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# Suppression of 2,3-Oxidosqualene Cyclase by High Fat Diet Contributes to Liver X Receptor-α-mediated Improvement of Hepatic Lipid Profile*[S]

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**The liver X receptors (LXRs) sense oxysterols and regulate genes involved in cholesterol metabolism. Synthetic agonists of LXRs are potent stimulators of fatty acid synthesis, which is mediated largely by sterol regulatory element-binding protein-1c (SREBP-1c). Paradoxically, an improved hepatic lipid profile by LXR was observed in mice fed a Western high fat (HF) diet. To explore the underlying mechanism, we administered mice normal chow or an HF diet and overexpressed LXRα in the liver. The HF diet with tail-vein injection of adenovirus of LXRα increased the expression of LXR-targeted genes involved in cholesterol reverse transport but not those involved in fatty acid synthesis. A similar effect was also observed with the use of 22R-hydroxycholesterol, an LXR ligand, in cultured hepatocytes. Consequently, SREBP-1c maturation was inhibited by the HF diet, which resulted from the induction of Insig-2a. Importantly, increased cholesterol level suppressed the expression of 2,3-oxidosqualene cyclase (OSC), which led to an increase in endogenous LXR ligand(s). Furthermore, siRNA-mediated knockdown of OSC expression enhanced LXR activity and selectively up-regulated LXR-targeted genes involved in cholesterol reverse transport. Thus, down-regulation of OSC may account for a novel mechanism underlying the LXR-mediated lipid metabolism in the liver of mice fed an HF diet.**

The liver X receptors (LXRs),[2] including LXRα and LXRβ, are members of the nuclear receptor superfamily of transcription factors. They play central roles in the transcriptional regulation of genes that participate in reverse cholesterol transport and lipid metabolism. LXRs form obligate heterodimers with the retinoid X receptor to interact with LXR response elements in the regulatory regions of target genes. LXRβ is expressed ubiquitously, but LXRα predominantly distributes in tissues such as the liver, intestine, adipocytes, and macrophages that play important roles in lipid homeostasis (1). The natural ligands for both LXRα and -β include oxysterols such as 22(*R*)-hydroxycholesterol [22(*R*)-HC], 27-HC (2), and 24-(*S*),25-epoxycholesterol (24,25-EC), the latter produced in parallel with cholesterol (3–4). LXR activation seems to prevent cholesterol overload by promoting cholesterol efflux to inhibit atherogenesis in mice. ApoE$^{-/-}$ or LDLR$^{-/-}$ mice receiving bone marrow from LXRα$\beta^{-/-}$ mice showed a marked increase in lesion size (5). The administration of a synthetic LXR ligand to these knock-out models decreased atherosclerotic lesions (6–7). In addition, synthetic LXR agonists confer an anti-diabetic effect in db/db diabetic mice (8). In light of these findings, LXR agonists could be pharmacological agents for treating vascular disease associated with hyperlipidemia. However, current synthetic LXR ligands markedly increase hepatic lipogenesis and plasma level of triglycerides, largely because of the induction of sterol regulatory element-binding protein-1c (SREBP-1c) and its downstream targets in the liver (9). The loss of LXR results in a different effect on the expression of ATP-binding cassette transporter A1 (ABCA1) and SREBP-1c, which indicates that LXRs interact differentially with the transcription machinery present in target gene promoters (10).

SREBPs, including SREPB-1a, -1c, and -2, are transcription factors that regulate genes involved in cholesterol and fatty acid metabolism. SREBP-1c activates genes involved in fatty acid synthesis. SREBP-2 augments genes regulating cholesterol biosynthesis, whereas SREBP-1a seems to be engaged in both pathways. In cells with abundant sterols, SREBPs bind to insulin-inducing gene (Insig)-1 and -2, together with SREBP cleavage-activating protein (SCAP), at the rough endoplasmic reticulum membrane. Upon sterol deprivation, SREBPs are cleaved in the Golgi apparatus to release their amino acid termini, which then translocate to the nucleus, where they bind to the sterol regulatory element (SRE) in the promoters of various target genes. Sharing 59% sequence homology, Insig-1 and Insig-2 bind to SCAP in a sterol-dependent fashion (11–12). Insig-2a is enriched in the liver and selectively down-regulated by insulin (13) and up-

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2 and Table S1.

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[2] The abbreviations used are: LXR, liver X receptor; GFP, green fluorescent protein; HF, high fat; SREBP-1c, sterol regulatory element-binding protein-1c; ABCA1, ATP-binding cassette transporter A1; OSC, 2,3-oxidosqualene cyclase; LDL, low density lipoprotein; MOI, multiplicity of infection; 24,25-EC, 24-(*S*),25-epoxycholesterol; RNAi, RNA interference.

