with viscosity of $2 \times 10^{16}$) produces an acceptable fit to the data (not shown) and general similarity with the inverted slip distribution (Fig. 3, C and D).
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# Regulation of Absorption and ABC1-Mediated Efflux of Cholesterol by RXR Heterodimers

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**Several nuclear hormone receptors involved in lipid metabolism form obligate heterodimers with retinoid X receptors (RXRs) and are activated by RXR agonists such as rexinoids. Animals treated with rexinoids exhibited marked changes in cholesterol balance, including inhibition of cholesterol absorption and repressed bile acid synthesis. Studies with receptor-selective agonists revealed that oxysterol receptors (LXRs) and the bile acid receptor (FXR) are the RXR heterodimeric partners that mediate these effects by regulating expression of the reverse cholesterol transporter, ABC1, and the rate-limiting enzyme of bile acid synthesis, CYP7A1, respectively. Thus, these RXR heterodimers serve as key regulators of cholesterol homeostasis by governing reverse cholesterol transport from peripheral tissues, bile acid synthesis in liver, and cholesterol absorption in intestine.**

Several orphan nuclear hormone receptors regulate cholesterol homeostasis. The liver X receptors (LXRα/NR1H3 and LXRβ/NR1H2) and farnesoid X receptor (FXR/NR1H4) (*1*) are bound and activated by oxysterols and bile acids, respectively, and regulate the expression of genes involved in cholesterol metabolism [reviewed in (*2*–*4*)]. The LXRs mediate oxysterol-regulated transcriptional induction of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme of the classic bile acid biosynthetic pathway. *Lxr*α-knockout mice fed a high cholesterol diet fail to up-regulate CYP7A1 expression and bile acid production, thereby accumulating cholesterol ester in their livers (*5*). This phenotype is exacerbated in *Lxr*α/β double-knockout mouse strains (*6*). Additional LXR target genes involved in lipid metabolism include the human cholesterol ester transfer protein (CETP), which translocates cholesterol ester between lipoprotein fractions (*7*), and the murine adenosine triphosphate–binding cassette transporter 8 (ABC8), which has been implicated in macrophage lipid flux (*8*). FXR mediates bile acid–dependent repression of the CYP7A1 gene (*9*, *10*) by inducing the transcription of small heterodimer partner (SHP) (*11*), an orphan receptor that is a transcriptional repressor of CYP7A1 expression (*12*). In a similar manner, FXR also regulates expression of several proteins involved in bile acid metabolism, including sterol 12α-hydroxylase (CYP8B1), which controls the relative production of the primary bile acids, the intestinal bile acid binding protein, and the phospholipid transfer protein (*9*, *13*, *14*).

LXRs and FXR form obligate heterodimers with retinoid X receptors (RXRs) and belong to a subclass of nuclear hormone receptors that are activated by RXR agonists. We exploited this characteristic in animal studies by administering rexinoid LG268, a highly specific RXR ligand (*15*), to identify nuclear receptor-mediated changes in cholesterol balance and the receptor-regulated genes responsible for those changes. This approach had several advantages: It allowed a more global analysis of nuclear receptor-mediated events by allowing simultaneous assessment of several permissive receptors including LXRs, FXR, and the peroxisome proliferator-activated receptors (PPARs); it avoided complications that would arise from the use of oxysterols to activate LXR, because these compounds also affect the SREBP signaling pathway (*16*); and the effective doses and pharmacokinetics of LG268 had been established previously (*17*). Here, we show that rexinoids affect cholesterol absorption, transport, and catabolism in rodents through at least two pathways that regulate the transcription of key target genes. In one pathway, activation of the RXR/FXR heterodimer repressed CYP7A1 expression, resulting in decreased bile acid synthesis and cholesterol absorption. In the second pathway, activation of the RXR/LXR heterodimer by either a rexinoid or LXR agonist effectively blocked cholesterol absorption and induced reverse cholesterol transport in peripheral tissues. This effect was coincident with the increased expression in intestine and macrophages of ABC1, a gene product responsible for efflux of cellular free cholesterol (*18*–*20*).

**Rexinoid effects on cholesterol homeostasis.** Administration of the rexinoid LG268 to mice resulted in dose-dependent inhibition of cholesterol absorption (Fig. 1A). A fecal dual-isotope method was used to monitor cholesterol absorption (*21*), which measures the ratio of fecal excretion of [$^3$H]sitostanol (which is not absorbed) to [$^{14}$C]cholesterol (which is variably absorbed). LG268 at a dose of 1.4 mpk (equivalent to 0.0007% of the diet) resulted in a 50% reduction in cholesterol absorption, and a dose of 14 mpk resulted in complete inhibition. In addition, rexinoid treatment prevented the appearance of [$^{14}$C]cholesterol in the serum, confirming that no dietary cholesterol was transported across the enterocyte into the circulation (*22*). LG268 also inhibited cholesterol absorption in the Golden Syrian hamster, with a reduction of 37% at 3 mpk LG268 and 76% at 30 mpk LG268. Thus, the effect of rexinoid on cholesterol absorption was not species specific.

