The liver X receptors (LXRs) are oxysterol-activated nuclear receptor family transcription factors that regulate the expression of genes involved in cholesterol metabolism (3). LXRA (NR1H3) is mainly localized in liver, adipose tissue, intestine, kidney, and macrophages, whereas LXRB (NR1H2) is ubiquitiously expressed. In the rodent liver, LXRA induces transcription of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the bile acid synthesis. Treatment with LXR agonists prevents the overaccumulation of sterols in the intestine and macrophages by the induction of ATP-binding cassette (ABC) transporters involved in sterol efflux along with acceptor proteins such as apoprotein E (4). In macrophages, activation of LXRs stimulates transcription of the ABC transporters, ABCA1 and ABCG1. Synthetic LXR ligand administration reduced the development of atherosclerosis in mice deficient in low density lipoprotein receptor or apoprotein E (5). Mice with macrophages lacking both LXRA and LXRB developed more atherosclerotic lesions than mice with wild-type macrophages (6). In the small intestine, ABCA1, ABCG5, and ABCG8 are induced by LXR activation, which results in reduced sterol absorption (7, 8). These studies suggest that LXRs play an important role in the regulation of cholesterol homeostasis.

In addition to their role in maintaining cholesterol homeostasis, LXRs also regulate fatty acid metabolism. Administration of synthetic LXR agonists to mice induced expression of fatty acid biosynthetic genes, such as acetyl CoA carboxylase, fatty acid synthase (FAS), and stearoyl CoA desaturase-1, in liver, and increased plasma triglyceride and phospholipid levels (9). LXRs regulate expression of sterol regulatory element-binding protein-1c (SREBP-1c), a transcription factor that regulates the expression of genes involved in fatty acid biosynthesis (10). LXRs also act on the promoters of FAS and angiotensin-like protein 3, a liver-specific secretory protein, which inactivates lipoprotein lipase and increases plasma triglyceride levels (11–14). Because LXRs have both antiatherogenic and lipogenic activities, the development of LXR agonists that could regulate cholesterol metabolism without adverse hypertriglyceremia-inducing effects is of great pharmacological interest.

The nuclear receptors liver X receptor (LXR) α and LXRβ serve as oxysterol receptors and regulate the expression of genes involved in lipid metabolism. LXR activation induces the expression of ATP-binding cassette (ABC) transporters, such as ABCG5 and ABCG8, which inhibit intestinal absorption of cholesterol and phytosterols. Although several synthetic LXR agonists have been generated, these compounds have limited clinical application, because they cause hypertriglyceremia by inducing the expression of lipogenic genes in the liver. We synthesized derivatives of phytosterols and found some of them to act as LXR agonists. Among them, YT-32 [(22E)-ergost-22-ene-1α,3β-diol], which is related to ergosterol and brassicasterol, is the most potent LXR agonist. YT-32 directly bound to LXRα and LXRβ and induced the interaction of LXRα with cofactors, such as steroid receptor coactivator-1, as effectively as the natural ligands, 22(R)-hydroxysterol and 24(S),25-epoxycholesterol. Although the nonsteroidal synthetic LXR agonist T0901317 induced the expression of intestinal ABC transporters and liver lipogenic genes, oral administration of YT-32 selectively activated intestinal ABC transporters in mice. Unlike T0901317 treatment, YT-32 inhibited intestinal cholesterol absorption without increasing plasma triglyceride levels. The phytosterol-derived LXR agonist YT-32 might selectively modulate intestinal cholesterol metabolism.
Phytosterols are naturally occurring sterols and are the plant equivalents of mammalian cholesterol. The effects of phytosterols in reducing blood cholesterol have been demonstrated for several decades, although the precise mechanism has not been elucidated (15). Recently, ABCG5 and ABCG8 have been proposed to function as transporters for cholesterol and phytosterols (16–18). The findings that the expression of ABCG5 and ABCG8 is induced by LXR activation (8) and that the treatment of intestinal cells with phytosterols increases the expression of LXR target genes (19) suggest that phytosterols, or their metabolites, act as LXR ligands and influence cholesterol metabolism. At present, no phytosterol has been identified as a LXR ligand. In this work, we examined the effects of several phytosterols and their derivatives on the activity of LXRs and found that a compound related to ergosterol and brassicasterol is a potent agonist for LXRs.