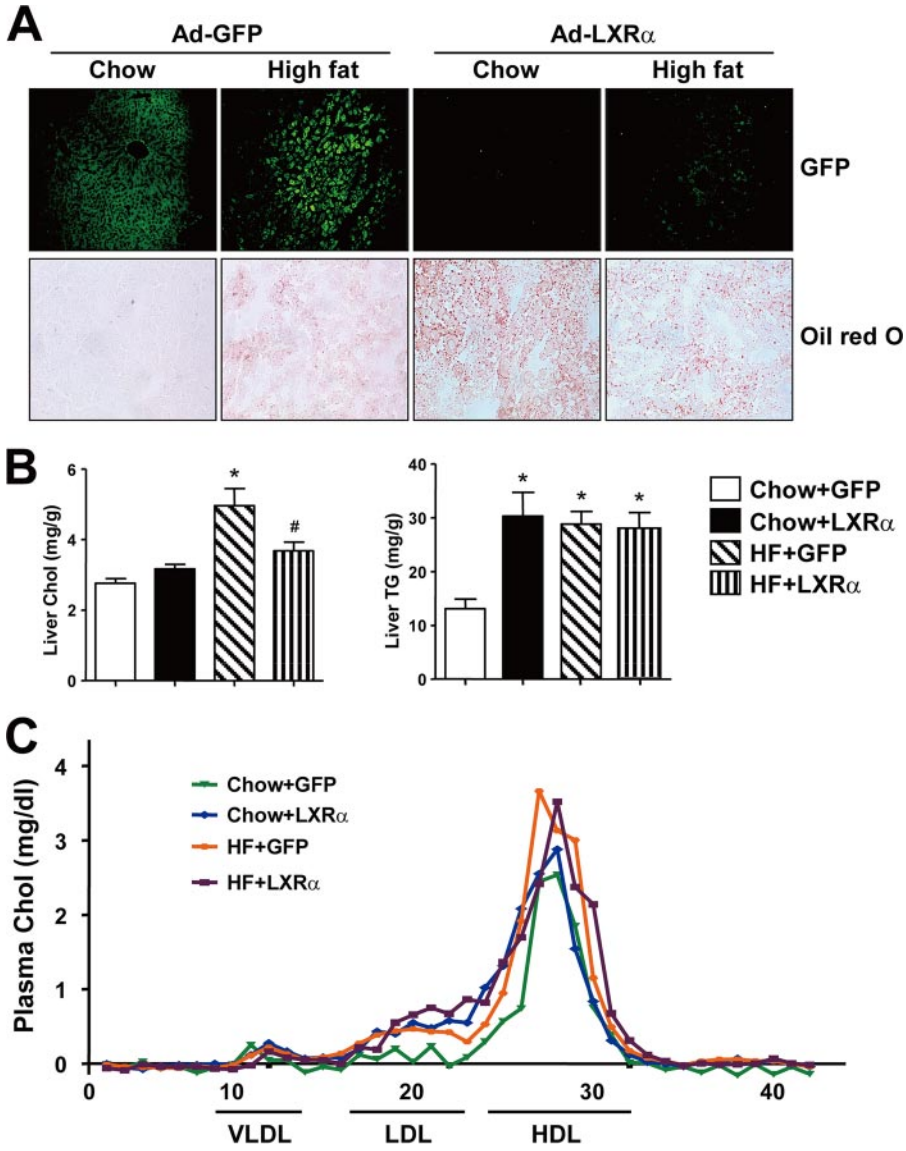


FIGURE 1. **Overexpression of LXRα in mouse liver and a high fat diet alter hepatic lipid and serum lipid profiles.** Eight-week-old male C57/BL6J mice were randomly separated into two groups fed regular chow or a Western high fat diet (HF; 0.15% cholesterol, 21% fat) for 8 weeks. The mice were then intravenously infected with $1 \times 10^9$ PFU of Ad-LXRα or Ad-GFP for 7 days ($n = 10$ in each group). *A*, frozen liver sections were stained with Oil-red O and observed by fluorescence microscopy. The results are representative of images for six mice in each group. *B*, liver lipids were extracted, and the level of cholesterol triglycerides was measured by automated clinical chemistry analysis. *C*, plasma lipoproteins were fractionated by FPLC, and the content of cholesterol in each fraction was determined by automated clinical chemistry analysis. The relative fractions of VLDL, LDL, and HDL are labeled. Data are expressed as mean ± S.E.

regulated by synthetic LXR agonists (14). Lehrke *et al.* (15) reported that a modest increase in hepatic LXRα worsened serum lipid profiles in LDLR$^{-/-}$ mice fed normal chow but had the opposite effect on lipid profiles and protected the animals against atherosclerosis with an HF diet. Kalaany *et al.* (16) also found LXR-null mice resistant to obesity on challenge with an HF, high-cholesterol diet. Remarkably, this phenotype depended on the presence of cholesterol in the diet.

2,3-Oxidosqualene cyclase (OSC) is a unique microsomal enzyme in the cholesterol biosynthetic pathway. OSC catalyzes not only the conversion of 2,3-monoepoxysqualene (MOS) to lanosterol but also the cyclization of 2,3;22,23-diepoxysqualene (DOS) to 24(*S*),25-epoxylanosterol, which is subsequently transformed into oxysterol 24,25-EC, a potent naturally occurring ligand of LXR (3, 17–18). Synthesis of 24,25-EC overrides cholesterol synthesis when OSC is partially inhibited because DOS has a lower $K_m$ for OSC than does MOS (17). Through its dual function, OSC inhibition can reduce cholesterol biosynthesis and enhance 24,25-EC synthesis. Recently, 24,25-EC was found to selectively upregulate LXR target genes involved in cholesterol efflux in macrophages, but had no effect on genes related to fatty acid synthesis or triglyceride accumulation (19). Thus, OSC becomes an attractive target for inhibition of cholesterol synthesis.

Given the paradoxical improvement of the hepatic lipid profile by LXR with an HF diet, we compared the role of LXRα in lipid metabolism in an overexpression mouse model fed normal chow or an HF diet. The HF diet with LXRα overexpression increased the expression of LXR-targeted genes involved in cholesterol reverse transport but not those involved in fatty acid synthesis. We showed that increased cholesterol level suppressed the expression of OSC, leading to an increase in endogenous LXR ligand(s). These results suggest that endogenous ligands caused by a Western HF diet upregulate the LXR target genes involved in cholesterol efflux but not those linked to fatty acid synthesis and triglyceride accumulation. Thus, the modulation of LXR function by modulating OSC may provide a paradigm for an alternative strategy in the treatment of fatty liver, atherosclerosis, and metabolic syndrome.

## EXPERIMENTAL PROCEDURES

*Cell Culture and Reagents*—The human hepatoma cell line HepG2 was maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Hyclone, Logan, UT). HEK293 cells were cultured in DMEM with 5% fetal bovine serum. Primary hepatocytes were isolated from male Sprague-Dawley rats by modified two-step collagenase liver perfusion (20). The isolated hepatocytes were seeded onto culture plates coated with rat-tail collagen I in RPMI 1640 media supplemented with 10% FBS and antibiotics. The adherent hepato-

cytes were cultured at 37 °C under 5% $CO_2$ overnight before various treatments. For the treatment with LXR agonists, 5 $\mu$M 22*R*-HC (Sigma) was included in the medium.

*Adenovirus Purification and Infection*—Recombinant adenoviruses encoding green fluorescence protein (GFP), LXR$\alpha$, and HA-nSREBP-1c were constructed and amplified as previously described (21–22). Virus particles were purified by cesium chloride gradient and concentrated by use of Sephadex-G-25 M columns (23). The titers of virus (PFU) were determined in HEK293 cells (24). HepG2 and rat primary hepatocytes were infected with recombinant adenoviruses at the indicated multiplicity of infection (MOI) and incubated for 24–48 h prior to experiments.

**TABLE 1**
**The index of insulin sensitivity and liver lipid of mice with HF diet and LXR$\alpha$ overexpression**

| | Chow | | High fat | |
|---|---|---|---|---|
| | Ad-GFP | Ad-LXR$\alpha$ | Ad-GFP | Ad-LXR$\alpha$ |
| Body weight (g) | 23.71 ± 1.63[a] | 23.33 ± 0.73 | 24.89 ± 2.32 | 24.31 + 0.87 |
| LW/BW (%) | 5.14 ± 0.17 | 6.77 + 0.24[b] | 4.91 ± 0.23 | 5.96± 0.35[c] |
| Liver chol (mg/g) | 2.44 ± 0.18 | 2.75 + 0.29 | 4.13 ± 0.40[b] | 3.25± 0.27[c] |
| Liver TG (mg/g) | 12.30 ± 0.85 | 23.55 + 3.22[b] | 26.94 ± 2.29[b] | 26.34 + 1.17 |
| Blood glucose (mmol/l) | 5.33 ± 0.69 | 5.08 + 0.35 | 5.71 ± 1.06 | 5.28 + 0.78 |
| Plasma insulin (mU/ml) | 29.37 ± 2.32 | 40.65 + 2.49[b] | 36.07 ± 1.84[d] | 34.95 + 3.18 |
| HOMA-IR | 7.01 + 1.96 | 8.27 + 2.49 | 9.11 ± 2.22 | 8.62 + 3.02 |

[a] Data are expressed as mean ± S.E.
[b] $p < 0.01$ vs Ad-GFP with chow diet.
[c] $p < 0.05$ vs Ad-GFP with HF diet.
[d] $p < 0.05$.