At all doses tested, decreased absorption elicited by rexinoid prevented accumulation of liver cholesterol, even when mice were fed a high (0.2%) cholesterol diet (Fig. 1B). In addition, total serum cholesterol levels were not elevated by rexinoid administration, but the relative level of high-density lipoprotein (HDL) cholesterol increased (Fig. 1C). The effect of LG268 was specific for cholesterol and had no consequence on general lipid absorption, mostly triacylglycerol (Fig. 1D). Decrease in lipid absorption observed at higher doses of rexinoid can be accounted for by the contribution of

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cholesterol to the total lipid measured in the assay. In addition, there was no evidence of steatorrhea. Inhibition of cholesterol absorption occurred at concentrations that fail to activate the PPARα signaling pathway and cause hepatomegaly in mice (Fig. 1E) (*23*). Therefore, inhibition of cholesterol absorption is the most potent activity yet observed for rexinoids, surpassing the dose-equivalent effects on glucose homeostasis, lipid metabolism, and cancer chemoprevention (*17*, *23*, *24*). Most other cholesterol absorption inhibitors, including surfomer, saponins, sitostanol, and neomycin, require much higher doses to be effective [reviewed in (*25*)]. The cholesterol inhibitors SCH58235 and SCH60663 are effective at very low doses, although their mechanism of action is unknown (*26*).

The rexinoid block in cholesterol absorption effectively deprived animals of exogenous cholesterol, and as a consequence, mice showed a compensatory increase in sterol synthesis to maintain normal physiological levels of tissue cholesterol (Fig. 2, A and B). This compensation is particularly impressive because these animals were provided diets containing cholesterol at an amount that is 10 times as high as that of standard rodent chow. This amount of cholesterol would normally suppress endogenous sterol synthesis. Nevertheless, in spite of high dietary cholesterol, sterol synthesis was increased, as was the expression of genes [HMG coenzyme A (CoA) synthase, HMG CoA reductase, and the LDL receptor] known to be up-regulated by cellular sterol depletion through the SREBP pathway (Fig. 2C) (*16*). The conclusion that up-regulation of these genes is due to sterol depletion and not direct action of rexinoid is supported by the finding that shorter term experiments of 6- to 12-hour rexinoid exposure did not cause up-regulation of gene expression, although other known primary target genes of LG268 were modulated (discussed below).

**Rexinoids affect cholesterol absorption by two mechanisms: repression of bile acid synthesis and induction of reverse cholesterol transport.** Inspection of the small intestine of mice treated with 30 mpk LG268 for 10 days showed no change in wet weight, and histological analyses did not reveal any alterations in villus length or architecture nor any accumulation of lipid (*22*). A structurally similar analog of LG268 that does not bind or activate RXR (*22*), which should similarly affect the physicochemical environment of the intestinal lumen, had no effect on cholesterol absorption or liver cholesterol accumulation, supporting the hypothesis that LG268 acts as an RXR agonist to modify the expression of genes involved in cholesterol absorption.

Inhibition of cholesterol absorption and compensatory increases in sterol synthesis have been observed previously in animals

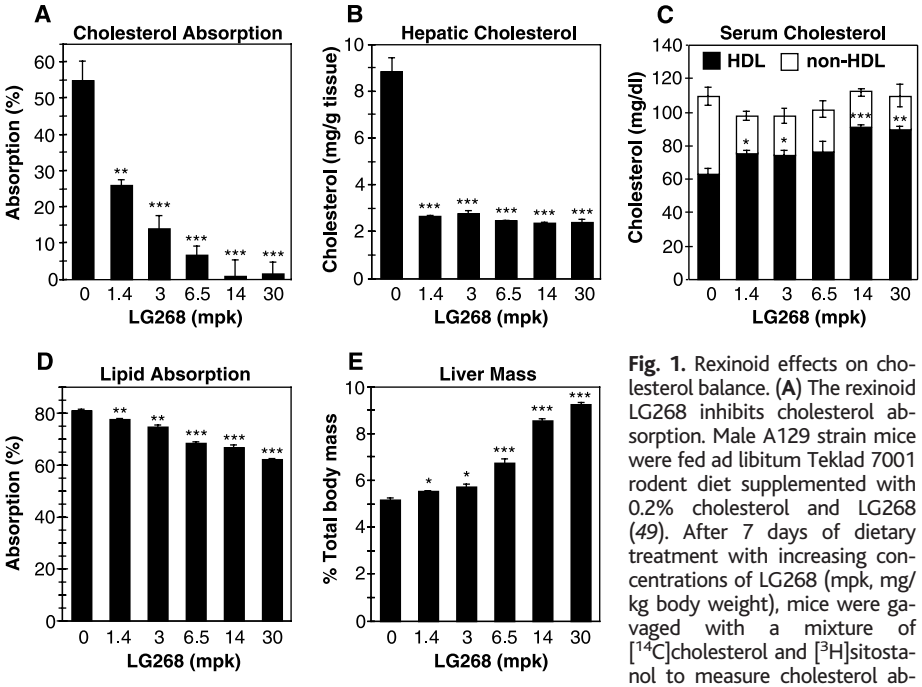


**Fig. 1.** Rexinoid effects on cholesterol balance. (**A**) The rexinoid LG268 inhibits cholesterol absorption. Male A129 strain mice were fed ad libitum Teklad 7001 rodent diet supplemented with 0.2% cholesterol and LG268 (*49*). After 7 days of dietary treatment with increasing concentrations of LG268 (mpk, mg/kg body weight), mice were gavaged with a mixture of [$^{14}$C]cholesterol and [$^{3}$H]sitostanol to measure cholesterol absorption by the fecal dual-isotope method, as described (*21*). (**B**) LG268 inhibits dietary cholesterol accumulation in the liver. Liver cholesterol levels were determined by gas chromatography against an internal standard (*21*). (**C**) LG268 increases HDL cholesterol concentrations in serum. Serum lipoprotein profiles were performed by size-exclusion chromatography followed by the determination of cholesterol levels in HDL (filled bar) and non-HDL (open bar) fractions. (**D**) LG268 inhibits absorption of cholesterol, but not other lipids. Total lipid absorption was determined gravimetrically (*21*). The decrease in total lipid absorption is due entirely to the absence of the cholesterol component in the total lipid measured. (**E**) LG268 effects on liver mass. Values in (A) to (E) reflect the mean ± SEM ($n = 5$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with the vehicle-treated control sample (Student's $t$ test). These observations were confirmed in a minimum of three experiments.

