JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Fucosterol Is a Selective Liver X Receptor Modulator That Regulates the Expression of Key Genes in Cholesterol Homeostasis in Macrophages, Hepatocytes, and Intestinal Cells

Minh-Hien Hoang,[†] Yaoyao Jia,[†] Hee-jin Jun,[†] Ji Hae Lee,[†] Boo Yong Lee,[‡] and Sung-Joon Lee*[,†]

[†]Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, South Korea

[‡]Department of Food Science and Biotechnology, CHA University, 22 Yatap-dong, Bundang-gu, Seongnam-si, Kyunggi-do 463-836, South Korea

**ABSTRACT:** Fucosterol, a sterol that is abundant in marine algae, has hypocholesterolemic activity, but the mechanism underlying its effect is not clearly understood. Because data suggest that fucosterol can increase plasma high-density lipoprotein concentrations, we investigated whether it could activate liver X receptors (LXRs), critical transcription factors in reverse cholesterol transport. Fucosterol dose-dependently stimulated the transcriptional activity of both LXR-$\alpha$ and -$\beta$ in a reporter gene assay, responses that were attenuated by the LXR antagonist $As_2O_3$. Fucosterol also activated co-activator recruitment in cell-free time-resolved fluorescence resonance energy transfer analysis. In THP-1-derived macrophages, it induced the transcriptional activation of ABCA1, ABCG1, and ApoE, key genes in reverse cholesterol transport, and thereby significantly increased the efflux of cholesterol. Fucosterol also regulated intestinal NPC1L1 and ABCA1 in Caco-2 cells. Notably, fucosterol did not induce cellular triglyceride accumulation in HepG2 cells, primarily because of its upregulation of Insig-2a, which delays nuclear translocation of SREBP-1c, a key hepatic lipogenic transcription factor. These results suggest that fucosterol is a dual-LXR agonist that regulates the expression of key genes in cholesterol homeostasis in multiple cell lines without inducing hepatic triglyceride accumulation.

**KEYWORDS:** *Fucosterol, LXR, anti-atherosclerosis*

## INTRODUCTION

Atherosclerosis is a leading cause of death worldwide, accounting for an estimated 72 million deaths each year.[1] Epidemiologic studies have identified elevated concentrations of low-density lipoprotein cholesterol (LDL-C) and reduced high-density lipoprotein cholesterol (HDL-C) concentrations as major contributors to atherogenesis.[2] Current treatments aim to reverse these profiles, in part by modifying hepatic cholesterol biosynthesis and dietary intake. It was recently demonstrated that liver X receptors (LXRs), ligand-activated transcription factors of nuclear receptor superfamily, play a critical role in protection against atherosclerosis.[3] LXR activation, by upregulating the expression of adenosine triphosphate-binding cassette (ABC) proteins A1, G1, and apolipoprotein E (ApoE), increases cholesterol efflux, stimulates reverse cholesterol transport (RCT) from peripheral tissues, and elevates HDL-C levels, thereby providing anti-atherogenic potential by inhibiting the progression and even promoting the regression of atherosclerosis.[4−6]

LXRs also impact systemic cholesterol levels by reducing intestinal cholesterol absorption and increasing biliary cholesterol excretion through regulation of the transporters ABCG5 and ABCG8.[7] Mutations in human ABCG5 or ABCG8 lead to sitosterolemia (abnormal absorption of sitosterols and hyperabsorption of cholesterol) and the development of premature cardiovascular disease.[8] Moreover, LXR activation inhibits hepatic gluconeogenesis and lowers plasma glucose levels, indicating the potential application of LXR activation in the treatment of type II diabetes mellitus, which worsens dyslipidemia and inflammation and, thus, accelerates atherosclerosis.[9]

These results highlight the cardioprotective effects of LXRs. However, studies have also shown that LXR agonists can cause liver steatosis and increase serum triglyceride levels in rodents by activating hepatic sterol regulatory element-binding protein 1c (SREBP-1c).[10] Thus, specific LXR ligands that do not induce fatty acid synthesis in the liver are of interest. Numerous groups have described agents that have beneficial effects on lipid metabolism. Hoang et al.,[11,12] Quinet et al.,[13] and Kratzer et al.[14] identified several novel LXR agonists, taurine, ethyl 2,4,6-trihydroxybenzoate, WAY-252623, and *N*,*N*-dimethyl-3$\beta$-hydroxy-cholenamide, that reduce atherosclerosis without activating SREBP-1c or increasing hepatic lipogenesis. This raised the possibility that some of the anti-atherosclerotic effects of LXR agonists may be independent of systemic lipid metabolism in hepatocytes and may be attributable to direct actions on the vascular wall that activate RCT. Thus, LXRs are attractive targets for novel pharmaceutical agents.

