTGGAACAGA	TGTCTTCGATGTCGTTCAAAACC	TGTGTCCAGTTCGCACATCTCGGC
Srebp1c	B1656094	GGAGCCATGGATTGCACATT	CCTGTCTCACCCCCAGCATA	CAGCTCATCAACAACCAAGACAGTGACTTCC
Srebp2	AF374267	CTGCAGCCTCAAGTGCAAAG	CAGTGTGCCATTGGCTGTCT	CCATCCAGCAGCAGGTGCAGACG
LXRα (NR1H3)	AF085745	GCTCTGCTCATTGCCATCAG	TGTTGCAGCCTCTCTACTTGGA	TCTGCAGACCGGCCCAACGTG
Hmgcr	BB664708	CCGGCAACAACAAGATCTGTG	ATGTACAGGATGGCGATGCA	TGTCGCTGCTCAGCACGTCCTCTTTC
Cyp7a1	NM_007824	CAGGGAGATGCTCTGTGTTCA	AGGCATACATCCCTTCCGTGA	TGCAAAACCTCCAATCTGTCATGAGACCTCC
Cyp27	AK004977	GCCTTGCACAAGGAAGTGACT	CGCAGGGTCTCCTTAATCACA	CCCTTCGGGAAGGTGCCCCAG
Acat1	NM_009230	TGGGTGCCACTTCGATGACT	TGAGTGCACACCCACCATTG	CCAACCTCATTGAAAAGTCCGCATCGC
Acat2	NM_011433	GGTGGAACTATGTGGCCAAGA	CCAGGATGAAGCAGGCATAGA	CAAACAGCCCAGGACCTGGGCAAAG
Lp1	NM_008509	AAGGTCAGAGCCAAGAGAAGCA	CCAGAAAAGTGAATCTTGACTTGGT	CCTGAAGACTCGCTCTCAGATGCCCTACA
Abca1	NM_013454	CCCAGAGCAAAAAGCGACTC	GGTCATCATCACTTTGGTCCTTG	AGACTACTCTGTCTCTCAGACAACACTTGACCAAG
Abcg5	AF312713	TCAGGACCCCAAGGTCATGAT	AGGCTGGTGGATGGTGACAAT	CCACAGGACTGGACTGCATGACTGCA
Abcg8	AK004871	GACAGCTTCACAGCCCACAA	GCCTGAAGATGTCAGAGCGA	CTGGTGCTCATCTCCCTCCACCAG
Bsep (*Abcb11*)	NM_021022	CTGCCAAGGATGCTAATGCA	CGATGGCTACCCTTTGCTTCT	TGCCACAGCAATTTGACACCCTAGTTGG
Mdr2 (*Abcb4*)	NM_008830	GCAGCGAGAAACGGAACAG	GGTTGCTGATGCTGCCTAGTT	AAAGTCGCCGTCTAGGCGCCGT
Ntcp (*Slc10a1*)	AB003303	ATGACCACCTGCTCCAGCTT	GCCTTTGTAGGGCACCTTGT	CCTTGGGCATGATGCCTCTCCTC
Oatp1 (*Slc21a1*)	NM_013797	CAGTCTTACGAGTGTGCTCCAGAT	ATGAGGAATACTGCCTCTGAAGTG	TGGATTTGCCAGTACATTTACCTTCTTGCCC
SR-BI	NM_016741	TCAGAAGCTGTTCTTGGTCTGAAC	GTTCATGGGGATCCCAGTGA	ACCCAAAGGAGCATTCCTTGTTCCTAGACA
18 S rRNA	X00686	CGGCTACCACATCCAAGGA	CCAATTACAGGGCCTCGAAA	CGCGCAAATTACCCACTCCGA

TABLE II
Bile flow and biliary secretion rates of C57BL/6J mice treated with the LXR agonist T0901317 or its solvent

Male C57BL/6J mice (2–3 months old) were treated with the LXR agonist T0901317 or solvent only as described under "Experimental Procedures" (n = six per group). Bile was collected for 30 min. Values represent means ± S.D.

	Control	T0901317
Bile flow (μl/min/100 g body weight)	8.2 ± 2.8	7.8 ± 2.6
Bile salts (nmol/min/100 g body weight)	584 ± 229	477 ± 200
Cholesterol (nmol/min/100 g body weight)	3.8 ± 1.4	10.3 ± 3.1[a]
Phospholipids (nmol/min/100 g body weight)	52.7 ± 10.8	44.2 ± 9.7
Cholesterol/phospholipid ratio	0.07 ± 0.03	0.23 ± 0.04[b]

[a] Indicates significant difference (Mann-Whitney U test, $p < 0.05$).
[b] Indicates significant difference (Mann-Whitney U test, $p < 0.001$).

TABLE III
mRNA expression levels in liver tissue of C57BL/6J mice treated with the LXR agonist T0901317 or its solvent measured by real-time reverse transcription-PCR

Male C57BL/6J mice (2–3 months old) were treated with the LXR agonist T0901317 or solvent only as described under "Experimental Procedures" (n = four per group). Quantitative real-time PCR was performed as described under "Experimental Procedures" with the primers and probes given in Table I. All data were standardized for 18 S rRNA. Expression in control mice was set to 1.00. Values represent means ± S.D.

mRNA	Control	T0901317
Srebp1a	1.00 ± 0.18	1.22 ± 0.06
Srebp1c	1.00 ± 0.15	2.64 ± 0.54[a]
Srebp2	1.00 ± 0.17	1.00 ± 0.06
LXR	1.00 ± 0.09	0.84 ± 0.11
Hmgcr	1.00 ± 0.10	1.55 ± 0.36[a]
Cyp7a1	1.00 ± 0.42	1.45 ± 0.74
Cyp27	1.00 ± 0.16	0.94 ± 0.08
Acat2	1.00 ± 0.12	1.24 ± 0.27
Abca1	1.00 ± 0.55	2.38 ± 0.96[a]
Abcg5	1.00 ± 0.42	2.81 ± 1.19[a]
Abcg8	1.00 ± 0.56	1.54 ± 0.51
Bsep	1.00 ± 0.18	1.01 ± 0.06
Mdr2	1.00 ± 0.10	1.13 ± 0.17
Ntcp	1.00 ± 0.04	0.97 ± 0.10
Oatp1	1.00 ± 0.41	0.63 ± 0.09

[a] Indicates significant difference (Mann-Whitney U test, $p < 0.05$).