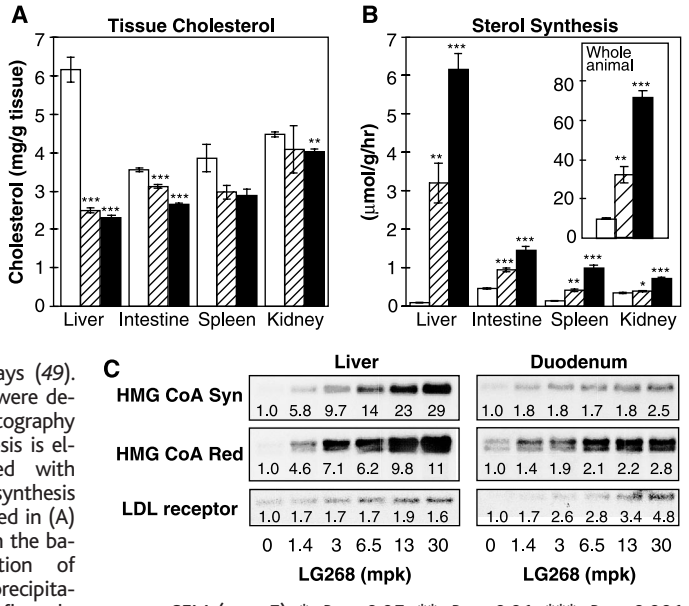


**Fig. 2.** Rexinoid inhibition of cholesterol absorption leads to a compensatory increase in tissue sterol synthesis. (**A**) LG268 prevents the accumulation of dietary cholesterol in peripheral tissues. Male A129 strain mice were fed diets supplemented with 0.2% cholesterol plus vehicle (open bar) or LG268 at 3 mpk (hatched bar) or 30 mpk (filled bar) for 7 days (*49*). Tissue cholesterol levels were determined by gas chromatography (*21*). (**B**) Sterol biosynthesis is elevated in mice treated with LG268. Rates of sterol synthesis for the same mice depicted in (A) were measured in vivo on the basis of the incorporation of [$^{3}$H]$H_2O$ into digitonin-precipitable sterols (*21*). Values reflect the mean ± SEM ($n = 5$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with the vehicle-treated sample (Student's $t$ test). (**C**) The mRNA expression levels of genes involved in cholesterol biosynthesis and uptake are similarly increased in the liver and duodenal mucosa of LG268-treated mice. Northern analyses were performed as described (*5*) with [$^{32}$P]-labeled cDNA probes (*48*). Results were quantified by phosphorimager, standardized against actin (*22*), and mathematically adjusted to yield a unit of 1 for the group receiving the control diet. Fold inductions are shown under each lane.

with deficient bile acid production such as the *Cyp7a1*−/− and *Cyp27*−/− mice (*21*, *22*). Indeed, LG268 treatment altered the composition and size of the bile acid pool to disfavor cholesterol absorption (column 2, Fig. 3, A and B). LG268 also repressed expression of CYP7A1 and CYP7B1 (Fig. 3C), the two rate-limiting enzymes in bile acid synthesis. RXR/FXR is the permissive nuclear receptor heterodimer that mediates rexinoid- and bile acid–induced repression of CYP7A1 (*12*) (see also below). This suggested that one mechanism of rexinoid inhibition of cholesterol absorption is through activating the RXR/FXR heterodimer, thereby depleting bile acids, which are essential for cholesterol absorption. To determine the contribution of the FXR pathway to the inhibition of cholesterol absorption, we supplied rexinoid-treated mice with diets containing 0.1% cholic acid, a primary bile acid with superior cholesterol solubilization properties (*27*). This concentration of cholic acid increased bile acid pool size to more than compensate for the loss due to rexinoid treatment (third and fourth columns, Fig. 3A). However, restoring the bile acid pool only alleviated 50% of the inhibitory effect of LG268 on cholesterol absorption (fourth column, Fig. 3B). Therefore, although one effect of LG268 on cholesterol uptake is due to a decrease in bile acid synthesis through RXR/FXR, other pathways must exist.

To identify other rexinoid-regulated pathways involved in the uptake and processing of cholesterol in the small intestine, we performed short-term experiments (12 hour, 30 mpk LG268) in mice and assessed the expression of candidate genes (Fig. 4). None of the proteins implicated in intestinal cholesterol uptake (SR-B1 and caveolin), esterification (ACAT-1 and 2), and packaging into chylomicrons (apo B and MTP) exhibited a change in transcriptional regulation by rexinoid (Fig. 4) (*22*, *28*). In contrast, expression of ABC1 mRNA increased along the cephalocaudal axis of the intestine in a distinct pattern that correlates with the localization of cholesterol absorption in the rodent small intestine (*29*). ABC1 is an energy-dependent transporter located in the plasma membrane and is involved in the cellular efflux of unesterified cholesterol (*30*, *31*). These data suggest that the induction of ABC1 expression results in the inhibition of cholesterol absorption by exporting free cholesterol from inside the enterocyte into the intestinal lumen. This hypothesis is supported by two recent findings that show that mice lacking *Abc1* have increased cholesterol absorption efficiency (*32*) and chickens with an ABC1 mutation accumulate cholesterol esters in their enterocytes (*33*).