Sterols are important structural components of cell membranes. Phytosterols are known to reduce the serum cholesterol concentration in both humans and animals.[15−18]

Received: May 2, 2012
Revised: October 30, 2012
Accepted: November 1, 2012
Published: November 1, 2012


© 2012 American Chemical Society

dx.doi.org/10.1021/jf3019084 | J. Agric. Food Chem. 2012, 60, 11567−11575

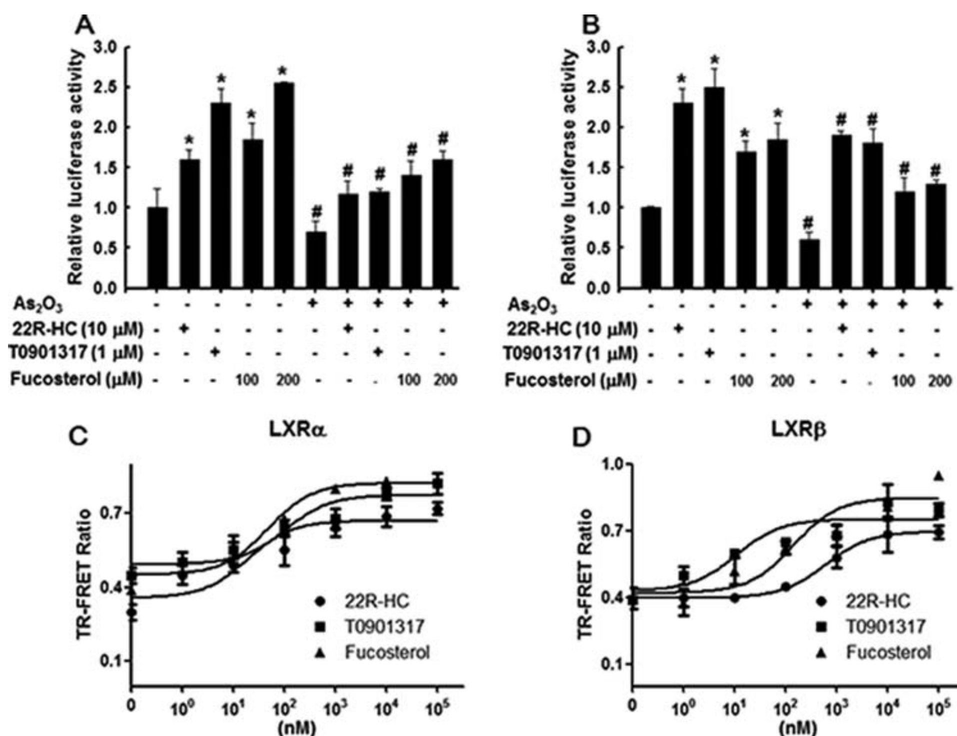


**Figure 1.** Fucosterol activates LXR-$\alpha$ and -$\beta$ by interacting directly with their LBDs. Fucosterol activates (A) LXR-$\alpha$ and (B) LXR-$\beta$ transactivation activity. HEK 293 cell cultures were cultured with or without 100 nM $As_2O_3$. After 1 week, cells were transfected with expression plasmids for receptors and pSV-$\beta$-galactosidase, together with the reporter plasmid pGL4.35[luc2P/9XGAL4UAS/Hygro]. The cells were exposed to fucosterol (100 and 200 $\mu$M), 10 $\mu$M 22*R*-HC, or 1 $\mu$M T0901317 for 24 h and then assayed for luciferase activity. Activation of (C) LXR-$\alpha$ and (D) LXR-$\beta$ by 22*R*-HC, T0901317, and fucosterol was assessed by the TR-FRET assay. Data represent the means ± SE ($n$ = 4−5). (*) $p < 0.05$ versus the control. (#) $p < 0.05$ versus the absence of $As_2O_3$.

Current National Cholesterol Education Program guidelines for cholesterol management suggest phytosterol consumption as a therapeutic option to reduce plasma cholesterol levels and atherosclerosis.[19] A daily intake of 2−2.5 g of phytosterol results in an average reduction in the cholesterol level of up to 10%.[16,20] The cholesterol-lowering action of phytosterols is thought to occur, at least in part, through competition with dietary and biliary cholesterol for intestinal absorption by NPC1L1.[16] Moreover, after intestinal uptake, plant sterols and their derivatives in the circulation have been shown to act as signaling molecules, notably LXR ligands (*in vivo* and *in vitro* experiments), suggesting that phytosterols may regulate cholesterol homeostasis by multiple mechanisms.[21−23] Specifically, ergosterol and brassicasterol (YT-32), administered orally to mice (at concentrations of 50 and 250 mg/kg, respectively), were found to be potent agonists for LXR and induced the expression of ABC transporters in the mouse intestine.[21] In line with these observations, phytosterols from the 4-desmethylsterol family have been shown to be ligands of both LXR-$\alpha$ and -$\beta$ in a co-activator peptide recruitment assay.[23]

The most abundant sterol in marine algae, fucosterol, has been shown to inhibit absorption of cholesterol from the intestine.[24] In addition, the fucosterol derivatives 4-methylsterols from seaweed *Crypthecodinium cohnii* did not alter serum or liver lipid concentrations but increased the serum HDL-C concentration by 25%.[25] Kritchevsky et al. reported that rats fed with cholesterol and cholesterol plus bile salt diets have shown a significant increase in total serum cholesterol and a decreased HDL-C level. In contrast, the cholesterol−bile salt plus 4-methylsterols diet significantly raised the amount of the HDL-C level, but no other serum or liver lipid parameters were affected.[25] Collectively, these previous data led us to hypothesize that fucosterol may increase plasma HDL concentrations by activating LXR activity. In the present study, we confirmed that fucosterol is indeed a LXR agonist and regulates cholesterol homeostasis in multiple cells by activating LXR target genes.