**EXPERIMENTAL PROCEDURES**

**Chemical Compounds**—T0901317 was purchased from Cayman Chemical (Ann Arbor, MI), 22(R)-Hydroxycholesterol, 24(S),25-epoxycholesterol, fucosterol, and fucostanol were from Steraloids (Newport, RI), ergosterol was from Nacalai (Kyoto, Japan), and brassicasterol, campesterol, β-sitosterol, and stigmasterol were from Wako (Osaka, Japan). YT compounds were synthesized as reported previously (20).

**Plasmids**—Fragments of hLXRα (GenBank™ accession number NM_005693), hLXRβ (accession number NM_007121), mLXRα (accession number NM_013839), and mLXRβ (accession number U09419) were inserted into pCMX vector to make pCMX-hLXRα, pCMX-hLXRβ, pCMX-mLXRα, and pCMX-mLXRβ, respectively (21). The ligand-binding domains of hLXRα, hLXRβ, and h retinoid X receptor (RXR)α were inserted into pCMX-GAL4 vector to make pCMX-GAL4-hLXRα, pCMX-GAL4-hLXRβ, and pCMX-GAL4-hRXRα, respectively (22), and full-length fragment of hLXRα was inserted into pCMX-VP16 vector to make pCMX-VP16-hLXRα (23). The amino acid 1–436 fragment of hLXRα was inserted into pCMX to generate pCMX-hLXRα-dAF-2. Nuclear hormone receptor-interacting domains of steroid receptor coactivator-1 (SRC-1) (amino acids 595–771; GenBank™ accession number U90661), ACTR (601–780; accession number AF036892), DRIP205 (578–728; accession number Y13467), and RIP140 (480–600; accession number X84373) were inserted into pCMX-GAL4 vector for pCMX-GAL4-SRC-1, pCMX-GAL4-ACTR, pCMX-GAL4-DRIP205, and pCMX-GAL4-RIP140, respectively (24). LXR-responsive rCYP7A-DR-4x3-tk-LUC and GAL4-responsive MH100(UAS)x4-tk-LUC reporters were utilized to evaluate the activities of LXRs and GAL4-chimera receptors (22, 25). All plasmids were sequenced prior to use to verify DNA sequence fidelity.

**Cell Culture and Cotransfection Assay**—Human embryonic kidney 293 cells were cotransfected with GAL4-hLXRα expression vector in combination with GAL4-responsive MH100(UAS)x4-tk-LUC reporter. Cells were then incubated with vehicle control (EtOH), phytosterols or their derivatives (10 μM) and assayed for luciferase activity. Luciferase activity of the reporter is expressed as -fold induction of compound treatment relative to vehicle control. The values represent means ± S.D.

![Phytosterol Derivative Acting as LXR Agonist](image)

**FIG. 1. Phytosterol derivatives activate LXRs.** Human embryonic kidney 293 cells were cotransfected with GAL4-hLXRα expression vector in combination with GAL4-responsive MH100(UAS)x4-tk-LUC reporter. Cells were then incubated with vehicle control (EtOH), phytosterols or their derivatives (10 μM) and assayed for luciferase activity. Luciferase activity of the reporter is expressed as -fold induction of compound treatment relative to vehicle control. The values represent means ± S.D.

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FIG. 1. Phytosterol derivatives activate LXRs. Human embryonic kidney 293 cells were cotransfected with GAL4-hLXRα expression vector in combination with GAL4-responsive MH100(UAS)x4-tk-LUC reporter. Cells were then incubated with vehicle control (EtOH), phytosterols or their derivatives (10 μM) and assayed for luciferase activity. Luciferase activity of the reporter is expressed as -fold induction of compound treatment relative to vehicle control. The values represent means ± S.D.