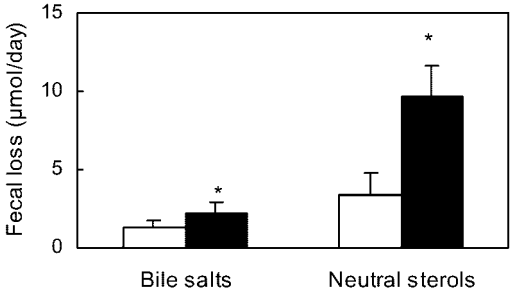

FIG. 1. **Fecal loss of neutral sterols and bile salts of C57BL/6J mice treated with the LXR agonist T0901317 or its solvent.** C57BL/6J mice were treated with T0901317 (*black bars*) or solvent only (*white bars*) for 4 days (n = 10 per group); feces were collected during the last 24 h of the experiment and analyzed as described under "Experimental Procedures." The *asterisks* indicate significant difference (Student's t test, $p < 0.001$).

RESULTS

LXR Activation by T0901317 Increases Plasma HDL and Induces Hepatic Steatosis in C57BL/6J Mice—Treatment with the LXR agonist T0901317 resulted in profound changes in plasma and liver lipid homeostasis in C57BL/6J mice, as previously reported by ourselves (41) and others (23, 29). On the treatment protocol employed in this study, mice developed significantly elevated plasma levels of cholesterol, particularly in the esterified fraction, and phospholipids. HDL cholesterol was elevated by 59% upon treatment. Administration of T0901317 increased liver weight by 40% without any change in body weight. Hepatic total cholesterol content was decreased in treated mice (−15%), partly as a result of a significantly diminished cholesteryl ester concentration (−32%), whereas the concentration of phospholipids was not affected. In treated animals,

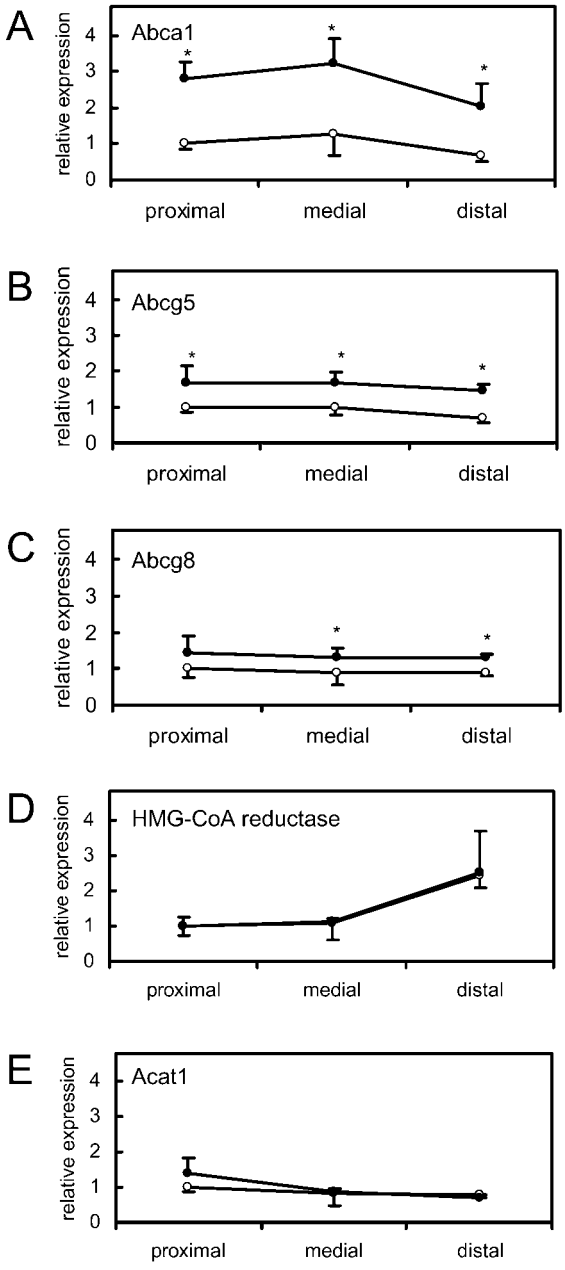

FIG. 2. **mRNA expression levels in the intestines of C57BL/6J mice treated with the LXR agonist T0901317 or its solvent measured by real-time PCR.** C57BL/6J mice were treated with T0901317 (●) or solvent only (○) for 4 days (n = four per group); the intestine was removed, rinsed with cold phosphate-buffered saline, divided into three equal parts, and analyzed as described under "Experimental Procedures." All data were standardized for 18 S rRNA. Expression in the proximal part of the small intestine in animals receiving the solvent only was set to 1. The *asterisks* indicate significant difference (Mann-Whitney U test, $p < 0.05$). *A–E*, relative expression of *Abca1*, *Abcg5*, *Abcg8*, *Hmgcr*, and *Acat1*, respectively.

TABLE IV

Plasma lipid levels in DBA/1 wild-type and Abca1$^{-/-}$ mice treated with the LXR agonist T0901317 or its solvent

Male and female DBA/1 wild-type and *Abca1*$^{-/-}$ mice (6–8 months old) were treated with the LXR agonist T0901317 or solvent only as described under "Experimental Procedures" (n = five to six per group). Blood was collected by cardiac puncture. Values represent means ± S.D.

	Wild-type		*Abca1*$^{-/-}$	
	Control	T0901317	Control	T0901317
Total cholesterol (mM)	1.12 ± 0.55	1.64 ± 0.69	0.50 ± 0.30	1.11 ± 0.33[a]
Free cholesterol (mM)	0.35 ± 0.14	0.62 ± 0.10[a]	0.29 ± 0.08	0.72 ± 0.25[a]
Cholesteryl ester (mM)	0.77 ± 0.42	1.02 ± 0.69	0.21 ± 0.28	0.39 ± 0.14
Phospholipids (mM)	1.26 ± 0.61	2.07 ± 1.28	0.75 ± 0.43	1.34 ± 0.28
Triglycerides (mM)	0.73 ± 0.33	0.93 ± 0.28	0.83 ± 0.38	1.88 ± 1.39

[a] Indicates significant difference (Mann-Whitney U test, $p < 0.05$).