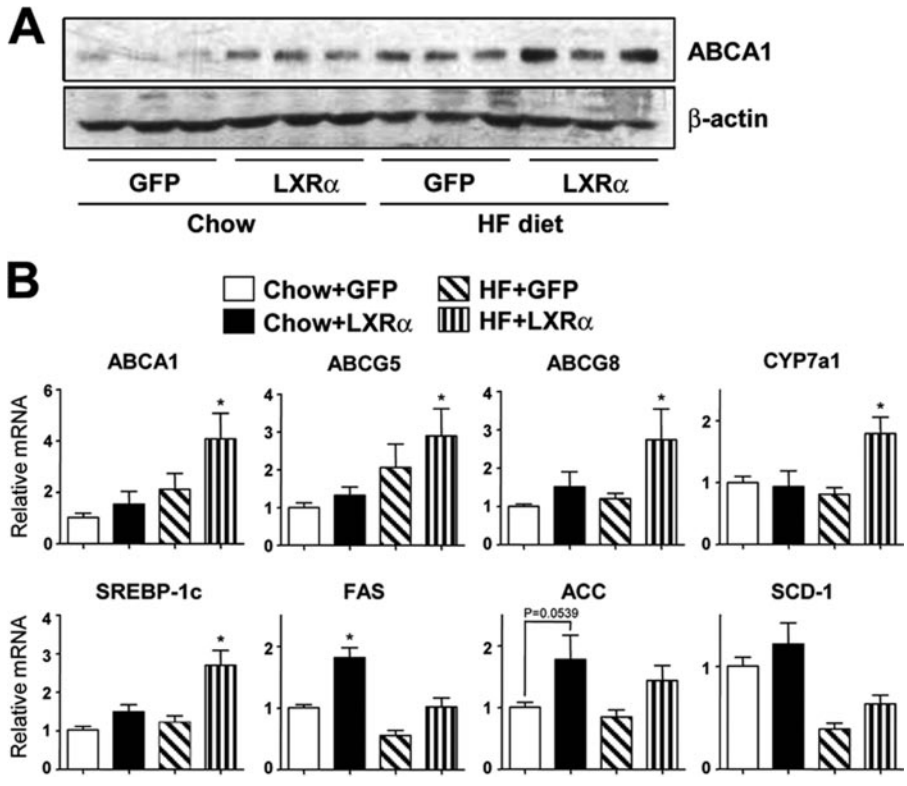


FIGURE 2. **High fat diet alters the expression of LXR target genes in mouse liver.** *A*, liver extracts underwent Western blot analysis with antibodies against ABCA1 or $\beta$-actin. *B*, mRNA levels of LXR$\alpha$ target genes, including *ABCA1*, *ABCG5*, *ABCG8*, *Cyp7a1*, *SREBP-1c*, *FAS*, *ACC*, and *SCD-1*, were examined by quantitative RT-PCR (qRT-PCR) of RNA samples from livers of treated mice ($n = 10$). Data are mean ± S.E. of the mRNA levels normalized to that of $\beta$-actin and expressed as fold of that of GFP-infected specimens (*, $p < 0.05$).

*Animal Model and Treatment*—C57BL/6J mice were bred at the breeding facility of the Peking University Health Science Center. In accordance with the guidelines of the Protection of Laboratory Animals, all animal procedures were approved by the Institutional Animal Care and Use Committee of the Peking University Health Science Center. The mice were maintained under a 12-h light/12-h dark cycle and fed standard laboratory chow and tap water *ad libitum*. Eight-week-old male mice were fed a chow diet or a Western HF diet (0.15% cholesterol, 21% fat) for 8 weeks. For adenoviral infection, the mice under different diets were separated into various groups of GFP ($n = 10$), LXR$\alpha$ ($n = 10$), and SREBP-1c(N) ($n = 4$), according to treatment with Ad-GFP, Ad-LXR$\alpha$, or Ad-SREBP-1c(N) at $1 \times 10^9$ PFU in 0.1 ml of saline administered through tail-vein injection. Levels of fasting blood glucose were measured after a 6-h fast by use of a portable glucometer (ACCU-CHEK II; Roche Applied Science). Seven days after adenovirus injection, mice were anesthesized and killed. The blood samples were withdrawn from the mouse saphenous vein, and plasma insulin concentrations were measured by use of an ultrasensitive $I^{125}$-linked immunosorbent assay kit (Fu Rui Inc., Beijing, China). Liver tissues were dissected and stored at −80 °C until analysis.

*Western Blot Analysis*—Liver tissue was ground in a lysis buffer (150 mM NaCl, 1% Triton X-100, 100 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 $\mu$g/ml aprotinin, and 10 $\mu$g/ml pepstatin A). After ultrasonication, specimens were centrifuged and the supernatant was collected. Equal amounts of lysates (50 $\mu$g/lane) were separated on 10% SDS-PAGE. Western blot analysis involved antibodies against LXR$\alpha$, ABCA1 (Novus Biologicals, Littleton, CO), SREBP-1 (Santa Cruz Biotechnology, Santa Cruz, CA), and $\beta$-actin (Biosynthesis Biotech, Beijing, China).

*Real-time PCR Analysis*—Total RNA was isolated using the TRIzol reagent (Applygen, Beijing, China) and reverse-transcribed using the Reverse Transcription System kit (Promega). The forward and reverse primers are listed in supplemental Table S1. The synthesized cDNA was mixed with the primers in the TaqMan PCR Master Mix (Tiangen, Beijing, China). SYBR Green I was used to monitor amplification of DNA detected by the MX3000P QPCR system (Stratagene). The relative amount of mRNA was calculated by use of the comparative $C_T$ method. Gene expression was normalized to $\beta$-actin levels.

*Histological and Oil-red O Staining*—Histochemical analysis of mouse liver sections, preparation