## ■ MATERIALS AND METHODS

**Reagents.** Cell culture reagents and supplies were obtained from Hyclone (Logan, UT). Fucosterol, T0901317, 22*R*-hydroxylcholesterol (22*R*-HC), $\beta$-mercaptoethanol, $As_2O_3$, fatty acid-free albumin (FAFA), 22-NBD-cholesterol, and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma (St. Louis, MO). Human HDL was purchased from Calbiochem (La Jolla, CA). $As_2O_3$ stock solution (100 mM) was prepared with 0.1 N sodium hydroxide and subsequently diluted with phosphate-buffered saline (PBS). Total RNA extraction reagent (RNAiso Plus) and real-time polymerase chain reaction (PCR) premix (SYBRPremix Ex Taq) were obtained from Takara (Otsu, Japan). Oligo (dT)15 primer was purchased from Promega (Madison, WI). Primary (anti-SREBP 1 and $\beta$-actin) and secondary (anti-rabbit and anti-mouse immunoglobulin G) antibodies were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were purchased from Sigma.

**Cell Culture and Treatments.** HEK 293, human monocytic THP-1, H4IIE, HepG2, and Caco2 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). HEK 293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (PES) and were used in luciferase reporter assays. THP-1 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, 0.05 mM $\beta$-mercaptoethanol, and 1% PES. The cells were differentiated in the presence of 50 ng/mL PMA

for 72 h prior to treatment and RNA extraction. HepG2 and H4IIE cells were cultured in DMEM and minimum essential medium (MEM), respectively, supplemented with 10% FBS and 1% PES. Caco2 cells were maintained in MEM supplemented with 20% FBS, 1% non-essential amino acids, 2.5 mM hydroxyethyl piperazineethanesulfonic acid, and 1% PES. All cell lines were grown in 5% $CO_2$ at 37 °C. For experiments, HEK 293 and THP-1 cell cultures were cultured with or without 100 nM $As_2O_3$. After 1 week, cells were incubated in medium containing 22*R*-HC (10 $\mu$M), T0901317 (1 $\mu$M), or fucosterol (100 or 200 $\mu$M) for 48 h. H4IIE and Caco-2 cells were pre-incubated in DMEM and MEM, respectively, for 24 h. The following day, after removing the media, the cells were incubated for an additional 48 h in 2 mL of medium containing 22*R*-HC (10 $\mu$M), T0901317 (1 $\mu$M), or fucosterol (100 or 200 $\mu$M). Stock solutions of fucosterol (20 mM), 22*R*-HC (1 mM), and T0901317 (0.1 mM) were prepared in ethanol. Vehicle control cells were treated with the corresponding amount of ethanol (1%) (as used for the highest substance concentration). Each treatment was carried out in at least triplicate.

**Transfection and Luciferase Assay.** The vectors used in luciferase assays included pGL4.35[luc2P/9XGAL4UAS/Hygro] (Promega, Madison, WI), pSV-*β*-galactosidase (kindly provided by Dr. Soo-Jong Um, Sejong University, Seoul, Korea), pFN26AhLXR*α*, and pFN26AhLXR*β*. The pFN26AhLXR*α* and pFN26AhLXR*β* clones were constructed by subcloning the ligand-binding domains (LBDs) of hLXR-*α* (amino acids 137−102) and hLXR-*β* (amino acids 209−468), respectively, into pFN26A(BIND)hRluc-neo Flexi (Promega), digested with *Sgf*I and *Pme*I.

Experiments were performed according to the method reported previously.[12] HEK 293 cells were cultured with or without 100 nM $As_2O_3$. After 1 week, cells ($2 \times 10^5$/mL) were plated in 24-well culture plates and then incubated in DMEM without antibiotics. On the following day, cells were co-transfected with pGL4.35[luc2P/9XGAL4UAS/Hygro], pSV-*β*-galactosidase, and either pFN26AhLXRα or pFN26AhLXR*β*, using the HilyMax transfection reagent (Dojindo Molecular Technologies, Gaithersburg, MD), according to the protocol of the manufacturer. At 24 h post-transfection, the transfected cells were cultured in DMEM containing 22*R*-HC (10 $\mu$M), T0901317 (1 $\mu$M), fucosterol (100 or 200 $\mu$M), or vehicle (1% ethanol) in the presence or absence of the LXR antagonist $As_2O_3$[26] for an additional 24 h. Next, the cells were lysed and assayed for luciferase and *β*-galactosidase activities using a firefly luciferase assay kit (Biotium, Hayward, CA) and a *β*-galactosidase enzyme assay system (Promega), respectively, according to the protocols of the manufacturers. In each experiment, luciferase assay results were normalized to *β*-galactosidase and expressed as relative luciferase activity.

**Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) Assay for LXR-*α*/-*β* Ligand-Binding Activity.** TR-FRET assays for LXR-*α* or -*β* were performed using a commercial kit (Invitrogen, Carlsbad, CA). The LBD of LXR-*α* or -*β* in the assay included the glutathione *S*-transferase (GST) domain, which is recognized by the terbium-tagged GST antibody. The ligand-mediated conformational change of LXR LBD recruits the fluorescein-labeled co-activator peptide, fluorescein-TRAP220/DRIP-2 or fluorscein-D22, for LXR-*α* or -*β*, respectively. The terbium fluorescence on the anti-GST antibody is excited at 340 nm, and then energy is transferred to the fluorescein label on the co-activator peptide and detected as an emission at 520 nm by FRET, instead of emitting at 495 nm directly emitted from terbium. When running the assay, LBD is added to the test compounds, followed by the addition of a mixture of the fluorescein−co-activator peptide and terbium anti-GST antibody. After an incubation period at room temperature, the TR-FRET ratio of 520:495 is calculated and can be used to determine the $EC_{50}$ from a dose−response curve of the compound. Thus, the vertical axis in panels C and D of Figure 1 shows the TR-FRET ratio of 520:495. After incubation for 2 h at room temperature, the samples were analyzed using a SpectraMax instrument with time-resolved fluorescence laser excitation at 340 nm, emission at 495 nm, and LXR-*α* or -*β* binding detection at 520 nm. The ratio of the emission signals at 520 and 495 nm was plotted against the log of the ligand concentration to generate a binding curve. To determine the concentration required to produce a 50% effect ($EC_{50}$), data were fitted to a sigmoidal dose−response curve using GraphPad Prism, version 5.0 (GraphPad Software, Inc., La Jolla, CA).

**Cellular Cholesterol Efflux Experiments.** Cholesterol experiments were performed according to the method by Atshaves et al.,[27] with some modifications. Briefly, THP-1 cells were plated in 6-well plates at a density of $10^6$/well and differentiated into macrophages through incubation in the presence of 50 ng/mL PMA for 72 h. The following day, the medium was removed and the cells were incubated for an additional 48 h in 2 mL of medium containing 10 $\mu$M 22*R*-HC and 1 $\mu$M T0901317 (positive control), fucosterol (100 or 200 $\mu$M), or vehicle (1% ethanol). They were then loaded with 1.5 $\mu$M of 22-NBD-cholesterol (Invitrogen) in medium containing 2.5% FBS for 1 h in a 37 °C $CO_2$ incubator. After loading, the cells were washed twice with Puck's buffer [1 mM $Na_2HPO_4$, 0.9 mM $H_2PO_4$, 5.0 mM KCl, 1.8 mM $CaCl_2$, 0.6 mM $MgSO_4$, 6 mM glucose, 138 mM NaCl, and 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)] and placed in serum-free medium. HDL (10 $\mu$g/mL) was added to start the efflux experiment. After incubation for 3 h, the medium was removed and the cells were resuspended in FACS buffer and analyzed using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) by determining FL-1 fluorescence.[25] The data were analyzed using Cell Quest Pro software (BD Biosciences). The HDL-dependent efflux of 22-NBD-cholesterol was calculated as the following equation. Each efflux assay was performed in triplicate.

$$\text{percent efflux} = (\text{total fluorescent from the cells in the absence of HDL} - \text{total fluorescent from the cells in the presence of HDL}) / \text{total fluorescent from the cells in the absence of HDL}$$

**Cellular Triglyceride (TG) Measurements.** Cellular lipids were extracted as described previously.[28,29] Cellular TG content was quantified by an enzymatic method using a Cobas C111 automatic analyzer (Roche, Basel, Switzerland).

**Oil Red O Staining.** Cells were washed with ice-cold PBS and fixed through incubation overnight with formalin (10%, v/v). Next, fixed cells were washed with water and isopropanol (60%, v/v) and stained with Oil Red O (0.35%, v/v) for 1 h. After washing with water, images were acquired using an inverted microscope (Eclipse Ti-S, Nikon).

**Quantitative (q)PCR.** Total RNA was extracted from THP-1-derived macrophages and Caco2 and HepG2 cells, using an RNAiso Plus kit, according to the protocol of the manufacturer, after treatment for 2 days with 22*R*-HC, T0901317, and fucosterol or vehicle. Real-time qPCR was performed with Bio-Rad iQ SYBR Green Supermix reagent and a Bio-Rad iQ5 Cycler System. The primers used are shown in the study by Hoang et al.[11] Expression levels were normalized to those of glyceraldehyde 3-phosphate dehydrogenase or cyclophilin by the normalized expression (CT) method, according to the guidelines of the manufacturer.