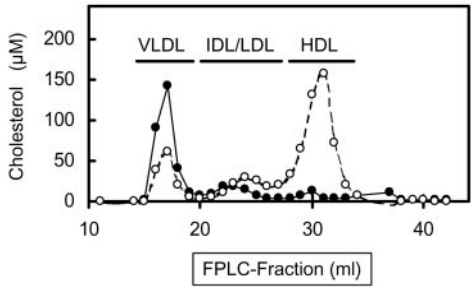

FIG. 3. **FPLC analysis of plasma cholesterol of DBA/1 and *Abca1*$^{-/-}$ mice treated with the LXR agonist T0901317.** DBA/1 wild-type mice (○) and *Abca1*$^{-/-}$ mice (●) were treated with T0901317 for 4 days (n = five per group). Blood was collected via cardiac puncture and pooled before FPLC analysis. Analysis was performed as described under "Experimental Procedures." *IDL*, intermediate density lipoprotein; *LDL*, low density lipoprotein.

we found a >8-fold increase in hepatic triglyceride content, in accordance with recently published studies (30). Histologically, these animals presented with profound hepatic fat deposits, but no signs of liver damage were noticed (data not shown).

LXR Activation by T0901317 Induces Biliary Hypersecretion of Cholesterol in C57BL/6J Mice—Bile flow was unaffected by T0901317 treatment when calculated on the basis of body weight (Table II). Biliary cholesterol output was 2.7-fold higher upon treatment, whereas biliary bile salt and phospholipid output was not affected. As a consequence, the ratio of cholesterol to phospholipids increased from 0.07 to 0.23 upon treatment, indicative of uncoupling of biliary cholesterol from phospholipid secretion.

Gene expression profiles of key regulatory, metabolic, and transporter-encoding genes involved in hepatic cholesterol metabolism were analyzed by real-time PCR (Table III). As expected (42, 43), the gene encoding sterol regulatory element-binding protein 1c (*Srebp1c*) was the only regulatory gene with a modified expression (2.6-fold up) upon T0901317 treatment. This predicted increase is indicative of the overall stimulatory action of the agonist on hepatic gene expression, also supported by an ~5-fold increase in expression levels of the LXR target gene *Lpl* encoding lipoprotein lipase (data not shown). The gene encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*), the key enzyme in cholesterol synthesis, was up-regulated by 55%, whereas the 45% up-regulation of the bile salt synthesis gene *Cyp7a1* did not reach statistical significance. T0901317 treatment increased expression of *Abca1* and *Abcg5* 2.4- and 2.8-fold, respectively; expression of hepatic *Abcg8* showed a high variation in its expression levels. Expression of transporters involved in bile salt uptake (*Ntcp* (Na/taurocholate-cotransporting polypeptide) and *Oatp1* (organic anion-transporting polypeptide-1)) and secretion (*Bsep* (bile salt export pump)) and in phospholipid secretion (*Mdr2* (multidrug resistance P-glycoprotein-2)) remained unaffected.

LXR Activation by T0901317 Accelerates Fecal Sterol Loss in C57BL/6J Mice—Fecal bile salt loss was increased by 84% upon activation of LXR with T0901317 (Fig. 1), reflecting increased hepatic bile salt synthesis. In addition, neutral sterol output was enhanced by 187% in T0901317-treated mice. Increased expression of *Abca1* in the intestine has been proposed to reduce the efficacy of cholesterol (re)absorption and hence to enhance fecal cholesterol disposal (23). Indeed, treatment of mice with the LXR agonist T0901317 resulted in an ~3-fold increase in *Abca1* mRNA abundance along the entire length of the small intestine (Fig. 2*A*). Likewise, expression of *Abcg5* and *Abcg8*, recently implicated in control of cholesterol absorption (44–46), was induced in treated animals, albeit less pronounced than that of *Abca1* (Fig. 2, *B* and *C*). In contrast, mRNA levels of *Hmgcr* and *Acat1* (encoding acyl-coenzyme A:cholesterol acyltransferase-1), indicative of intestinal cholesterol synthesis and cholesterol esterification, respectively, were similar in treated and control animals (Fig. 2, *D* and *E*). No changes in intestinal morphology were noted upon microscopic examination of hematoxylin/eosin- and oil red O-stained sections (data not shown).

To elucidate the specific role of ABCA1 in the observed LXR-mediated stimulation of cholesterol disposal in mice, we subsequently conducted a series of similar experiments in *Abca1*$^{-/-}$ mice and adequate wild-type controls on a DBA/1 background. Both male and female mice were used in these studies; no specific gender effects on the parameters studied were noted unless otherwise stated. Therefore, outcome is, in most cases, presented as average values per group.

LXR Activation by T0901317 Increases Cholesterol in Very Low Density Lipoprotein (VLDL)-sized Fractions in Abca1$^{-/-}$ Mice—Upon treatment with T0901317, plasma concentrations of free cholesterol were increased in both *Abca1*$^{-/-}$ and DBA/1 wild-type mice (Table IV). Cholesteryl ester concentrations were not significantly affected, resulting in an increase in total cholesterol in *Abca1*$^{-/-}$ mice only. Both plasma phospholipid and triglyceride levels were not significantly changed upon T0901317-mediated activation of LXR in DBA/1 mice. FPLC separation of plasma lipoproteins revealed that, as anticipated, the increase in plasma cholesterol in wild-type mice was in the HDL-sized lipoprotein fraction. Fig. 3 illustrates the lipoprotein pattern in T0901317-treated wild-type and *Abca1*$^{-/-}$ mice. Upon LXR activation, wild-type mice showed elevated cholesterol concentrations in the HDL-sized fractions, whereas *Abca1*$^{-/-}$ mice showed increased cholesterol concentrations in the VLDL-sized fractions.

Hepatobiliary Cholesterol Secretion Is Increased upon T0901317 Treatment in Both Wild-type and Abca1$^{-/-}$ Mice—As shown in Table V, treatment with T0901317 did not change bile flow in wild-type or *Abca1*$^{-/-}$ mice. Bile salt secretion remained unchanged in wild-type mice, but slightly decreased in T0901317-treated *Abca1*$^{-/-}$ mice. Phospholipid output rates were not altered in *Abca1*$^{-/-}$ mice and were moderately lowered in wild-type mice upon LXR activation. Expression of genes involved in hepatic cholesterol metabolism and of ATP-binding cassette transporters known to be involved

TABLE V

Bile flow and biliary secretion rates in DBA/1 wild-type and Abca1$^{-/-}$ mice treated with the LXR agonist T0901317 or its solvent

Male and female DBA/1 wild-type and *Abca1*$^{-/-}$ mice (6–8 months old) were treated with the LXR agonist T0901317 or solvent only as described under "Experimental Procedures" (n = five to six per group). Bile was cannulated for 30 min. Values represent means ± S.D.