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2 Y. Tachibana, unpublished data.
Glutathione S-transferase (GST) Pull-down Assays—Nuclear receptor interaction domain of SRC-1 (amino acids 601–771) was cloned into the GST fusion vector pGEX-4T1 (Amersham Biosciences). GST-SRC-1 fusion protein was expressed in BL21 DE3 cells (Promega, Madison, WI). 35S-Labeled LXRs were generated using the TNT Quick Coupled Transcription/Translation System (Promega). GST pull-down assays were performed as reported previously (26, 27). Approximately 1/9262 g of GST-SRC-1 was bound in glutathione-Sepharose beads (Amersham Biosciences) and equilibrated in binding buffer (20 mM Tris-HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40). Bound GST proteins were then incubated with labeled LXR and test ligand for 1.5 h at 4 °C. After binding, beads were washed three times with washing buffer (20 mM Hepes, pH 7.7, 50 mM KCl, 20% glycerol, 0.1% Nonidet P-40), resuspended in SDS-PAGE sample buffer, and loaded onto a 10% SDS-polyacrylamide gel. After electrophoresis, bound proteins were visualized by autoradiography and quantified utilizing BAS2500 system (Fujifilm, Tokyo, Japan).

Animal Studies—C57BL/6J mice were obtained from Charles River (Yokohama, Japan) and housed in a room under controlled temperature (23 ± 1 °C) and humidity (45–65%) and had free access to water and chow (Oriental Yeast, Tokyo). Experiments were conducted when the mice (males) were between 8 and 9 weeks of age. Mice were treated orally with YT-32, YT-33, or T0901317 in a polyethylene glycol/Tween 80 (4/1) formulation or vehicle alone. Mice were analyzed 12 h after treatment under non-fasting conditions. Plasma triglyceride levels were determined with Triglycerides E-test Wako (Wako). To evaluate intestinal cholesterol absorption, mice were administered 20 Ci of [1,2-3H] cholesterol (Amersham Biosciences) suspended in 150 μl of oil via gavage 12 h after treatment with YT-32, YT-33, or T0901317, and radioactivities in 10 μl of plasma were measured with a liquid scintillation spectrometer. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University.

Figure 2. YT-32 is a potent agonist for LXRs. A, effects of YT-32 on hLXRβ and hRXRa. Cells were cotransfected with GAL4-hLXRβ or GAL4-hRXRa vector in combination with MH100(UAS)x4-tk-LUC reporter and treated with vehicle control (EtOH), ergosterol, YT-32, or 22(R)-hydroxycholesterol (22(R)-HC) at 10 μM. B, effect of YT-32 on mLXRa and mLXRb. Cells were cotransfected with CMX control vector, CMX-mLXRa, or CMX-mLXRb in combination with rCYP7A-DR-4x3-tk-LUC reporter. C, AF-2-dependent activation of LXRs by YT-32. Cells were cotransfected with CMX control vector, CMX-hLXRa, or CMX-hLXRb-dAF-2 and rCYP7A-DR-4x3-tk-LUC and treated with EtOH control or 10 μM YT-32. D, association of LXRs with cofactors induced by YT-32. Cells were cotransfected with GAL4 control vector or GAL4-chimera vector for SRC-1, ACTR, DRIP205, or RIP140, in combination with VP16 control or VP16-hLXRa vector and MH100(UAS)x4-tk-LUC reporter and treated with EtOH control, 10 μM YT-32, 22(R)-hydroxycholesterol, 24(S),25-epoxycholesterol (24(S),25-EC), or 100 nM T0901317. The values represent means ± S.D.
Identification of Phytosterol Derivatives as LXR Agonists—The effects of phytosterols including ergosterol, brassicasterol, campesterol, β-sitosterol, stigmasterol, and fucosterol on LXRα were examined by using the GAL4-chimeric receptor assay. The ligand-binding domain of hLXRα was fused to the DNA-binding domain of the yeast transcription factor GAL4 (28). The GAL4-hLXRα expression plasmid was cotransfected with a GAL4-responsive luciferase reporter into human embryonic kidney 293 cells. Because this reporter is activated only by the GAL4-chimera receptor, the potentially confounding effects of endogenous receptors are eliminated. As reported previously (9, 29), the oxysterol 24(S),25-epoxycholesterol and the synthetic ligand T0901317 induced the activation of LXRα (Fig. 1). None of the naturally occurring phytosterols were able to activate LXRα. Next, we tested a panel of synthetic phytosterol derivatives (YT compounds) (Fig. 1). Ergosterol derivatives (YT-6 and YT-32), a campesterol derivative (YT-4), a stigmasterol derivative (YT-17), and a poriferasterol derivative (YT-34) induced LXRα activation. Among the test compounds, YT-32 [(22R)-ergost-22-ene-1α,3β-diol] was the most efficacious LXRα agonist at 10 μM. Interestingly, at this concentration, YT-32 activated GAL4-hLXRα more effectively than an endogenous LXR ligand, 24(S),25-epoxycholesterol. Therefore, YT-32 is identified as a potent sterol agonist for GAL4-hLXRα.