**Immunoblotting Analysis.** HepG2 cells were lysed in ice-cold lysis buffer [10 mM Tris-HCl (pH 7.4), 0.1 M ethylenediaminetetraacetic acid (EDTA), 10 mM NaCl, 0.5% Triton X-100, and protease inhibitor cocktail (Roche, Mannheim, Germany)]. The resulting lysate was clarified by centrifugation at 14 000 rpm for 10 min at 4 °C. To quantify SREBP-1, proteins were isolated from the nuclear and membrane fractions using a kit (Cayman Chemical, Ann Arbor, MI), according to the protocol of the manufacturer. The protein concentration was determined using a Bio-Rad protein kit, with bovine serum albumin (Sigma, St. Louis, MO) as the standard. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and immunoblotting were performed as described previously.[30]

**Statistical Analysis.** All data are expressed as the means ± standard error (SE). Pairs of groups were compared using Student's *t* test. Differences were considered to be statistically significant at $p < 0.05$.

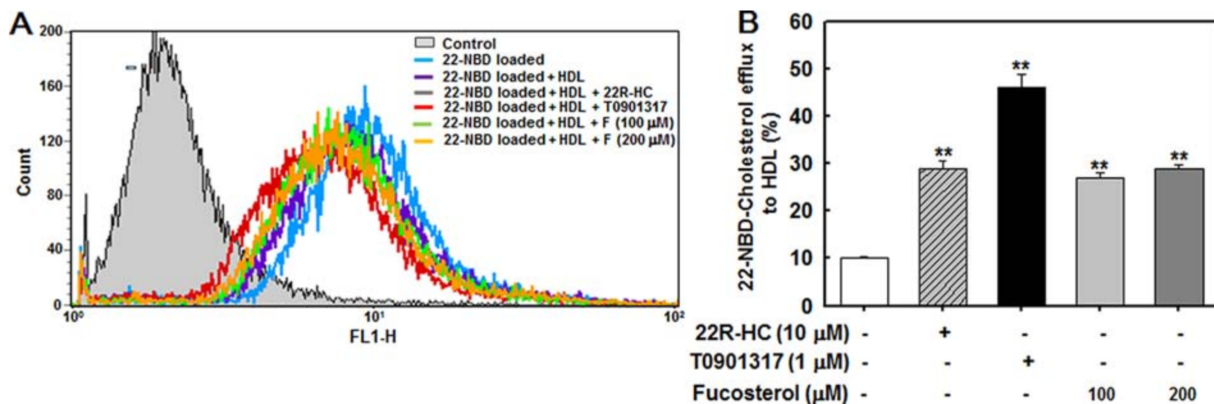


**Figure 2.** Fucosterol induces cholesterol efflux in a THP-1-derived macrophage. Cholesterol efflux was quantified using 22-NBD-cholesterol and by FACS analysis. (A) FACS profile of cholesterol efflux in THP-1-derived macrophages treated with HDL. (B) Relative 22-NBD-cholesterol efflux to HDL. Data represent the means ± SE ($n$ = 3–5). (*) $p < 0.05$ and (**) $p < 0.01$ versus the control.