	Wild-type		*Abca1*$^{-/-}$	
	Control	T0901317	Control	T0901317
Bile flow (μl/min/100 g body weight)	7.2 ± 0.9	7.4 ± 1.2	7.8 ± 1.6	7.9 ± 1.8
Bile salts (nmol/min/100 g body weight)	392 ± 68	307 ± 119	472 ± 213	251 ± 120[a]
Cholesterol (nmol/min/100 g body weight)	8.0 ± 1.1	17.0 ± 3.2[a]	8.4 ± 2.5	19.5 ± 7.5[a]
Phospholipids (nmol/min/100 g body weight)	63.1 ± 7.0	38.1 ± 7.7[a]	67.6 ± 16.2	48.6 ± 22.3
Cholesterol/phospholipid ratio	0.13 ± 0.02	0.46 ± 0.13[a]	0.12 ± 0.03	0.42 ± 0.08[a]

[a] Indicates significant difference (Mann-Whitney U test, $p < 0.05$).

TABLE VI

mRNA expression levels in liver tissue of DBA/1 wild-type and Abca1$^{-/-}$ mice treated with the LXR agonist T0901317 or its solvent measured by real-time reverse transcription-PCR

Male and female DBA/1 wild-type and *Abca1*$^{-/-}$ mice (6–8 months old) were treated with the LXR agonist T0901317 or solvent only as described under "Experimental Procedures" (n = five per group). cDNA synthesis and real-time PCR were performed as described under "Experimental Procedures" with the primers and probes given in Table I. All data were standardized for 18 S rRNA. Expression in wild-type mice receiving solvent only was set to 1.00. Values represent means ± S.D.

mRNA	Wild-type		*Abca1*$^{-/-}$	
	Control	T0901317	Control	T0901317
Srebp1c	1.00 ± 0.31	2.04 ± 1.22	1.17 ± 0.49	2.49 ± 0.78[a]
Hmgcr	1.00 ± 0.25	0.90 ± 0.36	0.60 ± 0.22	1.18 ± 0.43[a]
Cyp7a1	1.00 ± 0.36	0.60 ± 0.26	0.92 ± 0.40	0.46 ± 0.20
SR-BI	1.00 ± 0.14	0.73 ± 0.19[a]	0.81 ± 0.20	0.69 ± 0.12
Abca1	1.00 ± 0.41	0.80 ± 0.38	0.53 ± 0.17	0.52 ± 0.08
Abcg5	1.00 ± 0.14	1.75 ± 0.85	0.75 ± 0.28	1.91 ± 0.67[a]
Abcg8	1.00 ± 0.16	1.65 ± 0.68	0.81 ± 0.16	1.51 ± 0.48[a]
Bsep	1.00 ± 0.14	1.07 ± 0.45	0.86 ± 0.16	1.02 ± 0.40
Mdr2	1.00 ± 0.27	0.83 ± 0.21	0.88 ± 0.16	0.76 ± 0.14

[a] Indicates significant difference (Mann-Whitney U test, $p < 0.05$).

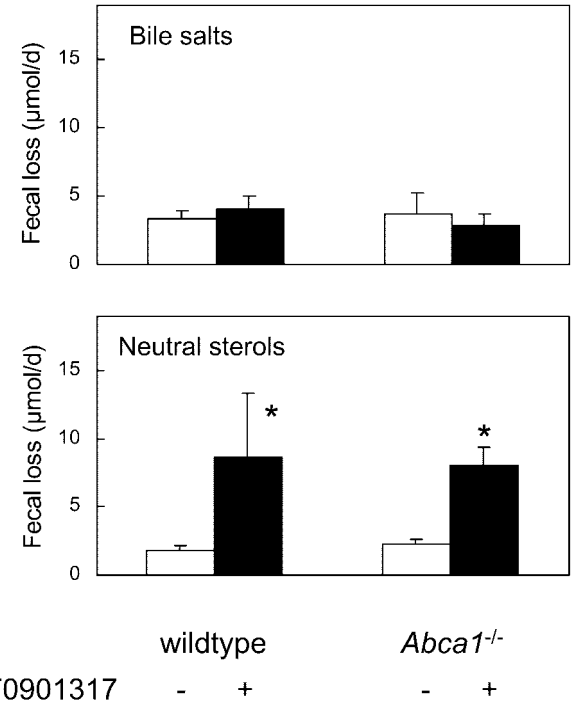

FIG. 4. **Fecal loss of neutral sterols and bile salts of DBA/1 and *Abca1*$^{-/-}$ mice treated with the LXR agonist T0901317 or its solvent.** DBA/1 wild-type and *Abca1*$^{-/-}$ mice were treated with T0901317 (*black bars*) or solvent only (*white bars*) for 4 days (*d*) (n = five per group). Feces were collected for the last 24 h of the experiment and analyzed as described under "Experimental Procedures." The *asterisks* indicate significant difference (Mann-Whitney U test, $p < 0.05$).

in bile formation (*i.e. Bsep* and *Mdr2*) was not affected by ABCA1 deficiency or LXR activation (Table VI). Biliary cholesterol secretion was markedly increased in T0901317-treated DBA/1 mice and, despite the absence of HDL, to the same extent in treated *Abca1*$^{-/-}$ mice. In both strains, a >3 times higher cholesterol/phospholipid ratio was found in bile, indicative of cholesterol hypersecretion induced by LXR activation that is independent of ABCA1 functioning.

Fecal Neutral Sterol Excretion Is Stimulated by LXR Activation in the Absence of ABCA1—Fecal sterol loss during the final 24 h of the experiment is shown in Fig. 4. Fecal bile salt output was similar in DBA/1 wild-type and *Abca1*$^{-/-}$ mice both under control conditions and upon LXR stimulation (Fig. 4, *upper panel*). As expected, neutral sterol loss in wild-type mice was dramatically increased upon treatment (Fig. 4, *lower panel*). Surprisingly, *Abca1*$^{-/-}$ mice showed a very similar response upon LXR activation, *i.e.* a significantly higher neutral sterol output.