**YT-32 Is a Potent Agonist for LXRαs**—The effects of YT-32 on hLXRβ and the LXR heterodimerization partner hRXRα were examined. YT-32, but not ergosterol, activated GAL4-hLXRβ, whereas both compounds had no effect on RXRα (Fig. 2A). Although RXR ligands have been reported to induce the activation of LXR/RXR heterodimers (22), the data indicate that YT-32 activates LXRα/RXR and LXRβ/RXR heterodimers by acting on LXRα. YT-32 did not activate PPARα, PPARβ, PPARγ, FXR, VDR, PXR, or CAR (data not shown). Because some nuclear receptors are known to exhibit species-specific ligand selectivity (30), we next examined the effects of YT-32 on mouse LXRα by cotransfecting full-length mouse LXRα or LXRβ vectors and a luciferase reporter containing the DR-4 element (LXRE) of the rat CYP7A promoter (25). YT-32 activated mLXRα and mLXRβ to levels equivalent to or greater than 22(R)-hydroxysterol, another physiological LXR agonist (31), whereas ergosterol had no activity on mouse LXRα (Fig. 2B).

Upon ligand bindings, nuclear receptors undergo a conformational change that induces C-terminal activation function-2 (AF-2)-dependent recruitment of coactivators such as SRC-1 (32). We examined the effect of YT-32 on an LXRα AF-2 deletion mutant. YT-32-dependent activation of LXRα was completely abolished by truncation of the AF-2 domain (Fig. 2C). To assay ligand-dependent interactions of LXRs with coactivators, the receptor-interacting domains of SRC-1, ACTR, DRIP205, and RIP140 were fused to the GAL4 DNA-binding domain (24). Cotransfection of GAL4 cofactors with LXRs fused to the transactivation domain of herpesvirus VP16 protein allowed for detection of ligand-dependent cofactor recruitment (28). YT-32, 22(R)-hydroxysterol, 24(S),25-epoxycholesterol, and T0901317 induced LXRα-SRC-1 interaction (Fig. 2D). YT-32 and the other agonists also induced the association of LXRα with ACTR, DRIP205, and RIP140. Interestingly, YT-32 stimulated interaction of LXRα with ACTR and DRIP205 more efficiently than the oxysterol agonists 22(R)-hydroxysterol and 24(S),25-epoxycholesterol at 10 μM.

To determine whether YT-32 binds directly to LXRα, GST pull-down analysis was performed. We generated a fusion protein of GST and the nuclear receptor-interacting domain of SRC-1 and evaluated the ligand-dependent interaction between GST-SRC-1 and isotope-labeled hLXRα (Fig. 3). YT-32 binding to LXRα was completely abolished by truncation of the AF-2 domain (Fig. 3E). These interactions were not observed when the AF-2 domain of LXRα was deleted (Fig. 3C). YT-32 also induced the interaction of hLXRβ with SRC-1 as effectively as T0901317 (Fig. 3F). The data demonstrate that YT-32 activates LXRs by direct binding.