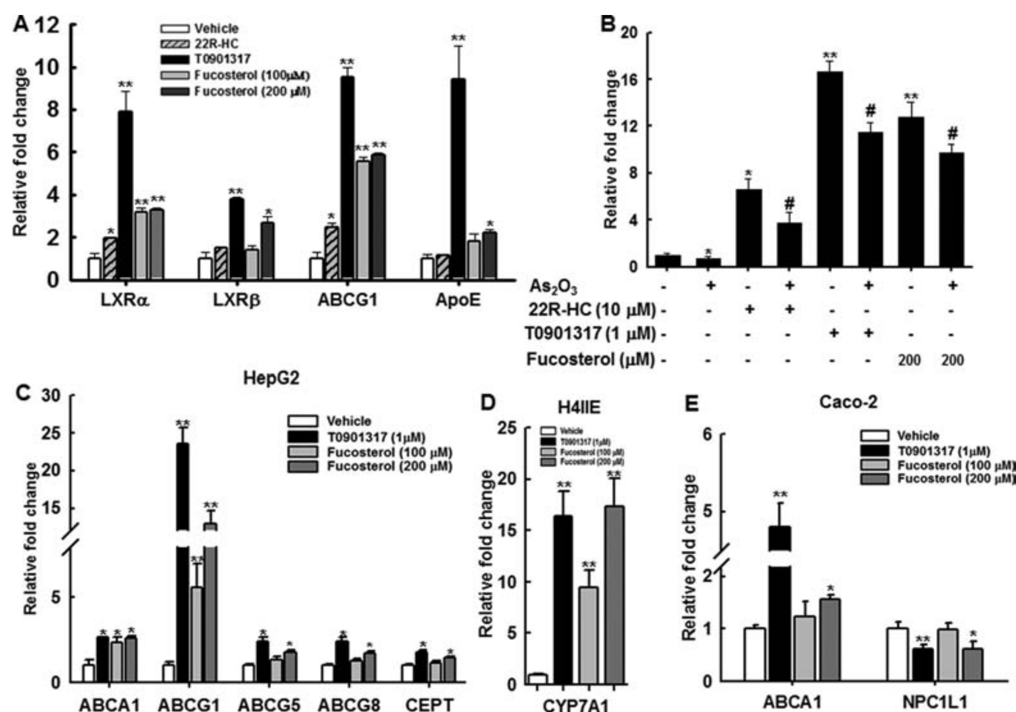


**Figure 3.** Induction of LXRs and their responsive genes by fucosterol in multiple cell types. THP-1 monocytes were incubated with PMA (50 ng/mL) for 3 days to induce their differentiation into adherent macrophages. The macrophages and HepG2, H4IIE, and Caco2 cells were then treated with fucosterol (100 or 200 $\mu$M), 10 $\mu$M 22$R$-HC, 1 $\mu$M T0901317, or vehicle (1% ethanol) for 48 h. Total RNA was extracted, and mRNA expression of (A) LXR-$\alpha$, ABCG1, and ApoE and (B) ABCA1 in macrophages, (C) ABCA1, ABCG1, ABCG5, ABCG8, and CETP in HepG2 cells, (D) CYP7A1 in H4IIE cells, and (E) ABCA1 and NPC1L1 in Caco2 cells was measured by qPCR. Data represent the means ± standard error of the mean (SEM) ($n$ = 3–5). (*) $p < 0.05$ and (**) $p < 0.001$ versus the control. (#) $p < 0.05$ versus the absence of $As_2O_3$.

## ■ RESULTS

**Fucosterol Is an Agonistic of Both LXR-$\alpha$ and -$\beta$ and Stimulates LXR Transactivation.** The effect of fucosterol on LXR transactivation was assessed by luciferase reporter gene assays. Fucosterol significantly dose-dependently induced the transactivation of both LXR-$\alpha$ (+155% at 200 $\mu$M; $p < 0.05$; Figure 1A) and LXR-$\beta$ (+83% at 200 $\mu$M; $p < 0.05$; Figure 1B). In the presence of $As_2O_3$, the effects of 22$R$-HC, T0901317, and fucosterol on transactivation of LXR-$\alpha$ and -$\beta$ were attenuated.

Fucosterol could modulate LXR activity directly by interacting with the LBDs of LXRs or indirectly by inducing the synthesis of an endogenous ligand. To confirm the direct binding of fucosterol to LXR LBDs, a TR-FRET assay was performed. TR-FRET assays showed that the endogenous and synthetic LXR ligands 22$R$-HC and T0901317 strongly enhanced the recruitment of Trap 220/Drip-2 and D22 co-activator peptides (panels C and D of Figure 1). $EC_{50}$ values of 22$R$-HC were 5882 ± 299 nM for LXR-$\alpha$ and 464 ± 51 nM for LXR-$\beta$. $EC_{50}$ values of T0901317 were 1026 ± 280 nM for LXR-$\alpha$ and 105 ± 32 nM for LXR-$\beta$. Similarly, fucosterol dose-dependently induced recruitment of the Trap 220/Drip-2 co-activator peptide to LXR-$\alpha$ LBD and the D22 co-activator peptide to LXR-$\beta$ LBD, with $EC_{50}$ values of 464 ± 37 and 1391 ± 475 nM for LXR-$\alpha$ and -$\beta$, respectively.

**Fucosterol Promotes Cholesterol Efflux in THP-1-Derived Macrophage Cells and Regulates the Expres-**

**sion of LXRs and Their Responsive Genes in Multiple Cell Lines.** The activation of LXR-$\alpha$ promotes cholesterol efflux, stimulates RCT in macrophages, and inhibits the accumulation of cholesterol in hepatocytes *in vitro* and *in vivo*.[9,31] We next determined the effect of fucosterol on the efflux of cholesterol from THP-1-derived macrophages. LXR ligands (22*R*-HC and T0901317) and fucosterol significantly increased the efflux of cholesterol from HDL-treated THP-1-derived macrophages. Fucosterol dose-dependently increased the efflux of cholesterol from HDL-treated macrophages (Figure 2).

Fucosterol dose-dependently increased LXR-$\alpha$ and -$\beta$ mRNA levels in macrophages (Figure 3A). The expression of several LXR-responsive genes, including ABCG1 and ApoE, was significantly altered by incubation with fucosterol. Similar but stronger trends were observed in cells stimulated with T0901317. Fucosterol (200 $\mu$M) increased the ABCA1 mRNA level by 14-fold; this response was attenuated by $As_2O_3$, a LXR antagonist (Figure 3B).