The entry of cholesterol into the intestinal lumen consists of at least three components: delivery via bile; dietary intake; and direct intestinal secretion by enterocytes into the lumen, including shedding of cells. The rate of fecal cholesterol excretion (loss) is determined by the cumulative rate of cholesterol entry and the rate of (re)absorption of luminal cholesterol. From earlier studies (38), we know that dietary intake is ~4 μmol/100 g/day, which, under basal conditions, is similar to the biliary cholesterol flux in C57BL/6J mice. The basal rate of fecal cholesterol excretion in C57BL/6J mice is ~17 μmol/100 g/day, which is higher than the sum of dietary and biliary cholesterol influx into the intestine. By inference, at least ~50% of fecal cholesterol must originate in the intestine. Assuming no major effect of T0901317 treatment on dietary cholesterol intake, the treatment did not affect the relative contribution of cholesterol from the intestine to the amount of cholesterol excretion via feces, again at least ~50%. This implies, however, that T0901317 treatment significantly increased the absolute amount of cholesterol in feces originating in the intestine. In DBA/1 wild-type and *Abca1*$^{-/-}$ mice under

TABLE VII

mRNA expression levels in intestines of DBA/1 wild-type and Abca1$^{-/-}$ mice treated with the LXR agonist T0901317 or its solvent measured by real-time reverse transcription-PCR

Male and female DBA/1 wild-type and *Abca1*$^{-/-}$ mice (6–8 months old) were treated with the LXR agonist T0901317 or solvent only as described under "Experimental Procedures" (n = five per group). RNA was isolated from three parts of the small intestine; equal amounts of RNA were pooled for cDNA synthesis and analyzed as described under "Experimental Procedures" with the primers and probes given in Table I. All data were standardized for 18 S rRNA. Expression in wild-type mice receiving solvent only was set to 1.00. Values represent means ± S.D.

mRNA	Wild-type		*Abca1*$^{-/-}$	
	Control	T0901317	Control	T0901317
Hmgcr	1.00 ± 0.13	0.98 ± 0.16	1.12 ± 0.30	0.87 ± 0.24
Acat1	1.00 ± 0.18	1.84 ± 0.49[a]	1.19 ± 0.61	1.47 ± 0.55
Acat2	1.00 ± 0.19	1.14 ± 0.29	1.21 ± 0.60	1.01 ± 0.22
Abca1	1.00 ± 0.35	2.59 ± 1.16[a]	0.42 ± 0.12	1.26 ± 0.40[a]
Abcg5	1.00 ± 0.09	2.02 ± 0.31[a]	0.94 ± 0.16	1.72 ± 0.48[a]
Abcg8	1.00 ± 0.12	1.84 ± 0.33	1.08 ± 0.33	1.90 ± 0.53[a]
SR-BI	1.00 ± 0.20	2.31 ± 0.89[a]	1.47 ± 0.57	1.76 ± 0.47

[a] Indicates significant difference (Mann-Whitney U test, $p < 0.05$). For *Abca1*, ~40% of the remaining, putatively inactive mRNA was detectable in *Abca1*$^{-/-}$ mice, as the PCR primers are located outside the deleted exons (11, 31).

basal conditions, the sums of dietary (assumed) and biliary cholesterol influx into the intestine were higher than the fecal rate of cholesterol excretion, indicating net cholesterol absorption by the intestine. After T0901317 treatment, however, the sum of biliary and dietary (assumed) cholesterol influx into the intestine was *lower* than the fecal rate of cholesterol excretion, implying that the net intestinal cholesterol flux had shifted from absorption from the lumen to excretion into the lumen (data not shown).

The absence of any effect of genotype or treatment on intestinal expression of *Hmgcr* indicates that changes in fecal neutral sterol excretion are unlikely due to effects on intestinal cholesterol synthesis (Table VII). Expression of *Abca1* was clearly induced in the intestines of wild-type mice after treatment with T0901317. The intestinal expression of *Abcg5* and *Abcg8* was similar in wild-type and *Abca1*$^{-/-}$ mice and, in both strains, up-regulated upon LXR activation. Due to high variation, there was no significant increase in *Abcg5* mRNA levels in the wild-type mice. This high variation was, at least partly, caused by less pronounced induction in male animals. In wild-type mice, expression of *Acat1* and *SR-BI* also increased significantly. This effect was less pronounced in *Abca1*$^{-/-}$ mice.

DISCUSSION

Activation of the nuclear receptor LXR influences multiple steps involved in maintenance of cholesterol homeostasis, particularly by inducing expression of genes that control key steps in removal of excess cholesterol from the body. It has been postulated that LXR agonists may combine three potentially anti-atherogenic effects, *i.e.* increased efflux from peripheral tissues toward HDL by up-regulation of ABCA1 and ABCG1, increased catabolism of cholesterol by up-regulation of bile salt synthesis, and inhibition of dietary cholesterol absorption via up-regulation of intestinal cholesterol transporters like ABCA1, ABCG5, and ABCG8 (15, 16). The present study demonstrated that short-term administration of the LXR agonist T0901317 elevates plasma HDL levels in wild-type mice, both of C57BL/6J and DBA/1 backgrounds, as previously described (29, 30). This was accompanied by a marked hypersecretion of cholesterol into bile and a strongly increased fecal excretion of neutral sterols. Biliary secretion of phospholipids and, in the wild-type mice, also of bile salts remained largely unaffected. Hence, LXR activation fully uncoupled cholesterol from other biliary lipids. Surprisingly, increased hepatobiliary and fecal cholesterol disposal was found to be totally independent of ABCA1-mediated HDL formation and the (putative) contribution of ABCA1 to the control of intestinal cholesterol absorption because the effects of LXR activation on these parameters were indistinguishable between DBA/1 wild-type and *Abca1*$^{-/-}$ mice. Moreover, the results of this study strongly indicate that pharmacological LXR activation stimulates direct efflux of cholesterol from the intestinal epithelium into the lumen. Based on the assumption that dietary intake of cholesterol was identical in all groups and an estimate of 24 h of biliary cholesterol excretion, this efflux was calculated as the difference between dietary and biliary input minus fecal output. LXR activation greatly increased this flux. From our data, however, we cannot draw conclusions concerning the relative contributions of decreased absorption, increased direct sterol excretion by intestinal cells, and accelerated shedding of enterocytes to this net loss of sterols. There were no indications of a compensatory increase in intestinal cholesterol synthesis because *Hmgcr* expression remained unaffected along the length of the small intestine. Independent of the mechanism, this finding delineates the important role of the intestine in cholesterol homeostasis, as suggested by us previously (19, 38, 47), and that the presence of ABCA1 is not required to fulfill this role. In line with the proposed role of the ATP-binding cassette half-transporters ABCG5 and ABCG8 in cholesterol efflux toward the intestinal lumen (44, 45) and the consistently induced expression of these genes in intestines of LXR agonist-treated mice, it is tempting to speculate that these half-transporters have a crucial role in LXR-induced changes in intestinal cholesterol metabolism.