**Structure-function Relationship of YT-32 on LXRs**—YT-32 is a sterol compound with a saturated cholesterol structure and the same side chain as ergosterol and brassicasterol (see Fig. 1 and Fig. A4). YT-33 differs from YT-32 only in the absence of the 1α-hydroxyl group. Interestingly, whereas YT-32 is a potent ligand for LXRs, YT-33 completely lacks agonist activity, indicating that the presence of 1α-hydroxyl group is necessary for LXRα activation by YT-32 (Fig. 1A). To further examine the structure-function relationship between YT-32 and LXRs, we...
synthesized several YT-32 derivatives containing the 1α-hydroxyl group (Fig. 4A). YT-32 is a 22-ergosten with two hydroxyl groups at 1α and 3β positions. Although YT-56 and YT-57 have the same hydroxyl groups and side chain configuration as YT-32, they are 5,22-dien and 5,7,22-trien, respectively. YT-59 has a saturated cholesterol ring structure like YT-32 but also has a saturated bond at C22. We compared dose-response curves of these compounds on full-length hLXRα (Fig. 4B). Among these compounds, YT-32 was the most potent LXRα activator (effective concentration for 50% maximal activation, EC₅₀ = 0.41 μM), followed in rank order by YT-59 (EC₅₀ = 1.3 μM) and YT-56 (EC₅₀ = 15 μM). YT-57 had no activity on LXRα. Interestingly, YT-32 and YT-59 were able to activate LXRα more effectively than the natural oxysterol, 24(S),25-epoxycholesterol (EC₅₀ = 0.81 μM). The data indicate that saturated cholesterol structure is important for LXRα activation, because activity decreases as saturation decreases (YT-32 > YT-56 > YT-57). Comparing YT-32 with YT-59, saturation at C22 slightly impairs LXRα activation. We next compared the effects of YT-32 and its related compounds on LXRβ (Fig. 4C). YT-32 induced activation of LXRβ similarly to 24(S),25-epoxycholesterol (EC₅₀ = 1.1 μM for YT-32, 1.1 μM for
24(S),25-epoxycholesterol). YT-59 activated LXRβ less potently than YT-32 and 22(R)-hydroxycholesterol (EC50 = 8.8 μM for YT-59, 3.2 μM for 22(R)-hydroxycholesterol). YT-56 and YT-33 were not effective LXRβ agonists. Taken together, these data indicate that the 1α-hydroxyl group and saturated ring structure are important for LXRα and LXRβ activation.

**Fig. 5. LXR target gene expression in intestine and liver of mice treated with YT-32.** Mice (n = 3) were orally administrated with 50 or 250 mg/kg of YT-32, 10 mg/kg of T0901317, or 250 mg/kg of YT-33. Twelve h after administration, total RNA was extracted from liver and intestinal mucosa. **A**, quantitative real-time PCR from intestinal RNA for ABCA1, ABCG5, ABCG8, LXRα, and LXRβ after treatment with YT-32 or T0901317. YT-32 and T0901317 significantly induced the intestinal expression of ABCA1, ABCG5, and ABCG8. **B**, quantitative real-time PCR from liver RNA for ABCA1, ABCG5, ABCG8, SREBP-1c, and FAS after treatment with YT-32 or T0901317. Although T0901317 induced the expression of SREBP-1c, as well as ABCG5 and ABCG8, YT-32 was not effective in inducing these genes in the liver. **C**, quantitative real-time PCR from intestinal RNA for ABCA1, ABCG5, ABCG8, LXRα, and LXRβ after treatment with YT-33. The values represent means ± S.D. *, p < 0.05 compared with the vehicle control.
YT-32 Induces Intestinal ABC Transporter Genes—To investigate the in vivo effects of YT-32, we administered YT-32 orally to mice and examined the mRNA expression of target genes including ABC transporters in intestine and liver. YT-32 and T0901317 induced the expression of ABCA1, ABCG5, and ABCG8 in the intestine (Fig. 5A). The level of LXRα and LXRβ expression in the intestine were not changed by treatment with these compounds. As reported previously (8, 9), T0901317 induced the expression of ABCG5, ABCG8, SREBP-1c, and FAS but did not significantly induce that of ABCA1 in the liver. In contrast to the effects in intestine, YT-32 did not effectively increase the liver mRNA levels of LXR target genes (Fig. 5B). Expression of the LXRα and LXRβ genes in the liver was not changed after treatment with YT-32 or T0901317 (data not shown). YT-33, which lacks the 1α-hydroxyl group present in YT-32 and does not activate LXRs (see Figs. 1 and 4), did not change the expression of ABCA1, ABCG5, ABCG8, LXRα, or LXRβ in the intestine (Fig. 5C). Therefore, treatment with YT-32 induced the expression of intestinal ABC transporters but had slight or no observable effect on the expression of liver lipogenic genes.