In HepG2 cells, fucosterol (200 $\mu$M) increased ABCA1, ABCG1, ABCG5, ABCG8, and cholesteryl ester transfer protein (CETP) mRNA expression by 2.4-, 13.2-, 1.5-, 1.3-, and 0.8-fold ($p < 0.05$), respectively (Figure 3C). Cholesterol 7$\alpha$-hydroxylase (CYP7A1) gene expression is regulated by LXR in rodents but not in humans because the human CYP7A1 gene promoter lacks a LXR-responsive element.[32] Thus, we confirmed the effect of fucosterol in rat hepatocytes (H4IIE cells). As expected, incubation with fucosterol increased CYP7A1 mRNA levels in a dose-dependent manner in H4IIE rat hepatocytes (Figure 3D).

In Caco-2 cells, fucosterol dose-dependently induced intestinal ABCA1 mRNA expression (Figure 3E). In comparison to controls, the expression of Niemann-Pick C1 Like 1 (NPC1L1) mRNA was reduced by 40.2% in Caco-2 cells stimulated with fucosterol at a concentration of 200 $\mu$M.

**Fucosterol Regulates the Expression of Genes Involved in Gluconeogenesis and Glucose Storage in Hepatocytes.** The effects of fucosterol on the expression of LXR-responsive genes involved in glucose metabolism, including the genes encoding glucose-6-phosphatase (G6 Pase), phosphoenolpyruvate carboxykinase 1 (PEPCK-1), and glucokinase (GCK), were assessed in HepG2 cells (Figure 4). Fucosterol reduced G6 Pase mRNA levels in a dose-dependent manner but had no effect on the expression of PEPCK-1. In comparison to controls, fucosterol induced the expression of GCK in a dose-dependent manner.

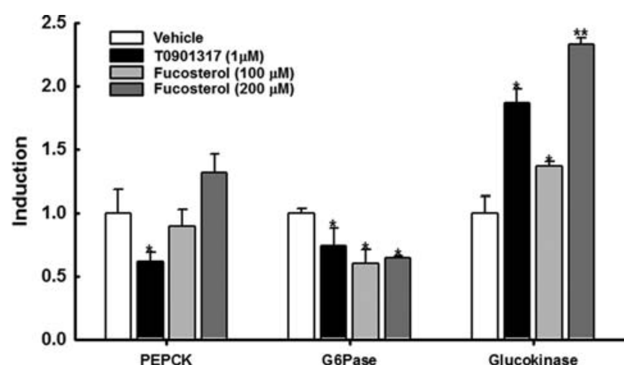


**Figure 4.** Effects of fucosterol on the mRNA expression of LXR-responsive genes involved in gluconeogenesis. HepG2 cells were treated with fucosterol (100 or 200 $\mu$M), 1 $\mu$M T0901317, or vehicle (1% ethanol) for 48 h. Total RNA was then extracted, and mRNA expression levels were measured by qRT-PCR. Data represent the means ± SEM ($n = 3$). (*) $p < 0.05$ versus the control.

**Fucosterol Had No Effect on Cellular TG Concentrations Because It Suppressed the Nuclear Translocation of SREBP-1c in Hepatocytes.** The activation of LXRs frequently promotes hepatic lipogenesis and hyperlipidemia *in vitro* and *in vivo* through the induction of SREBP-1c, a critical transcription factor that promotes hepatic lipogenesis.[10] In comparison to controls, T0901317 significantly increased the cellular TG concentration (Figure 5A). In contrast, fucosterol did not alter the cellular TG concentration in hepatocytes, despite the fact that it induced LXR activation (Figure 5A). Oil Red O staining showed similar results (Figure 5B).

The endogenous LXR agonist, 22*R*-HC, and fucosterol (200 $\mu$M) increased SREBP-1c gene expression 2.7- and 1.8-fold compared to the control, respectively; however, they did not affect the expression of fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD-1), key LXR-responsive genes, whose expression levels were significantly increased by T0901317 (Figure 5C). We further assessed SREBP-1 protein expression in hepatocytes to investigate the hypolipidemic mechanism of fucosterol in hepatocytes. Fucosterol increased levels of precursor SREBP-1 (pSREBP-1) in HepG2 cells in a dose-dependent manner, in accordance with its transcriptional activation. At concentrations of 100 and 200 $\mu$M, fucosterol increased pSREBP-1 levels by 30 and 150%, respectively ($p < 0.05$). However, the level of nuclear SREBP-1 (nSREBP-1), an active form of SREBP-1, was not altered by fucosterol (Figure 5D). In comparison to the controls, expression of nSREBP-1 was not induced and was significantly reduced by 18% ($p < 0.05$) in cells stimulated with 22*R*-HC. These findings suggest that fucosterol inhibited the nuclear translocation of SREBP-1c in hepatocytes and, thus, did not stimulate FAS and SCD-1 gene expression. This may be the result of induction of insulin-induced gene 2a (Insig-2a), which suppresses the nuclear translocation of SREBP-1c. Incubation with fucosterol (200 $\mu$M) induced the Insig-2a gene expression 2.6-fold ($p < 0.05$) compared to the control (Figure 5C).