The classical view of RCT (1) predicts that, under steady-state conditions, all cholesterol synthesized in peripheral organs is eventually transported by HDL to the liver for excretion into bile, followed by its disposal via feces. ABCA1 is considered a crucial factor in this process because the absence of a functional protein in Tangier disease (7–9) and in *Abca1*$^{-/-}$ mice (31) is associated with a complete lack of HDL. The validity of the RCT concept to explain mass cholesterol flux from the periphery to the liver has been questioned by a series of studies in mice showing that the magnitude of RCT (or "centripetal cholesterol flux") is not determined by plasma HDL levels (48–53) and is not affected by stimulation of individual key steps in the process, such as cholesterol efflux from peripheral tissues, SR-BI-mediated uptake of HDL cholesterol by the liver, and conversion of cholesterol into bile salts (54). In addition, we have recently shown that the absence of HDL due to ABCA1 deficiency does not alter hepatic cholesterol synthesis, which would be anticipated if HDL accommodates a quantitatively important cholesterol flux toward the liver, and does not affect biliary cholesterol excretion and fecal sterol loss (19). These observations, together with the fact that macrophages contribute only modestly to HDL cholesterol (20), indicate that the relevance of ABCA1 and high HDL in protection from

atherosclerosis is related to events at the level of the vessel wall rather than to stimulation of mass cholesterol flux. This, in turn, implies that the absence of HDL in patients with Tangier disease and *Abca1*$^{-/-}$ mice is not due to the absence of the protein in macrophages. Because specific overexpression of ABCA1 in hepatocytes and macrophages of *Abca1*$^{-/-}$ mice using an adenoviral construct driven by the *Apoe* promoter normalizes plasma HDL (14), it is tempting to speculate that hepatic ABCA1 has a role in formation of nascent HDL particles. Thus, it is highly likely that elevated HDL levels in T0901317-treated mice are attributable to increased hepatic ABCA1 expression and function. Elevated VLDL cholesterol in treated *Abca1*$^{-/-}$ mice may be due to enhanced formation of VLDL particles by the liver. Recent data from our laboratory show that LXR activation strongly promotes the production of large, triglyceride-rich VLDL particles by the liver in wild-type mice (41). Whether or not there are qualitative or quantitative differences in this respect between wild-type and *Abca1*$^{-/-}$ mice remains to be established.

There are data to indicate that HDL cholesterol is an important source for both biliary cholesterol and bile salt synthesis (17, 18). The similar increase in biliary cholesterol excretion in *Abca1*$^{-/-}$ mice and their controls upon LXR activation shows, at least, that other sources are able to fully compensate for the lack of HDL cholesterol in *Abca1*$^{-/-}$ mice. As hepatic cholesteryl ester concentrations were diminished by ~50–85% in treated mice independently of the presence of ABCA1, part of the excess biliary cholesterol may have been derived from hepatic stores. Expression of *Hmgcr* was slightly increased in T0901317-treated mice, indicating that a compensatory increase in synthesis also may contribute. Despite the fact that *Cyp7a1* has been identified as a *bona fide* LXR target gene *in vitro* (55), we observed only a modest (C57BL/6J mice) or no (DBA/1 mice) increase in fecal bile salt secretion upon LXR activation, indicating limited effects on total bile salt synthesis. Accordingly, no significant effects on *Cyp7a1* expression levels were found. The fact that, in contrast to other reports (23, 29, 43), we did not find an induction of *Cyp7a1* expression upon T0901317 administration is probably related to differences in treatment protocols (see below). It has been known for >10 years that *Cyp7a1* mRNA is relatively unstable, putatively related to the circadian rhythm of its expression (56). We therefore might have missed the LXR-induced peak expression measured by others. In any case, our data demonstrate that ABCA1-dependent HDL formation is dispensable for biliary routing of cholesterol both as free cholesterol and after conversion to bile salts.

Interestingly, LXR activation by T0901317 stimulated hepatobiliary cholesterol excretion without influencing biliary phospholipid excretion. The latter is in accordance with unaltered *Mdr2* expression upon LXR activation. Biliary bile salt secretion, which constitutes a major driving force for biliary cholesterol and phospholipid secretion (57), was either not affected or even slightly reduced in treated animals. Consequently, LXR activation leads to cholesterol hypersecretion into bile, as illustrated by the 3–4-fold increase in the biliary cholesterol/phospholipid ratio in all T0901317-treated groups of mice. This suggests LXR-mediated up-regulation of a specific process/transporter responsible for cholesterol disposal into bile. It has been suggested that ABCG5/ABCG8, ATP-binding cassette half-transporters defective in β-sitosterolemia (44, 45), may have a role in this process (15, 16). However, direct evidence for this role is still lacking. As recently reported (46) and confirmed in this study, expression of *Abcg5* and *Abcg8* is indeed induced in livers of LXR-treated mice. In a recent study, we found a relationship between rates of biliary cholesterol excretion and hepatic *Abcg5/Abcg8* expression in different mouse models of biliary cholesterol hypersecretion.[2] However, there are also models of cholesterol hypersecretion, most notably the diosgenin-fed mouse, in which *Abcg5/Abcg8* expression remains unaffected. Thus, direct proof for a role of these transporters in LXR-induced cholesterol hypersecretion will have to await studies in *Abcg5/Abcg8* knockout mice.