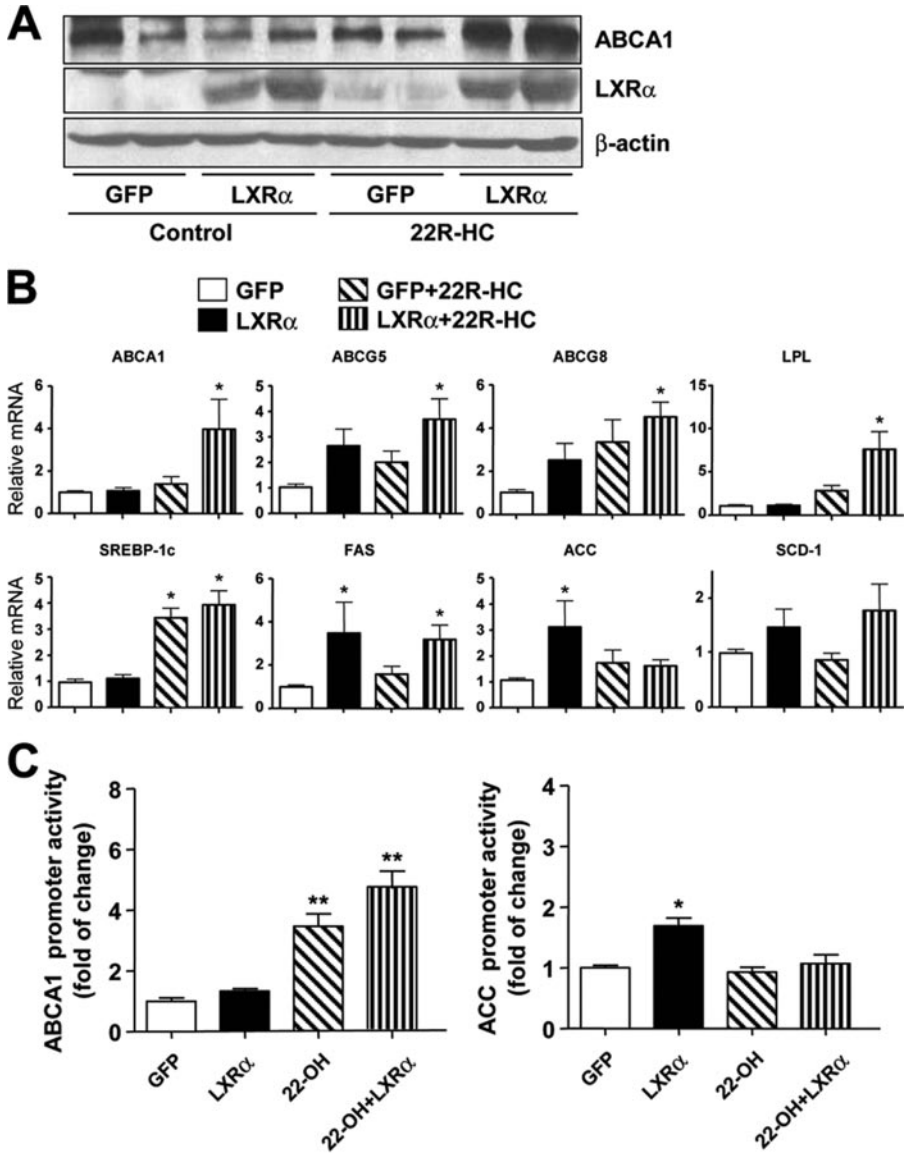


FIGURE 3. **The regulation of LXR target genes by LXR$\alpha$ and its ligand in HepG2 cells.** HepG2 cells were infected with Ad-LXR$\alpha$ (50 multiplicity of infection [MOI]) for 24 h, then treated with 22R-HC (5 $\mu$M) for another 24 h. *A*, proteins were extracted and underwent Western blot analysis with antibodies against LXR$\alpha$, ABCA1, and $\beta$-actin. *B*, mRNA levels of ABCA1, ABCG5, ABCG8, LPL, SREBP-1c, FAS, ACC, and SCD-1 were examined by qRT-PCR. Results were from three independent experiments, and the data are means $\pm$ S.E. of the relative mRNA normalized to that of $\beta$-actin and expressed as fold of that of GFP-infected samples (*, $p < 0.05$). *C*, HepG2 cells were transfected with pGL3-ACC1-Luc or pGL3-ABCA1(-928)-luc, then treated with 22R-HC (5 $\mu$M) for another 24 h. $\beta$-Galactosidase plasmid was co-transfected as a transfection control. Luciferase activities were normalized to that of $\beta$-galactosidase. Data are mean $\pm$ S.E. of the relative luciferase activities from three independent experiments, each performed in triplicate (*, $p < 0.05$; **, $p < 0.01$).

and Oil-red O staining of frozen or paraffin-embedded liver sections were performed as previously described (25–26). After staining, slides were washed three times in water and photographed using an Eclipse TE2000 inverted microscope system (Nikon Instruments, Inc.) at $\times$40 magnification.

*Serum and Liver Lipid Determination*—Samples of animal serum were collected, and lipoproteins were separated by fast performance liquid chromatography (FPLC) (Amersham Biosciences). The amount of plasma lipoproteins in serum or the FPLC fractions was detected by use of an automated clinical chemistry analyzer kit (Biosino Biotech). For quantification of liver cholesterol and triglycerides, liver sections were homogenized, which were extracted with chloroform:methanol (2:1). The lipid fractions were dried under nitrogen (15). For cholesterol and triglyceride measurement, the dried lipids of the aliquot were dissolved in 5% Triton X-100 in $H_2O$ and determined using the chemistry analyzer kit.

*Luciferase Activity Assay*—pABCA1 (-928)-luc, p-ACC1-luc, LXREx3, TK-luc, and CMX-GAL-hLXR$\alpha$ with a GAL4 reporter were described previously (27, 28). For transient transfection, plasmid DNA was transfected into HepG2 cells by use of the jetPEI method (PolyPlus, Illkirch, France), then cells underwent various treatments for 24 h. pRSV-$\beta$-gal was co-transfected as a transfection control. Twenty-four hours post-transfection, cells were lysed, and luciferase activities were measured and normalized to that of $\beta$-galactosidase.

*RNA Interference (RNAi)*—siRNA for OSC or scramble control siRNA were synthesized by Sigma, and the sequence of OSC siRNA was 5′-CTATGTGTCTCTCAGAATT-3′. siRNA was transfected into HepG2 cells by the N-TER method (Sigma) for 24 h. Then the siRNA-targeted cells underwent various treatments for another 24 h. Total RNA or lysis protein was collected for further analysis. For *in vivo* OSC knockdown experiment, pSIREN-OSC plasmid was constructed according to the manufacturer's instructions (RNAi-Ready pSIREN system, Clontech). pSIREN-OSC plasmid was delivered by use of a modified hydrodynamic transfection method as described previously (29). Briefly, 50 $\mu$g of plasmid dissolved in 2.5 ml of phosphate-buffered saline was rapidly injected into the mouse tail vein. An equal volume of vector control was injected in control mice. At 48 h after injection, mice were anesthesized and killed. Liver tissues were dissected and stored at $-80$ °C until analysis.

*24,25-EC Determination and Quantification*—Liver tissue lipids were extracted, and then high performance liquid chromatography (HPLC) was performed as described (30). Internal control 22S-HC (Sigma) was added into samples before the lipid extraction. 24,25-EC was analyzed by an HPLC instrument (Waters$^{TM}$ 600–2487) with a reverse-phase C18 column and a binary gradient solvent of 5 mM ammonium acetate in

methanol and water. The relative peak areas of 24,25-EC in the samples were used to calculate the amount of 24,25-EC by comparing with 22S-HC, the internal standard.

*Data Analysis*—The significance of variability was determined by an unpaired two-tailed Student's *t* test or analysis of variance. Each experiment involved triplicate measurements for each condition tested, unless indicated otherwise. All results are expressed as mean ± S.E. from at least three independent experiments. $p < 0.05$ was considered statistically significant.

## RESULTS

*Generation of Mice Models Overexpressing LXRα in Liver with Different Diets*—To investigate the effect of LXRα in the liver of mice under an HF diet, C57BL/6J mice were fed an HF diet, then intravenously injected with Ad-hLXRα or control Ad-GFP. The animals were sacrificed 7 days post-injection. Both LXRα mRNA and protein levels were higher in the livers of mice receiving Ad-LXRα than in Ad-GFP-infected controls (supplemental Fig. S1, *A* and *B*). The HF diet or LXRα overexpression alone was sufficient for lipid accumulation in the liver, as evidenced by Oil-red O staining (Fig. 1*A*). However, the HF diet seemed to reduce the effect of LXRα in lipid accumulation. Cholesterol content, but not triglyceride content, was higher with the HF diet than with LXRα overexpression (Fig. 1*B*). Serum lipoprotein analysis by FPLC showed that HF diet increased plasma cholesterol in all fractions. With hepatic LXRα overexpression, the fractions of low density lipoprotein (LDL) in mice fed the chow diet were higher than those in mice fed the HF diet (Fig. 1*C*). In addition, hyperinsulinemia in the LXRα-treated mice was reversed under the HF diet, which indicated improved insulin sensitivity (Table 1). Thus, the interplay between diet and hepatic LXR level seems to play a critical role in fatty liver and serum lipid profiles.