**RXR/LXR regulation of ABC1 expression.** To identify the RXR heterodimer partner responsible for inducing ABC1 expression, we performed short-term experiments using receptor-selective agonists. The receptors were chosen on the basis of their known expression in the intestine and their ability to function as permissive RXR partners. Dietary administration of agonists for RXRs (LG268), PPARα (fenofibrate), PPARγ (troglitazone), PXR (pregnenolone α-carbonitrile), FXR (chenodeoxycholic acid), and a nonsteroidal LXR agonist, *N*-(2,2,2-trifluoro-ethyl)-*N*-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide (T0901317) were used for 12-hour dosing experiments in mice. The identification of T0901317 as an LXR agonist was accomplished by cell reporter assays to establish receptor specificity (Fig. 5A) and potency [median effective concentration ($EC_{50}$) ~50 nM] (*22*) and coactivator interaction studies to demonstrate ligand binding [dissociation constant ($K_d$) ~ 50 nM] (*22*). The complete characterization of T0901317 will be described in detail elsewhere. The efficacy of the different agonists at doses used in these experiments was confirmed by measuring the expression of *Cyp7a1*, a gene that is known to be regulated by these nuclear receptors (Fig. 5B). Each agonist produced its predicted response, indicating that it was sufficiently ab-

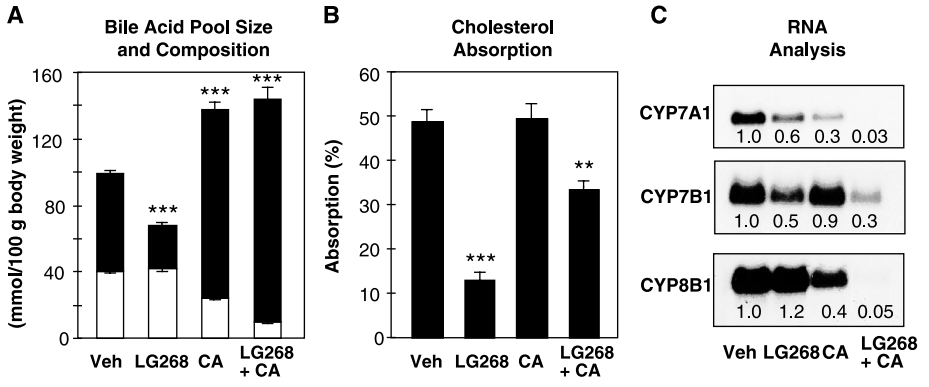


**Fig. 3.** Effects of LG268 and cholic acid treatment on bile acid pool size and cholesterol absorption. (**A**) LG268 decreases cholic acid pool size. Male, A129 strain mice received diets containing 0.2% cholesterol and vehicle (Veh), 3 mpk LG268, or 0.1% cholic acid (CA) for 10 days. Bile acids from the gall bladder, entire small intestine and its contents, and the bulk of the liver were extracted and analyzed by high-performance liquid chromatography, as described (*21*, *49*). The filled bars show the fraction of the bile acid pool that corresponds to cholic acid. (**B**) Inhibition of cholesterol absorption by LG268 is only partially reversed by restoring cholic acid levels. After 7 days of dietary treatment, cholesterol absorption was determined in animals of (A), as described (*21*). Values reflect the mean ± SEM ($n$ = 6). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with the vehicle-treated sample. (**C**) Northern analyses were performed on pooled liver mRNA (5 μg/lane) samples from animals in (A) as described in Fig. 1 with cited probes (*50*). Relative mRNA expression levels are shown under each lane.

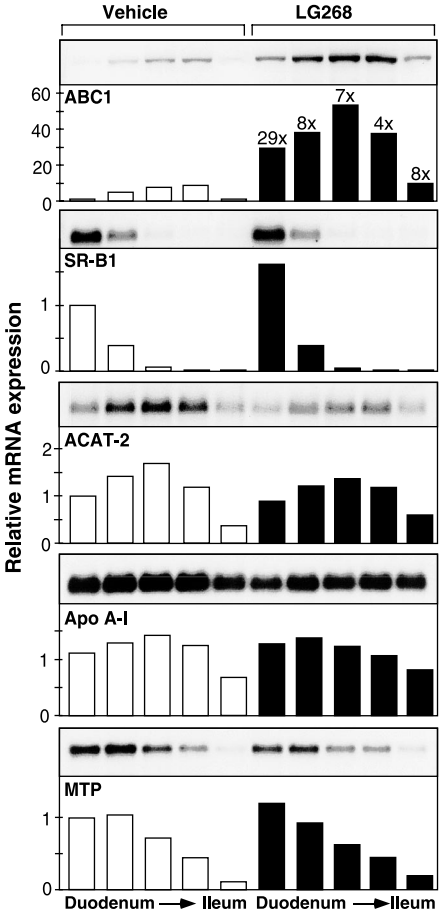


**Fig. 4.** Regulation of gene expression by LG268 in small intestine. Mice were fed diets containing 0.2% cholesterol plus vehicle (open bar) or 30 mpk LG268 (filled bar). Twelve hours later, mice were anesthetized and exsanguinated, and the small intestine was flushed and divided into five segments of equal length (from duodenum to ileum) for isolation of mucosal RNA. Equivalent amounts of mRNA from four mice per treatment were pooled and subjected to Northern blot analysis (5 μg/lane), as described (*5*), with the indicated probes (*51*). The bar graphs show relative mRNA levels standardized to actin controls (*22*). Numbers above bars in the ABC1 panel refer to fold increases in mRNA due to LG268 treatment for each intestine segment examined.

sorbed, circulated, and capable of affecting the expression of primary target genes within the 12-hour dosing regimen. ABC1 expression was selectively up-regulated only by the RXR and LXR agonists, suggesting that the RXR/LXR heterodimer regulates ABC1 transcription (Fig. 5C). Dietary cholesterol, a source of oxysterol ligands for LXR (*5*), also up-regulated expression of ABC1 mRNA in the intestine (*22*). Of interest, both LXRα and LXRβ mRNAs are detected in mouse small intestine in a cephalocaudal distribution that correlates with ABC1 expression and the localization of cholesterol absorption (*22*, *29*). RXRα mRNA levels are also abundant in the intestine, whereas RXRβ transcripts are present at low levels, and RXRγ is undetectable (*22*).