It has previously been reported that, besides its effects on genes involved in cholesterol transport, LXR activation also affects fatty acid metabolism by both SREBP1c-dependent (42) and -independent (30) mechanisms and causes hepatic steatosis (29). We did find dramatic increases in hepatic triglyceride content upon T0901317 treatment in all groups, including the *Abca1*$^{-/-}$ mice, at least partially accounting for the increases in liver weight/body weight ratios. Overall gene expression patterns were also similar to results reported by other groups (23, 29, 30, 43), although generally less pronounced induction upon T0901317 treatment was observed. However, for some genes (*e.g. Lpl* and phospholipid transfer protein), we did find high levels of induction (41). The modest increase in the other genes may be caused by a longer period of time between last dosage of the agonist and tissue sampling in our experiments or by different application modes (gavage *versus* diet). From the differences between C57BL/6J and DBA/1 wild-type mice and from quantitative differences between other studies (23, 29, 30, 43), it can furthermore be concluded that strain-specific factors may also be involved in reported differences in gene expression patterns upon LXR activation.

Because of its prominent position in controlling cholesterol homeostasis, pharmacological activation of LXR is currently being widely discussed as a promising tool to raise HDL, to improve RCT, and therefore to inhibit or prevent the development of atherosclerosis. In this study, we demonstrated that LXR activation by T0901317 leads to similarly increased rates of hepatobiliary cholesterol output and increased fecal sterol loss in wild-type and *Abca1*$^{-/-}$ mice. The underlying LXR-dependent mechanism is thus independent of (ABCA1-mediated) elevation of plasma HDL levels and the (putative) role of ABCA1 in intestinal cholesterol absorption. In addition, LXR activation is associated with stimulation of net cholesterol loss via the intestine, indicating the presence of additional pathways for direct removal of cholesterol from the body.

Acknowledgments—We thank Juul F. W. Baller, Renze Boverhof, Aldo Grefhorst, Anke ter Harmsel, Fjodor H. van der Sluijs, Henkjan J. Verkade, and Henk Wolters for excellent technical assistance and helpful discussions.

REFERENCES

1. Glomset, J. A., and Norum, K. R. (1973) *Adv. Lipid Res.* **11,** 1–65
2. Fielding, C. J., and Fielding, P. E. (1995) *J. Lipid Res.* **36,** 211–228
3. Barter, P. J., and Rye, K. A. (1996) *Curr. Opin. Lipidol.* **7,** 82–87
4. Dietschy, J. M., and Turley, S. D. (2002) *J. Biol. Chem.* **277,** 3801–3804
5. Attie, A. D., Kastelein, J. P., and Hayden, M. R. (2001) *J. Lipid Res.* **42,** 1717–1726
6. Oram, J. F. (2002) *Trends Mol. Med.* **8,** 168–173
7. Rust, S., Rosier, M., Funke, H., Real, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Duverger, N., Denefle, P., and Assmann, G. (1999) *Nat. Genet.* **22,** 352–355
8. Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Böttcher, A., Diederich, W., Drobnik, W., Barlage, S., Büchler, C., Porsch-Ozcurumez, M., Kaminski, W. E., Hahmann, H. W., Oette, K., Rothe, G., Aslanidis, C., Lackner, K. J., and Schmitz, G. (1999) *Nat. Genet.* **22,** 347–351
9. Brooks-Wilson, A., Marcil, M., Clee, S. M., Zhang, L. H., Roomp, K., van Dam, M., Yu, L., Brewer, C., Collins, J. A., Molhuizen, H. O., Loubser, O., Ouelette, B. F., Fichter, K., Ashbourne-Excoffon, K. J., Sensen, C. W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pimstone, S., Kastelein, J. J., and Hayden, M. R. (1999) *Nat. Genet.* **22,** 336–345
10. Bortnick, A. E., Rothblat, G. H., Stoudt, G., Hoppe, K. L., Royer, L. J., McNeish, J., and Francone, O. L. (2000) *J. Biol. Chem.* **275,** 28634–28640

[2] A. Kosters, R. J. J. M. Frijters, R. Ottenhoff, M. Jirsa, F. G. Schaap, I. M. de Cuyper, F. Kuipers, and A. K. Groen, submitted for publication.