YT-32 Inhibits Intestinal Cholesterol Absorption without Inducing Hypertriglycemia—The effects of YT-32 on intestinal cholesterol absorption and plasma triglyceride levels were examined. Mice were treated with YT-32, T0901317, or YT-33 for 12 h, and isotope-labeled cholesterol was administrated via gavage. As reported previously (33), plasma radioactivity increased gradually. Pretreatment with T0901317 and YT-32 decreased cholesterol absorption by 14 and 19%, respectively, 12 h after cholesterol administration, whereas YT-33 pretreatment did not change cholesterol absorption (Fig. 6A). The data strongly suggest that the cholesterol absorption-decreasing effect of YT-32 is mediated through LXRs. Treatment of mice with T0901317 increased plasma triglyceride as reported previously (Fig. 6B) (9). At a dose sufficient to effectively induce expression of ABCG5 and ABCG8 and inhibit intestinal cholesterol absorption, YT-32 did not alter plasma triglyceride levels (Fig. 6B). Treatment with YT-33 did not affect plasma triglycerides. Taken together, the LXR agonist YT-32 inhibited cholesterol absorption without inducing hypertriglycemia.

DISCUSSION

LXRs were originally identified as orphan members of the nuclear receptor superfamily and were later found to be receptors for endogenous oxysterols such as 22(R)-hydroxysterol and 24(S,25-epoxycholesterol (29, 31). The distribution of these natural ligands is tissue-specific. 24(S,25-Epoxycholesterol is formed in the liver via a shunt in the cholesterol biosynthetic pathway, whereas 22(R)-hydroxysterol is a transient intermediate in steroid hormone synthesis in the adrenal cortex. The limited systemic distribution of these oxysterols suggests the presence of other physiological LXRx ligands. The finding that cholesterol-treated macrophages express the LXR-responsive ABCA1 and ABCG1 genes and utilize the enzyme CYP27 to produce 27-hydroxysterol and cholestenoic acid suggests that 27-hydroxysterol may serve as an endogenous ligand for LXRs (34). Cholestenoic acid was also reported to be an LXRx agonist (35). Cholesterol feeding increased the intestinal expression of ABCG5 and ABCG8 in wild-type mice but not in LXRα/β null mice (8). Treatment with cholesterol or sitostanol increased the expression of the ABCA1 gene in intestinal cells (19). These findings suggest that LXRs are activated by metabolites of cholesterol and phytosterols in the intestine. However, the metabolic conversion of dietary sterols, such as cholesterol and phytosterols, has not been elucidated. In the present work, we show that an ergosterol derivative is a potent agonist for LXRs and induces the expression of ABC transporters in the mouse intestine. Our findings strongly suggest that the cholesterol-lowering effect of phytosterols is at least partly because of conversion of these compounds to LXRx agonists, which activate the expression of sterol efflux transporters such as ABCG5 and ABCG8.