**ABC1 regulation in macrophages.** To explore the possibility that LXR and RXR agonists caused a systemic effect in mice to elicit changes in ABC1 expression, we performed experiments on isolated macrophages obtained from mice of various *Lxr* genotypes (Fig. 6). Macrophages regulate ABC1 expression as a function of intracellular sterol status (*31*, *34*) and are dependent on reverse cholesterol transport to maintain sterol homeostasis (*35*). When mouse peritoneal macrophages were treated in culture with compactin (sterol-unloaded) or the physiologic LXR ligand 22(*R*)-hydroxycholesterol (sterol-loaded), ABC1 mRNA expression levels increased by greater than 30-fold in an oxysterol- and LXR-dependent manner (Fig. 6A). This induction was absent in macrophages devoid of both LXRα and β (LXRα/β−/−), demonstrating the essential role of LXRs in the sterol-mediated induction of ABC1 expression. Both LXRα and LXRβ are expressed in wild-type mouse macrophages (*8*), and the results seen in macrophages isolated from either *Lxr*α or *Lxr*β knockout mice suggest that the two LXR genes are functionally redundant in this cell type. Elimination of both LXRs resulted in an increase in the basal expression level of ABC1, suggesting that LXRs exert transcriptional repression of this gene in the absence of ligand, a phenomenon described for other nuclear hormone receptors (*36*).

Treating macrophages with the LXR-selective agonist T0901317 also resulted in the same LXR-dependent up-regulation of ABC1 expression (Fig. 6A). T0901317 treatment did not affect the intracellular sterol status of these macrophages because expression of HMG CoA synthase, a gene repressed by sterol loading through the SREBP signaling pathway, was not altered (Fig. 6B), yet ABC1 expression was induced (Fig. 6A). This suggests that in contrast to previous reports (*31*), sterol loading per se is not the condition that regulates ABC1 expression or sterol efflux in mac-

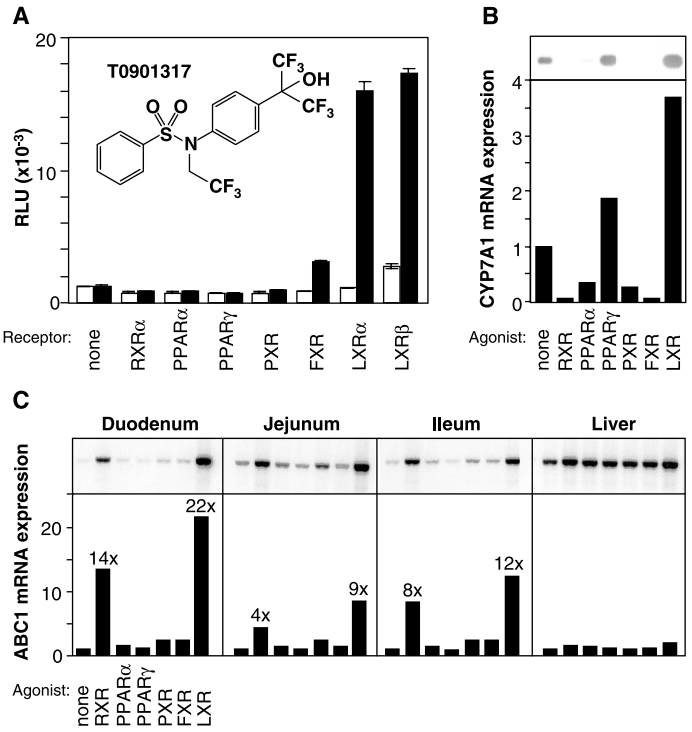


**Fig. 5.** The RXR/LXR heterodimer regulates ABC1 expression. (**A**) Structure of T0901317 and its characterization as an LXR-selective agonist. Transient transfection assays were performed in HEK293 cells with Gal4-receptor chimeras, as described (*9*). Cells were treated with 1 μM T0901317 (filled bar) or vehicle (dimethyl sulfoxide, open bar) for 16 hours and harvested to measure luciferase and β-galactosidase (β-Gal) activities. Transfection data were normalized to β-Gal, expressed as relative light units (RLU), and represent triplicate assays ± SEM. (**B**) Effects of various nuclear receptor agonists on CYP7A1 gene expression. Mice were fed diets containing 0.2% cholesterol plus vehicle (none) or the following agonists for 12 hours: 30 mpk LG268 (RXR), 0.5% fenofibrate (PPARα), 150 mpk troglitazone (PPARγ), 0.05% pregnenolone α-carbonitrile (PXR), 0.5% chenodeoxycholic acid (FXR), or 50 mpk T0901317 (LXR). Northern analysis was performed on mRNA pooled from four mice per treatment, as described in Fig. 2. (**C**) RXR/LXR regulates the expression of ABC1 in mouse small intestine. mRNA prepared from the mucosa of equal-sized intestine segments of the mice described in (B) were used for Northern analysis by the method detailed in Fig. 2. Numbers above bars refer to fold increases in mRNA due to treatment with RXR or LXR agonists.