11. Luciani, M. F., Denizot, F., Savary, S., Mattei, M. G., and Chimini, G. (1994) *Genomics* **21,** 150–159
12. Langmann, T., Klucken, J., Reil, M., Liebisch, G., Luciani, M. F., Chimini, G., Kaminski, W. E., and Schmitz, G. (1999) *Biochem. Biophys. Res. Commun.* **257,** 29–33
13. Wellington, C. L., Walker, E. K., Suarez, A., Kwok, A., Bissada, N., Singaraja, R., Yang, Y. Z., Zhang, L. H., James, E., Wilson, J. E., Francone, O., McManus, B. M., and Hayden, M. R. (2002) *Lab. Invest.* **82,** 273–283
14. Vaisman, B. L., Lambert, G., Amar, M., Joyce, C., Ito, T., Shamburek, R. D., Cain, W. J., Fruchart-Najib, J., Neufeld, E. D., Remaley, A. T., Brewer, H. B., Jr., and Santamarina-Fojo, S. (2001) *J. Clin. Invest.* **108,** 303–309
15. Edwards, P. A., Kast, H. R., and Anisfeld, A. M. (2002) *J. Lipid Res.* **43,** 2–12
16. Lu, T. T., Repa, J. J., and Mangelsdorf, D. J. (2001) *J. Biol. Chem.* **276,** 37735–37738
17. Schwartz, C. C., Halloran, L. G., Vlahcevic, Z. R., Gregory, D. H., and Swell, L. (1978) *Science* **200,** 62–64
18. Robins, S. J., and Fasulo, J. M. (1997) *J. Clin. Invest.* **99,** 380–384
19. Groen, A. K., Bloks, V. W., Bandsma, R. H. J., Ottenhoff, R., Chimini, G., and Kuipers, F. (2001) *J. Clin. Invest.* **108,** 843–850
20. Haghpassand, M., Bourassa, P. A., Francone, O. L., and Aiello, R. J. (2001) *J. Clin. Invest.* **108,** 1315–1320
21. Costet, P., Luo, Y., Wang, N., and Tall, A. R. (2000) *J. Biol. Chem.* **275,** 28240–28245
22. Venkateswaran, A., Laffitte, B. A., Joseph, S. B., Mak, P. A., Wilpitz, D. C., Edwards, P. A., and Tontonoz, P. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 12097–12102
23. Repa, J. J., Turley, S. D., Lobaccaro, J. A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R. A., Dietschy, J. M., and Mangelsdorf, D. J. (2000) *Science* **289,** 1524–1529
24. Schwartz, K., Lawn, R. M., and Wade, D. P. (2000) *Biochem. Biophys. Res. Commun.* **274,** 794–802
25. Willy, P. J., Umesono, K., Ong, E. S., Evans, R. M., Heyman, R. A., and Mangelsdorf, D. J. (1995) *Genes Dev.* **9,** 1033–1045
26. Teboul, M., Enmark, E., Li, Q., Wikstrom, A. C., Pelto-Huikko, M., and Gustafsson, J. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 2096–2100
27. Janowski, B. A., Willy, P. J., Devi, T. R., Falck, J. R., and Mangelsdorf, D. J. (1996) *Nature* **383,** 728–731
28. Janowski, B. A., Grogan, M. J., Jones, S. A., Wisely, G. B., Kliewer, S. A., Corey, E. J., and Mangelsdorf, D. J. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 266–271
29. Schultz, J. R., Tu, H., Luk, A., Repa, J. J., Medina, J. C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D., and Shan, B. (2000) *Genes Dev.* **14,** 2831–2838
30. Joseph, S. B., Laffitte, B. A., Patel, P. H., Watson, M. A., Matsukuma, K. E., Walczak, R., Collins, J. L., Osborne, T. F., and Tontonoz, P. (2002) *J. Biol. Chem.* **277,** 11019–11025
31. McNeish, J., Aiello, R. J., Guyot, D., Turi, T., Gabel, C., Aldinger, C., Hoppe, K. L., Roach, M. L., Royer, L. J., de Wet, J., Broccardo, C., Chimini, G., and Francone, O. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 4245–4250
32. Murphy, G. M., Billing, B. H., and Baron, D. N. (1970) *J. Clin. Pathol.* **23,** 594–598
33. Bligh, E., and Dyer, W. (1959) *Can. J. Biochem. Biophys.* **37,** 911–917
34. Böttcher, C., van Gent, C., and Pries, C. (1961) *Anal. Chim. Acta* **24,** 203–204
35. Gamble, W., Vaughan, M., Kruth, H. S., and Avigan, J. (1978) *J. Lipid Res.* **19,** 1068–1070
36. Arca, M., Montali, A., Ciocca, S., Angelico, F., and Cantafora, A. (1983) *J. Lipid Res.* **24,** 332–335
37. Setchell, K. D., Lawson, A. M., Tanida, N., and Sjovall, J. (1983) *J. Lipid Res.* **24,** 1085–1100
38. Voshol, P. J., Havinga, R., Wolters, H., Ottenhoff, R., Princen, H. M., Oude Elferink, R. P., Groen, A. K., and Kuipers, F. (1998) *Gastroenterology* **114,** 1024–1034
39. Bloks, V. W., Plösch, T., van Goor, H., Roelofsen, H., Baller, J. F. W., Havinga, R., Verkade, H. J., van Tol, A., Jansen, P. L. M., and Kuipers, F. (2001) *J. Lipid Res.* **42,** 41–50
40. Heid, C. A., Stevens, J., Livak, K. J., and Williams, P. M. (1996) *Genome Res.* **6,** 986–994
41. Grefhorst, A., Elzinga, B. M., Voshol, P. J., Plösch, T., Kok, T., Bloks, V. W., van der Sluijs, F. H., Havekes, L. M., Romijn, J. A., Verkade, H. J., and Kuipers, F. (July 3, 2002) *J. Biol. Chem.* 10.1074/jbc.M204887200
42. Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. (2000) *Genes Dev.* **14,** 2819–2830
43. Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., and Brown, M. S. (2002) *J. Biol. Chem.* **277,** 9520–9528
44. Berge, K. E., Tian, H., Graf, G. A., Yu, L., Grishin, N. V., Schultz, J., Kwiterovich, P., Shan, B., Barnes, R., and Hobbs, H. H. (2000) *Science* **290,** 1771–1775
45. Lee, M. H., Lu, K., Hazard, S., Yu, H., Shulenin, S., Hidaka, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A. K., Salen, G., Dean, M., and Patel, S. B. (2001) *Nat. Genet.* **27,** 79–83
46. Repa, J. J., Berge, K. E., Pomajzl, C., Richardson, J. A., Hobbs, H., and Mangelsdorf, D. J. (2002) *J. Biol. Chem.* **277,** 18793–18800
47. Bandsma, R. H. J., Stellaard, F., Vonk, R. J., Nagel, G. T., Neese, R. A., Hellerstein, M. K., and Kuipers, F. (1998) *Biochem. J.* **329,** 699–703
48. Osono, Y., Woollett, L. A., Herz, J., and Dietschy, J. M. (1995) *J. Clin. Invest.* **95,** 1124–1132
49. Jolley, C. D., Woollett, L. A., Turley, S. D., and Dietschy, J. M. (1998) *J. Lipid Res.* **39,** 2143–2149
50. Xie, C., Turley, S. D., and Dietschy, J. M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 11992–11997
51. Jolley, C. D., Dietschy, J. M., and Turley, S. D. (1999) *Am. J. Physiol.* **276,** G1117–G1124
52. Xie, C., Turley, S. D., Pentchev, P. G., and Dietschy, J. M. (1999) *Am. J. Physiol.* **276,** E336–E344
53. Osono, Y., Woollett, L. A., Marotti, K. R., Melchior, G. W., and Dietschy, J. M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 4114–4119
54. Alam, K., Meidell, R. S., and Spady, D. K. (2001) *J. Biol. Chem.* **276,** 15641–15649
55. 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) *J. Biol. Chem.* **272,** 3137–3140
56. Noshiro, M., Nishimoto, M., and Okuda, K. (1990) *J. Biol. Chem.* **265,** 10036–10041
57. Verkade, H. J., Vonk, R. J., and Kuipers, F. (1995) *Hepatology* **21,** 1174–1189
58. Shimomura, I., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) *J. Clin. Invest.* **99,** 838–845

Increased Hepatobiliary and Fecal Cholesterol Excretion upon Activation of the Liver X Receptor Is Independent of ABCA1

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J. Biol. Chem. 2002, 277:33870-33877.
doi: 10.1074/jbc.M206522200 originally published online July 8, 2002