Several synthetic LXRx agonists have been shown to modulate LXRx regulation of cholesterol metabolism (9, 36, 37). Unfortunately, potent LXRx agonists cause undesirable increases in liver and plasma triglyceride levels, because of the activation of LXRx-responsive genes in the liver (SREBP-1c, FAS, and angio-pointin-like protein 3), in addition to their beneficial effects on cholesterol metabolism (10–13). SREBP-1c activates transcription of the major genes involved in fatty acid synthesis (acyetyl CoA carboxylase, FAS, stearil CoA desaturase-1, and glycerol-3-phosphate acyltransferase), and SREBP-1c transgenic mice show markedly increased liver triglycerides (38). Therefore, the development of selective modulators that act specifically on cholesterol metabolism without adverse lipogenic effects is required to realize the therapeutic potential of LXRx pharmacophores.
We found that YT-32, a derivative of ergosterol or brassicasterol, activated LXRs more effectively than the known oxysterol agonists 24(S),25-epoxycholesterol and 22R-hydroxycholesterol and that it induced the expression of ABC transporter genes in the intestine. Interestingly, the induction of LXR-regulated genes in the liver was only modest or not observed. This selective activity of YT-32 may be because of its sterol-based structure. YT-32 acts as a potent LXR agonist and induces the sterol exporting ABC transporters such as ABCA1, ABCG5, and ABCG8. ABCG5 and ABCG8 are expressed exclusively in liver and small intestine and are localized to the apical (intestinal) membrane of cells (39). Overexpression of these genes results in an ~50% reduction in absorption of dietary cholesterol and a dramatic increase in the biliary secretion of sterols (17). On the other hand, disruption of Abcg5 and Abcg8 in mice causes increased absorption of dietary phytosterols and a decrease in biliary cholesterol concentration (18). Therefore, Abcg5 and Abcg8 play an important role in biliary cholesterol secretion and the regulation of intestinal cholesterol absorption. Repa et al. (7) reported that ABCA1 expression was increased in the intestines of mice treated with a synthetic LXR agonist and suggested that ABCA1 catalyzed efflux causes the associated decrease in cholesterol absorption. However, the role of ABCA1 in intestinal cholesterol absorption remains to be clarified (2). ABCA1 was dominantly expressed on the basolateral surface of intestinal cells (40), and Plosch et al. (41) reported that the effect of the LXR agonist on fecal sterol loss was observed in Abca1 null mice, as well as wild-type mice. Recently (42), ABCG5 and ABCG8 were demonstrated to mediate the LXR agonist-mediated reduction of cholesterol absorption. YT-32 may reduce the intestinal absorption of dietary sterols, including cholesterol and phytosterols, by inducing the expression ABCG5 and ABCG8, which might excrete the ester from the basolateral membranes of the mucosal cells. Some fraction of YT-32 may be incorporated in the blood circulation where it could be available to stimulate the reverse cholesterol transport on atherosclerotic lesions. Alternatively, YT-32 may be secreted into the bile by ABCG5 and ABCG8 before it would be able to induce liver lipogenic genes. In contrast to YT-32, synthetic nonsteroidal agonists such as T0901317 do not seem to be substrates of the ABC transporters and it is therefore reasonable to ask if the clear improvement in plasma triglycerides by inducing lipogenic genes in the liver (9). Another possibility is that tissue selectivity of YT-32 is mediated by another mechanism as is the case for selective estrogen receptor modulators.

The differential cofactor recruitment shown in Fig. 2 may contribute to tissue-specific agonist activity. Further analysis is required to elucidate the mechanism of tissue-specific LXR activation by YT-32. Therefore, our data suggest that LXR agonists on the structure of natural sterols should be useful to agents in therapeutically modulating cholesterol metabolism.

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**Induction of Intestinal ATP-binding Cassette Transporters by a Phytosterol-derived Liver X Receptor Agonist**

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