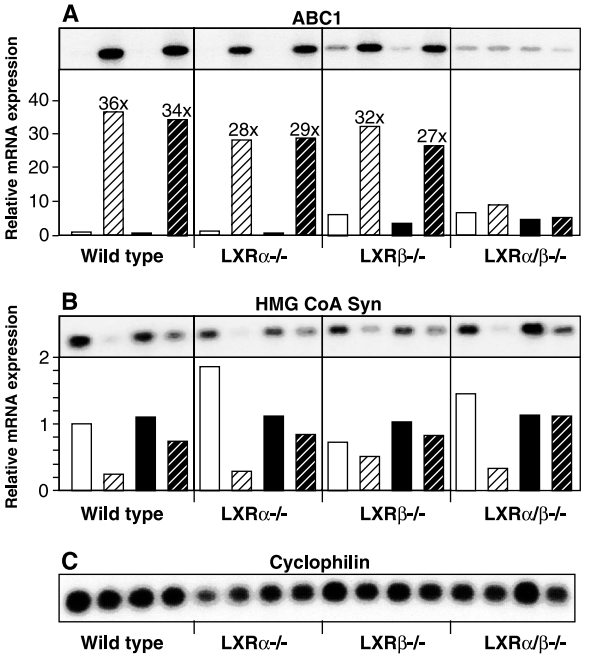


**Fig. 6.** LXR regulation of ABC1 expression in isolated mouse peritoneal macrophages. Peritoneal macrophages were obtained from thioglycolate-injected mice of wild-type, *Lxr*α−/−, *Lxr*β−/−, or *Lxr*α/β−/− genotypes (*5*, *6*, *8*). Cells were cultured for 42 hours in the presence of 10% lipoprotein-deficient serum containing 100 μM mevalonic acid as control (open bar) or mevalonic acid plus 5 μM compactin (filled bar), 10 μM 22(*R*)-hydroxycholesterol (open hatched bar), or 10 μM T0901317 (filled hatched bar). Total RNA was prepared and Northern analyses were performed. Results shown are representative of two independent experiments. (**A**) Oxysterol- and T0901317-dependent induction of ABC1 expression requires the presence of either LXRα or β. Numbers above bars refer to fold increases in mRNA due to treatment with oxysterols or T0901317. (**B**) HMG CoA synthase expression is repressed by oxysterol but is relatively unaffected by the LXR agonist T0901317. (**C**) Cyclophilin mRNA expression was determined for Northern blots in (A) and (B) and used as a loading control to establish relative mRNA levels. All mRNA bands were quantitated by phosphorimager.

rophages, but rather it is the availability of sterol-derived LXR ligands in lipid-loaded cells that regulates ABC1 expression. A putative RXR/LXR binding motif has been identified in the promoter of the human ABC1 gene (*37*), confirming that ABC1 is a direct target gene of RXR/LXR activation. Because lipid accumulation in macrophages can lead to the development of foam cells (*38*), our studies implicate the regulation of ABC1 expression by RXR/LXR as an important therapeutic target in preventing atherogenesis.