*HF Diet Increases the Expression of Genes Related to Reverse Cholesterol Transport*—LXR regulates genes involved in cholesterol efflux (*e.g. ABCA1*) and/or fatty acid synthesis (31). To study the mechanism by which LXRα differentially affects lipid metabolism in the liver under distinct diets, we examined the expression of these two types of LXR-targeted genes. Hepatic ABCA1 protein level was up-regulated by LXRα overexpression in conjunction with an HF diet (Fig. 2*A*). The synergistic effect was observed in mice receiving HF diet and Ad-hLXRα (HF+LXRα), which demonstrated the activation of hepatic LXR. Quantitative real-time PCR revealed that genes involved in reverse cholesterol transport, namely, *ABCA1*, *ABCG5*, *ABCG8*, and *Cyp7α1*, were moderately up-regulated by LXRα but significantly up-regulated by HF+LXRα. However, genes regulating lipid synthesis, such as *FAS* and *ACC1*, were greatly up-regulated by LXRα overexpression. A similar pattern was observed with SCD-1 but did not reach statistical significance. The HF diet had little effect on the mRNA level of these genes. SREBP-1c, the key transcriptional factor involved in fatty acid synthesis, is known to regulate FAS, ACC1, and SCD-1, but itself is a target gene of LXRα. Interestingly, SREBP-1c, similar to ABCA1, was up-regulated in the HF+LXRα group (Fig. 2*B*). These results suggest mechanisms other than SREBP-1c transcriptional regulation involved in the dysregulation of FAS, ACC1, and SCD-1 under the HF diet.

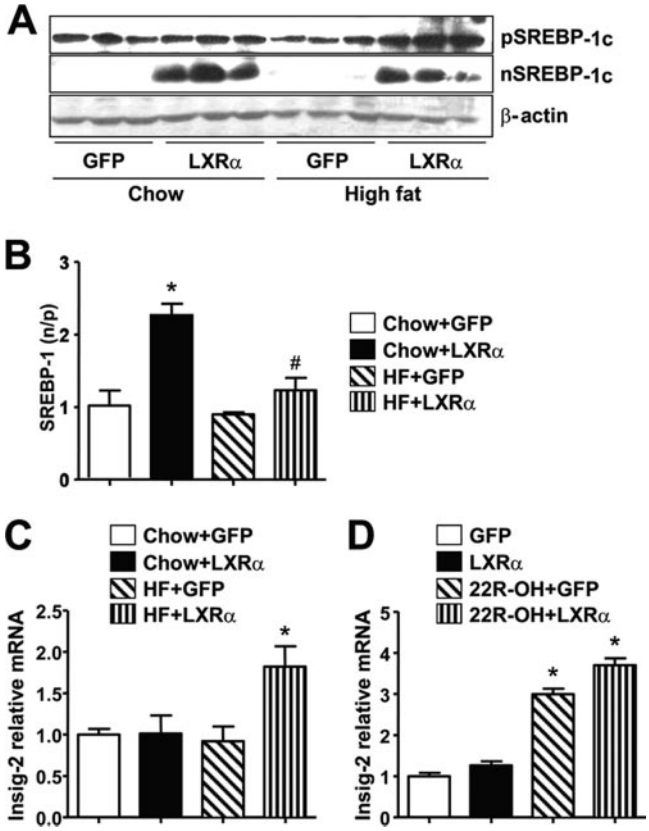


FIGURE 4. **The regulation of SREBP-1 and Insig-2 by LXRα and its ligand.** The grouping and treatment of animals were as in Fig. 1. *A*, isolated liver extracts underwent Western blot analysis with antibody against SREBP-1 or β-actin. The densities of the precursor (*p*) and nuclear form (*n*) of SREBP-1 were measured, and the ratio of SREBP-1(n/p) is shown in *B*. *C*, level of Insig-2a mRNA was examined by qRT-PCR with RNA samples from livers of different mouse groups. *D*, HepG2 cells were infected with Ad-LXRα (50 MOI) or Ad-GFP (50 MOI) for 24 h, then treated with 22R-HC (5 μM) for 24 h. Gene expression of Insig-2a was examined by qRT-PCR with total RNA samples from cell lysates. Data in *C* and *D* are mean ± S.E. of the relative mRNA normalized to that of β-actin and expressed as fold of that of GFP-infected samples (*, $p < 0.05$).

*LXRα and Its Ligand Differentially Regulate the Expression of LXRα-targeted Genes*—Given that an HF diet may provide endogenous ligand(s) of LXR to selectively up-regulate genes involved in reverse cholesterol transport, we examined the effect of LXRα and oxysterol, an endogenous LXR ligand, on the expression of LXRα-targeted genes. We infected HepG2 cells with Ad-LXRα or treated cells with 22R-HC, an LXR endogenous ligand. ABCA1 was synergistically up-regulated in cells expressing LXRα and treated with 22R-HC (Fig. 3*A*). The mRNA level was increased in genes encoding proteins related to reverse cholesterol transport but not those for lipid synthesis, which was similar to that found *in vivo* (Fig. 3*B versus* Fig. 2*B*). From these *in vitro* and *in vivo* results, we hypothesized that the LXR ligands differentially affect the promoter activities of these genes involved in reverse cholesterol transport and lipogenesis. The luciferase assay showed that LXRα increased the *ABCA1* promoter activity in HepG2 cells in a ligand-dependent manner (Fig. 3*C*, *left panel*). As expected, LXR overexpression in the absence of a ligand (22R-HC) increased the ACC promoter activity (Fig. 3*C*, *right panel*). When the ligand was present, the ACC promoter activity remained at the basal level. These