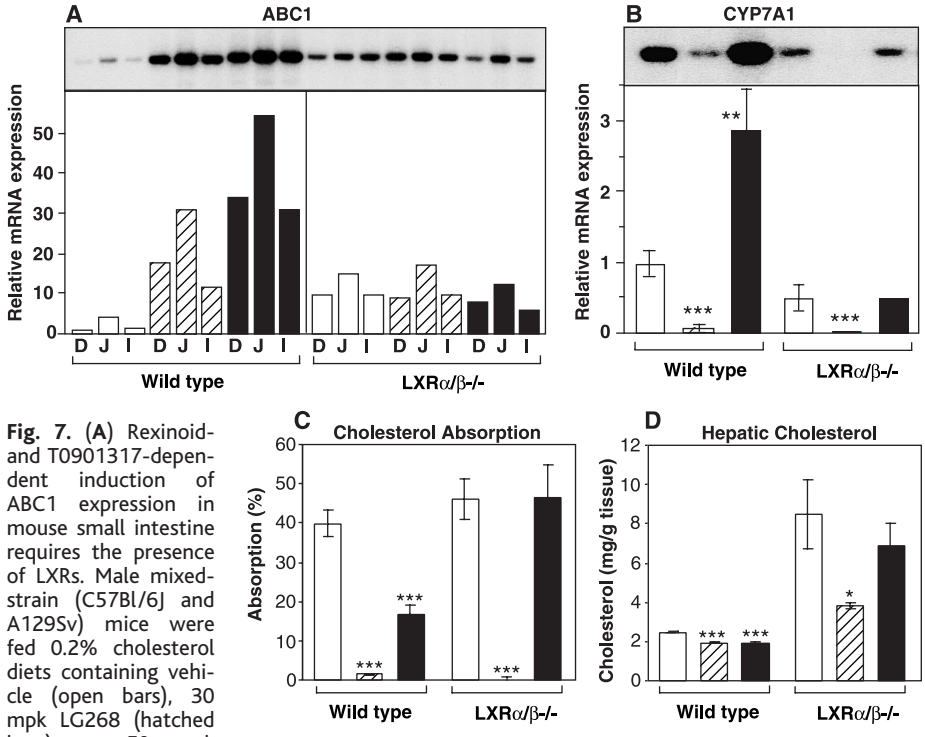


**Fig. 7.** (**A**) Rexinoid- and T0901317-dependent induction of ABC1 expression in mouse small intestine requires the presence of LXRs. Male mixed-strain (C57Bl/6J and A129Sv) mice were fed 0.2% cholesterol diets containing vehicle (open bars), 30 mpk LG268 (hatched bars), or 50 mpk T0901317 (filled bars) for 12 hours. mRNA was isolated from intestinal mucosa from the proximal third (duodenum, D), medial third (jejunum, J), and distal third (ileum, I) and pooled from the four mice of each treatment for Northern analysis, as described in Fig. 2. (**B**) Rexinoid-induced repression of CYP7A1 is independent of *Lxr* genotype. Northern analysis was performed on liver mRNA from individual mice as described in (A). (**C**) Inhibition of cholesterol absorption and (**D**) hepatic cholesterol accumulation by T0901317 requires LXRs. The RXR ligand LG268 continues to inhibit absorption by its ability to repress bile acid biosynthesis through an LXR-independent pathway. Male mice were fed 0.2% cholesterol diets containing 30 mpk LG268 or 50 mpk T0901317 for 10 days. Liver cholesterol concentrations and cholesterol absorption were determined (*21*). Values reflect the mean ± SEM ($n = 6$) and for relative measurements are compared with the vehicle-treated sample of wild-type mice [duodenum in (A)]. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

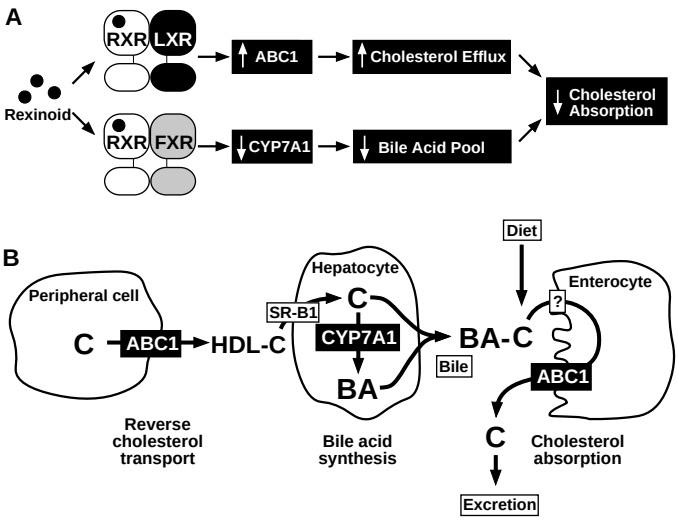


**Fig. 8.** (**A**) Rexinoids inhibit cholesterol absorption by two mechanisms, induction of ABC1 expression by RXR/LXR and repression of CYP7A1 expression by RXR/FXR. (**B**) Model of transcriptional control of cholesterol homeostasis by the RXR/LXR heterodimer through increased reverse cholesterol transport, enhanced bile acid biosynthesis, and inhibition of cholesterol absorption. LXR target genes (ABC1 and CYP7A1) are shown in black boxes. Localization of ABC1 to the apical membrane of the enterocyte is tentative.

**Cholesterol absorption and ABC1 expression in *Lxrα*/β-deficient mice.** To test the requirement for LXRs in the regulation of absorption and ABC1 expression in the mouse small intestine, we treated wild-type and *Lxrα*/β double-knockout mice with RXR- and LXR-selective agonists (Fig. 7). An increase in ABC1 expression was observed in the small intestine of only wild-type mice (Fig. 7A), and both LG268 and T0901317 inhibited cholesterol absorption (Fig. 7C) and prevented liver cholesterol accumulation (Fig. 7D) in these mice. The LXR agonist had no effect in the double-knockout mice, yet LG268 continued to show inhibition of absorption (discussed below). The increase in hepatic cholesterol levels observed in *Lxrα*/β double-knockout mice fed the 0.2% cholesterol diet (vehicle, Fig. 7D) results from the failure to up-regulate CYP7A1 expression and hence bile acid synthesis (Fig. 7B) (*5*, *6*). No cholesterol absorption inhibition was observed in *Lxrα*/β double-knockout mice treated with the LXR agonist (Fig. 7C). These studies support the hypothesis that the second mechanism by which rexinoid inhibits cholesterol absorption is mediated by the LXR-dependent up-regulation of ABC1 expression in small intestine. *Abc1* knockout mice show an increase in cholesterol absorption (*32*) that is diametrically equivalent to the decrease in absorption by the LXR agonist, supporting the hypothesis that increased intestinal ABC1 inhibits cholesterol absorption and that the RXR/LXR heterodimer modulates cholesterol absorption through the regulation of this gene. As in isolated macrophages, elimination of both LXRs resulted in increased basal expression of ABC1, which may explain why *Lxr* knockout mice do not show a greater difference in absorption from the wild-type mice when fed high dietary cholesterol (compare vehicle lanes in Fig. 7C).