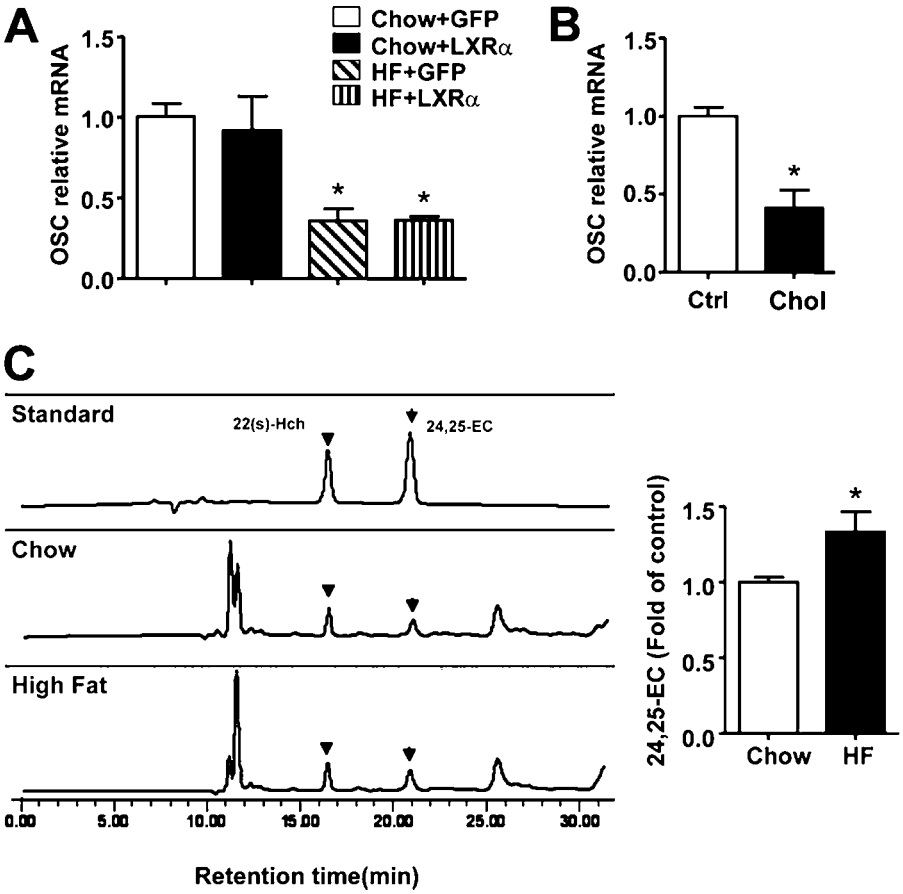


FIGURE 5. **Cholesterol suppresses hepatic OSC expression and increases 24,25-EC production.** *A* and *C*, animal treatments are the same as in Fig. 1. *B*, cholesterol was dissolved in ethanol in a stock solution (20 mg/ml). HepG2 cells were treated with cholesterol (Chol; 20 $\mu$g/ml, 1:1000 dilution) for 24 h. *A* and *B*, OSC mRNA was measured by qRT-PCR. Results are mean ± S.E. of the relative mRNA normalized to that of $\beta$-actin and expressed as fold that of control (*, $p < 0.05$). *C*, liver 24,25-EC was determined by HPLC. Representative results from 6 mice in each group are indicated (*left*). The relative peak areas of 24,25-EC in the samples were compared with the internal control 22S-HC (*right*) and expressed as fold of expression of control samples (*, $p < 0.05$).

results suggest that while ABCA1 up-regulated by LXR was ligand-dependent, that of ACC was ligand-independent.

*LXRα Increases SREBP-1c Transcription but Inhibits Its Maturation*—Although SREBP-1c mRNA level was up-regulated by LXRα overexpression in mice under the HF diet and with the endogenous ligand in cultured hepatocytes, the expression of genes involved in fatty acid synthesis exhibited a ligand-independent pattern. The activity of SREBPs is known to be controlled at both transcriptional and post-transcriptional levels. We studied whether LXRα also regulates SREBP-1c at the post-transcriptional level by detecting the level of the SREBP-1 precursor and its nuclear form. With LXRα and the HF diet, the level of both forms of SREBP-1c was increased in the liver (Fig. 4*A*), but the HF diet decreased the level of the mature form of SREBP-1c and conferred a lower ratio of nuclear to precursor form than with LXRα alone (Fig. 4*B*). Thus, the HF diet might inhibit cleavage via augmenting anchor proteins to prevent SREBP-1 translocation from the endoplasmic reticulum to the Golgi network.

Recently, TO901317, a synthetic LXR agonist, was reported to enhance the expression of Insig-2a, the major Insig variant in the liver (14). We therefore detected the expression of Insig-2a in mouse groups. LXRα, together with the HF diet, increased the level of hepatic insig-2a *in vivo* (Fig. 4*C*). Similar results were obtained in cultured HepG2 cells with the LXR ligand (Fig. 4*D*). Thus, Insig-2a was up-regulated by LXRα in a ligand-dependent manner, which, in turn, inhibited the maturation of SREBP-1c.

*Diet Had No Effect on the Outcome of Overexpressed Nuclear SREBP-1c*—SREBP-1 is the transcription factor governing hepatic lipogenesis, and SREBP-1c is the major form of SREBP-1 in rodents. To study whether diet directly affect SREBP-1c target genes, we injected mice with adenovirus encoding the nuclear form of human SREBP-1c (nSREBP-1c), then determined the protein and mRNA expression after 7 days. The overexpression of nSREBP-1C in the liver was confirmed at the protein level (supplemental Fig. S2*A*). Consistent with previous study (32), the nSREBP-1c overexpression induced severe lipid accumulation (supplemental Fig. S2*B*) and up-regulated its target genes FAS, ACC, and SCD-1 (supplemental Fig. S2*C*). However, diet had little effect on changes caused by overexpressing nSREBP-1c.

*HF Diet Reduced OSC Expression in the Liver to Produce the Ligand of LXR*—To further confirm that the HF diet provided endogenous ligands of LXR, we explored the possible endogenous LXR ligands induced by the HF diet. Partial inhibition of OSC could attenuate cholesterol synthesis and increase the production of 24,25-EC, an endogenous ligand of LXR (18–19). We thus measured hepatic OSC expression and the level of 24,25-EC in mice. The HF diet, but not LXR overexpression, significantly inhibited the expression of OSC in liver (Fig. 5*A*). Similarly, cholesterol treatment also inhibited the expression of OSC *in vitro* (Fig. 5*B*). Furthermore, 24,25-EC in mouse liver was increased under the HF diet (Fig. 5*C*). To test whether the inhibited OSC expression increased LXR ligand and LXR transcriptional activity, cellular 24,25-EC was measured by HPLC, and the plasmid LXREx3-luc or CMX-GAL-hLXR and a GAL4 reporter were co-transfected into HepG2 cells. The knockdown of OSC by siRNA transfection (Fig. 6*A*) increased the level of 24,25-EC (Fig. 6*B*). The luciferase activity of both LXRE-luc and GAL-hLXR/Gal-luc was also increased by OSC RNAi treatment (Fig. 6*C*). Consistently, OSC knocking down by siRNA increased ABCA1 and Insig-2 mRNA level in HepG2 cells, whereas level of SREBP1c was not affected (Fig. 6*D*), which suggests that the suppression of OSC may facilitate the production of the endogenous LXR ligand, 24,25-EC, which in turn activates LXR in cells. Similar to the effect of the HF diet,

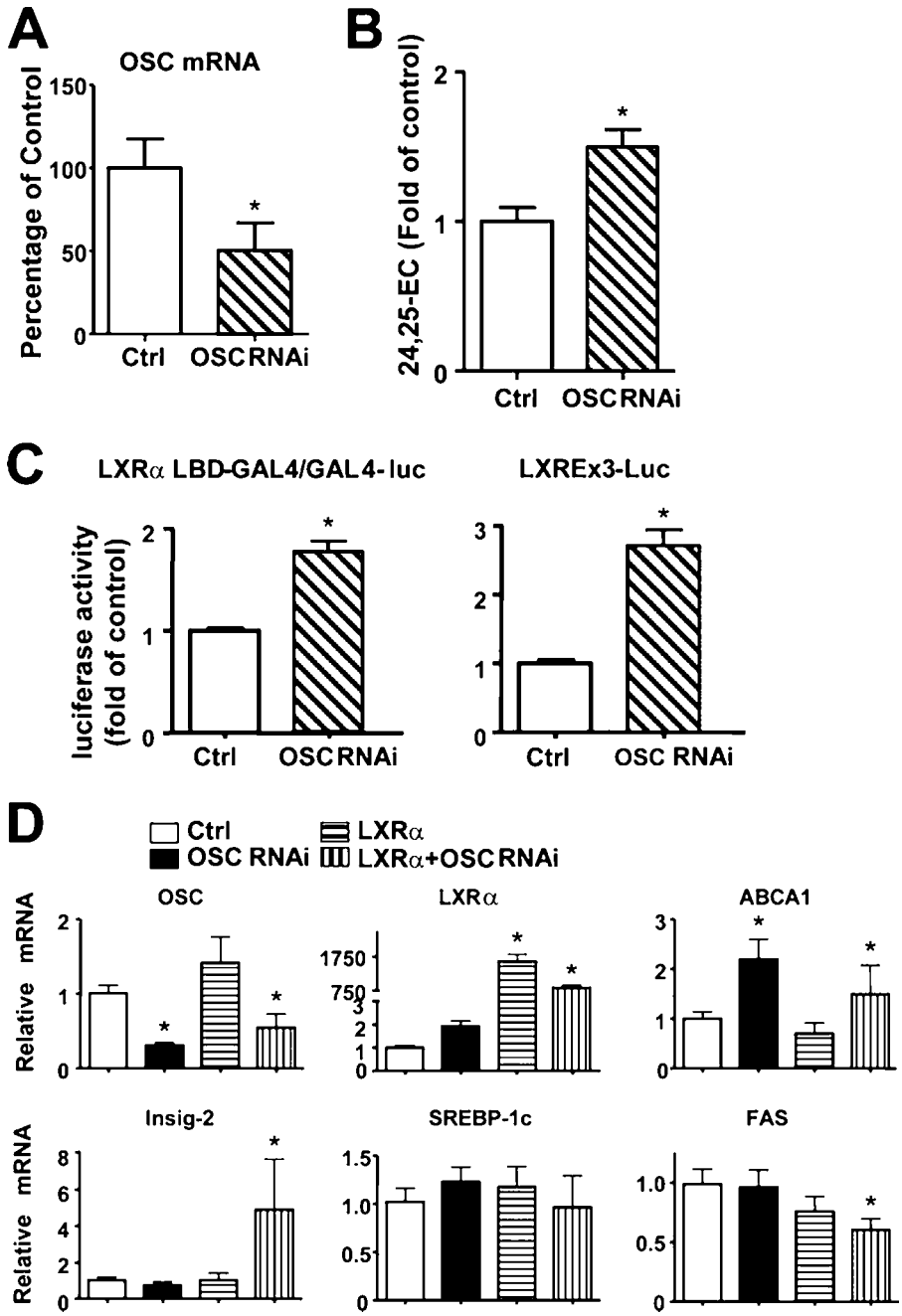


FIGURE 6. **OSC knockdown increases level of 24,25-EC and activates LXR *in vitro*.** HepG2 cells were treated with OSC siRNA or scramble siRNA for 24 h. *A*, OSC mRNA level and *B*, cellular content of 24,25-EC were determined. The relative peak areas of 24,25-EC in the samples were compared with the internal control 22S-HC and expressed as fold of control samples from three independent experiments (*, $p < 0.05$). *C*, cells were treated with OSC siRNA or scramble siRNA for 24 h and then transfected with the reporter plasmid CMX-Gal4-hLXR/TK-MH100X4-Luc or LXREx3-Luc for another 24 h. β-galactosidase plasmid was co-transfected in all experiments as a transfection control. Promoter activities were measured by use of luciferase, which was normalized to β-galactosidase activity. The results are expressed as relative luciferase activities. Data are mean ± S.E. of the relative luciferase activities from three independent experiments, each performed in triplicate (*, $p < 0.05$). *D*, HepG2 cells were infected with Ad-LXRα (50 MOI) and then transfected with OSC siRNA or scramble siRNA (20 nM) for 48 h. The levels of OSC, LXRα, and LXR-target gene mRNA were analyzed by real-time PCR. β-Actin cDNA was used as an internal control. Data are means ± S.E. from three independent experiments (*, $p < 0.05$; **, $p < 0.01$).

hepatic OSC knockdown by pSIREN-OSC plasmid via hydrodynamic tail-vein injection (Fig. 7*A*) (29) showed an increased level, although not significant, of 24,25-EC (Fig. 7*B*). The mRNA expression of LXR target genes such as Insig-2, ABCG5, and SREBP-1c was also increased (Fig. 7*C*). These results strongly suggest that the HF diet increased the production of 24,25-EC through the suppression of OSC by increased cholesterol content in the liver, which in turn, promoted the reverse cholesterol transport but did not increase the hepatic lipogenesis.

## DISCUSSION

The LXRs are a family of nuclear receptors that promote reverse cholesterol transport, limit inflammation, and improve glucose tolerance. Because of these beneficial effects, LXRs are targets for the development of drugs for treating cardiovascular, metabolic, and/or inflammatory diseases. Although LXR synthetic agonists can improve metabolism in many models, the observation that LXR synthetic ligands markedly increase hepatic lipogenesis and plasma triglyceride levels confines their clinical application (9). The increase in hepatic lipogenesis could be due to the induction of SREBP-1c by LXRs (1). However, LXR knock-out reveals that LXR is essential for the response to dietary cholesterol overload (33). Lehrke *et al.* (15) reported that overexpression of LXRα in liver with an HF diet improved dyslipidemia and atherosclerosis in LDLR$^{-/-}$ mice. In the current study, we studied the combinatory effect of LXR and an HF diet on fatty liver. Increased LXRα content promoted lipid accumulation in the liver. However, this accumulation could be attenuated by an HF diet. This finding supports the possibility of a Western HF diet causing an elevated level of endogenous ligands for LXR (15–16).

The LXR-targeted genes involved in reverse cholesterol transport were up-regulated by LXRα overexpression under an HF diet. Interestingly, the expression of LXR-targeted genes engaged in fatty acid synthesis was the same in mice fed regular chow or the HF diet; thus LXR exerts a distinct regulation on its target genes, which accounts for the attenuated lipid accumulation in the liver (Fig. 1*A*). Data from experiments with cultured hepatocytes confirmed that LXR regulates its target