**Dual mechanism of rexinoid action.** The difference in the inhibition of cholesterol absorption in wild-type versus *Lxr* double-knockout mice receiving the RXR agonist (Fig. 7C) deserves additional consideration. In the wild-type mouse, LG268 effectively induced ABC1 expression (Fig. 7A) and inhibited cholesterol absorption (Fig. 7C). LG268 also repressed CYP7A1 expression (Fig. 7B) and bile acid production (Fig. 3), which led to a failure to solubilize and absorb cholesterol. This latter effect of LG268 was LXR independent (Fig. 7B) and was responsible for the continued inhibition of cholesterol absorption in the *Lxr* double-knockout mouse (Fig. 7C). RXR/FXR is the permissive nuclear receptor heterodimer responsible for this

repression of CYP7A1, and recent studies have shown that RXR/FXR-mediated repression of CYP7A1 is dominant over RXR/LXR-mediated induction of CYP7A1, which explains why the rexinoid represses rather than activates CYP7A1 (*12*). In summary, the rexinoid LG268 inhibits cholesterol absorption by at least two mechanisms (Fig. 8A): (i) RXR/LXR-mediated up- regulation of ABC1 to increase cholesterol efflux by the intestine and (ii) RXR/FXR-mediated repression of CYP7A1 to effectively reduce the intestinal bile acid pool necessary to solubilize cholesterol for absorption.

**The role of LXRs in cholesterol homeostasis.** Cholesterol homeostasis is maintained by a series of regulated pathways controlling the acquisition of cholesterol from endogenous and exogenous sources and the elimination of cholesterol, through conversion to bile acids. Despite numerous advances that have led to an understanding of endogenous cholesterol homeostasis, the mechanism regulating net cholesterol uptake by the intestine has remained unknown. Several observations have suggested that a protein-facilitated mechanism for cholesterol uptake at the brush border membrane may exist: (i) cholesterol uptake appears to be a saturable process that is sensitive to protease treatment (*39*); (ii) sterol uptake shows exquisite selectivity whereby cholesterol is readily absorbed but structurally similar phytosterols are not (*40*); and (iii) the large variation in cholesterol absorption efficiency observed in the human population (*41*), and seen between various strains of mice (*42*), suggests a genetic component to the absorption process. These observations have sparked renewed interest in the possibility that cholesterol uptake by the enterocyte can be regulated by pharmacologic agents (*25*, *43*).

Results presented here strongly support the hypothesis that intestinal ABC1 serves to efflux free cholesterol from the enterocyte back into the intestinal lumen, thereby modulating net cholesterol absorption efficiency. Furthermore, cholesterol absorption and the transcriptional regulation of ABC1 gene expression are mediated by the RXR/LXR heterodimer. Hence, a broader role for RXR/LXR regulation of whole body cholesterol elimination can now be envisioned (Fig. 8B). Activation of the LXR signaling pathway results in the up-regulation of ABC1 in peripheral cells, including macrophages (Fig. 6A), to efflux free cholesterol for transport back to the liver through HDL (Fig. 1C), where it is converted to bile acids by the LXR-mediated increase in CYP7A1 expression (Figs. 5B and 7B) (*5*). Secretion of biliary cholesterol in the presence of increased bile acid pools would normally result in enhanced reabsorption of cholesterol; however, with the increased expression of ABC1 (Fig. 7A) and efflux of cholesterol back into the lumen, there is a reduction in cholesterol absorption (Fig. 7C) and net excretion of cholesterol and bile acids. Our results are consistent with the phenotype exhibited in three different species (human, mouse, and chicken) with *ABC1*-null mutations (*18*, *19*, *32*, *33*, *44*, *45*). Cumulatively, these phenotypes display decreased serum HDL levels, reduced cellular cholesterol efflux, and increased cholesterol absorption and deposition in enterocytes. One question that remains is whether ABC1 alone is sufficient and required or whether other proteins are integral to this process. Future studies combining nuclear receptor mutants with transgenic animals overexpressing ABC1 should provide useful tools for further dissecting this pathway.

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49. Mice were fed ad libitum Teklad 7001 rodent diet supplemented with 0.2% cholesterol and LG268 or vehicle. Animals were housed in temperature-controlled rooms (22°C) with 12-hour light/dark cycles. For studies involving the measurement of bile acids and enzymes/RNA of the bile acid biosynthetic enzymes, mice were killed 2 hours before the end of the dark cycle to minimize effects due to the diurnal rhythm of this system. Animal experiments were approved by the Institution Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center. A 30 mg/kg of body weight (mpk) dose was achieved by the addition of LG268 at 0.015% of the diet. LG268 was solubilized at 4.5 mg/ml in a vehicle containing 0.9% carboxymethylcellulose, 9% PEG-400, and 0.05% Tween 80. Control animals received diet supplemented with vehicle.
50. Northern blots were hybridized with [$^{32}$P]-labeled cDNA probes for cholesterol 7α-hydroxylase (CYP7A1) (*46*), oxysterol 7α-hydroxylase (CYP7B1) (*47*), and sterol 12α-hydroxylase [CYP8B1, generated by reverse transcription polymerase chain reaction (RT-PCR) with primers 5′-GGGTACCAGTCTGTAGATGG and 5′-AGTCTCTGGTGGAAGAGACG based on the mouse sequence detailed in GenBank accession number AF090317].
51. [$^{32}$P]-labeled cDNA probes were apolipoprotein A-I (Apo A-I) (*48*), scavenger receptor-B1 (SR-B1) from H. Hobbs; acyl CoA:cholesterol acyltransferase-2 (ACAT-2) from R. Farese Jr.; mouse ABC1 generated by RT-PCR with primers 5′-TCTCGCCTGTTCTCAGACGC and 5′-CTCCAGGTATACACAGAGCC to generate a probe that spans nucleotides 390 to 1537 (GenBank accession number X75926); and mouse microsomal triglyceride transfer protein (MTP) created by RT-PCR with primers 5′-CGTTGTGTTACTGTGGAGG and 5′-TCTTCTCTCCTCGAAGTCC to amplify a product spanning nucleotides 216 to 1051 (GenBank accession number L47970).


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# Regulation of Absorption and ABC1-Mediated Efflux of Cholesterol by RXR Heterodimers

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