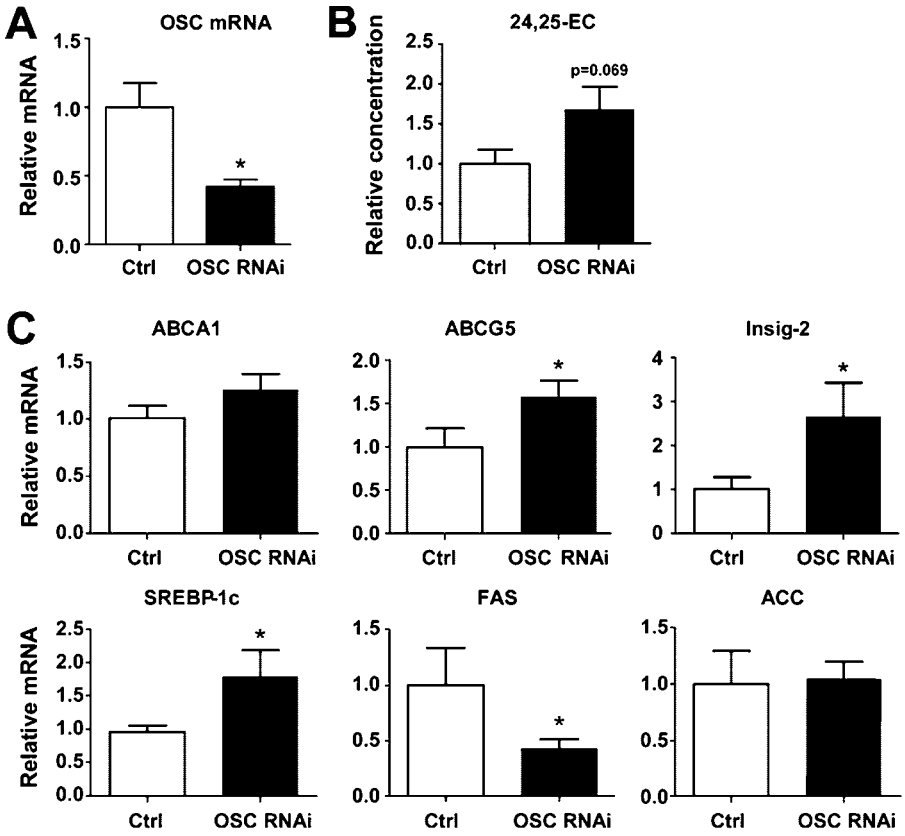


FIGURE 7. **OSC knockdown increases level of 24,25-EC and activates LXR *in vivo*.** Eight-week-old male C57/BL6J mice were injected with 50 $\mu$g of pSIREN-OSC plasmid or vector control plasmid in 2.5 ml of phosphate-buffered saline through tail-vein injection ($n = 6$). At 48-h post-injection, the liver OSC mRNA in *A* and LXR target genes mRNA in *C* were determined. Data are means $\pm$ S.E. of the mRNA levels normalized to that of $\beta$-actin and expressed as fold compared with controls. *B*, level of liver 24,25-EC was determined by HPLC, and the relative amount of 24,25-EC was expressed as fold of controls from six mice in each group (*, $p < 0.05$).

genes differently depending on endogenous ligands. Promoter analysis further revealed that LXR regulation of targets differs in the presence or absence of ligands. Thus, metabolic products from the HF diet may likely serve as endogenous ligands that upregulate target genes specifically involved in reverse cholesterol transport but not fatty acid synthesis.

SREBPs regulate the expression of more than 30 genes involved in cholesterol, triglycerides, and phospholipid biosynthesis and metabolism. SREBPs are synthesized as inactive precursors. Sterol depletion in the cell renders the translocation of SREBP precursors into the endoplasmic reticulum through Insigs and SCAP interaction (34–35). SREBP-1c preferentially activates fatty acid and triglyceride synthesis, whereas SREBP-2 governs cholesterol synthesis, although functional overlap may exist (35–36). The activation of SREBPs can occur with both transcriptional and post-translational modification. LXR activation plays a central role in SREBP-1c transactivation (37). Indeed, we found that the precursor of SREBP-1c was up-regulated by LXR in a ligand-dependent pattern (Fig. 2–4). However, Western blot analysis revealed the mature form of SREBP-1c increased by LXR but decreased in level with an HF diet. Insig-2 could be up-regulated by an LXR agonist, which suggests the post-translational regulation of SREBP-1c (14). Insig-2a, an anchor protein of SREBP, could be up-regulated by LXR in a ligand-dependent manner, similar to the up-regulation of other LXR targets involved in reverse cholesterol transport. As compared with LXR endogenous ligands, synthetic LXR agonists cause additional side effects (15). Of note, the HF diet alone did not significantly increase Insig-2a and ABCA1 mRNA level *in vivo*. This result suggests that high levels of both LXR and its ligand are needed to up-regulate such genes. Another explanation is that insig-2a is also regulated by insulin and other factors (13), which may be affected by an HF diet.

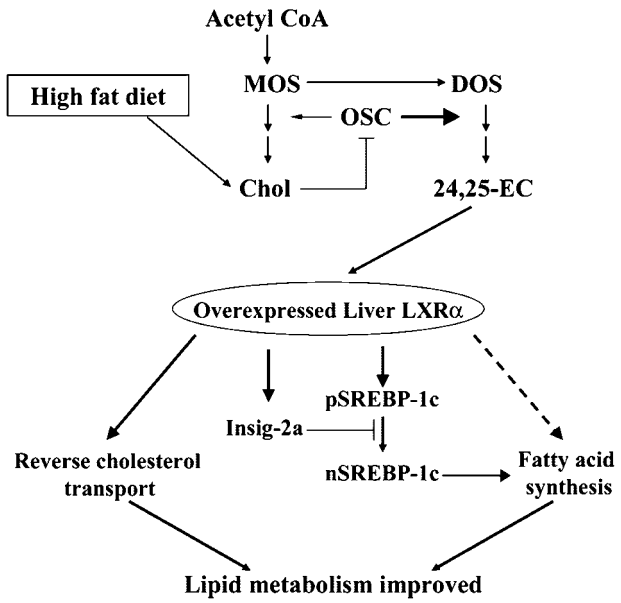


FIGURE 8. **Proposed model for the regulation of hepatic lipid metabolism by diet and LXR.** HF diet increases hepatic cholesterol content, which causes down-regulation of OSC. Partial inhibition of OSC decreases cholesterol synthesis but favors 24,25-EC synthesis, thus increasing production of 24,25-EC. With 24,25-EC as the endogenous ligand, LXR overexpression up-regulates *SREBP-1c* and *Insig-2a*, genes involved in reverse cholesterol transport. The increased expression of Insig-2a inhibits the activation of SREBP-1c, which in turn down-regulates SREBP-1c target genes for fatty acid synthesis. As a result, hepatic lipid metabolism is improved by LXR activation under the HF diet.

OSC plays a key role in the biosynthesis of cholesterol and 24,25-EC, an endogenous ligand of LXR. Because the substrate to synthesize 24,25-EC has a lower $K_m$ for OSC than that to synthesize cholesterol, partial inhibition of OSC would lead increased production of 24,25-EC (3, 17). Indeed, the OSC inhibitor increases ABCA1 expression and decreases SREBP-1c cleavage to result in an improved lipid metabolism *in vivo* and *in vitro* (3, 17–18). We found that the HF diet *in vivo* and increased cholesterol level *in vitro* significantly downregulated the expression of OSC and increased the amount of 24,25-EC.

The OSC knockdown by RNAi could generate increased 24,25-EC to mimic the effect of the HF diet and had a similar impact on LXR targets as an endogenous ligand. These results may explain, in part, the increase in LXR endogenous ligands responding to the HF diet in the liver. However, OSC RNAi did not have an identical regulation with the LXR ligand, which suggests that increased cellular cholesterol content could be oxidized to other oxysterols to activate LXR. Nonetheless, the direct evidence of the cause-and-effect linkage between the OSC suppression and LXR activation by the HF diet needs to be established. A gain-of-function approach with liver-specific OSC overexpression would be needed in future study.

We propose a model for lipid metabolism in the liver regulated by an HF diet and/or LXR (Fig. 8). An HF diet increases hepatic cholesterol content, leading to the down-regulation of OSC and therefore an increase in production of 24,25-EC, as an endogenous LXR ligand. The increase in 24,25-EC production further up-regulates SREBP-1c and Insig-2a involved in reverse cholesterol transport. The increased Insig-2a expression inhibits SREBP-1c activation, which, in turn, down-regulates SREBP-1c target genes involved in fatty acid synthesis. As a result, hepatic lipid metabolism is improved by LXR activation with the HF diet. Synthesized OSC inhibitors have a protective effect on many metabolic diseases. Thus, LXR mediating a protective role in the liver under the HF diet may provide a paradigm for developing an LXR$\alpha$ modulator to treat fatty liver, atherosclerosis, and metabolic syndrome.

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