## DISCUSSION

Phytosterols are known to reduce serum LDL-C levels but do not affect TG concentrations, and food products containing plant sterols are widely used as dietary options to reduce plasma cholesterol levels and the risk of atherosclerosis. Recently, Plat et al.[23] and Yang et al.[33] reported that the plant sterols stigmasterol, desmosterol, and campesterol increased the expression of LXR target genes and regulated cholesterol homeostasis via a LXR pathway. In this study, we examined the effect of fucosterol, a sterol abundant in marine algae, on LXR activation.

LXR-$\alpha$ and -$\beta$ are nuclear receptors and targets for the prevention and treatment of cardiovascular diseases. Because several relevant genes (CETP, CYP7A1, ABCA1, and ABCG5/G8) are regulated in a LXR-dependent manner, they could contribute to prevent hypercholesterolemia. In luciferase and co-activator peptide recruitment assays, we demonstrated that fucosterol activated LXR-$\alpha$ and -$\beta$ by binding directly to their ligand-binding domains and could thereby stimulate the transcription of target genes involved in cholesterol homeostasis in macrophages, hepatocytes, and intestinal cells. Although fucosterol has been classified as a strong activator

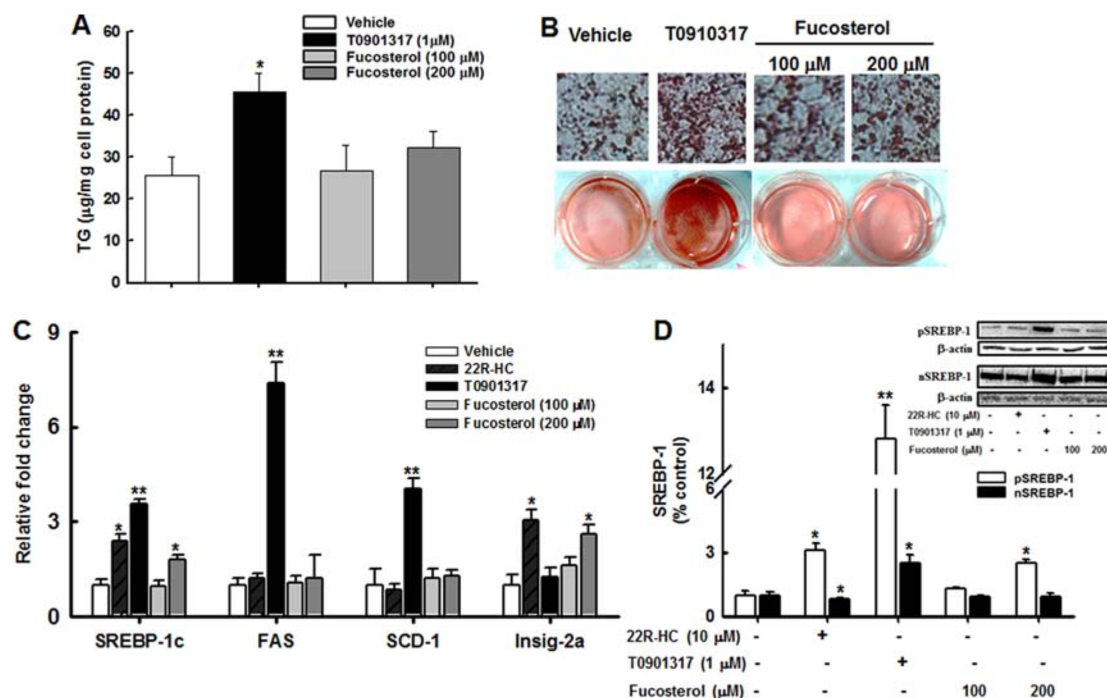


**Figure 5.** Fucosterol has modest effects on intracellular TG levels in hepatocytes and on the expression of hepatic lipogenesis genes. (A) Intracellular TG levels in hepatocytes. (B) Lipid staining of hepatocytes incubated with fucosterol and T0901317, showing fat accumulation in cells stained with Oil Red O. (C) Expression of the genes encoding SREBP-1c, FAS, SCD-1, and Insig-2a in HepG2 cells incubated with fucosterol, T0901317, or 22*R*-HC, as assessed by qPCR. (D) Protein expression of SREBP-1 in HepG2 cells, as determined by immunoblotting with an anti-SREBP-1 antibody. Data represent the means ± SEM ($n$ = 3−5). (*) $p < 0.05$ and (**) $p < 0.001$ versus the control.

of LXRs, with estimated $EC_{50}$ values in a co-activator peptide recruitment assay of 464 ± 37 and 1391 ± 475 nM for LXR-$\alpha$ and -$\beta$, respectively, it does not activate LXRs at low micromolar concentrations, as assessed in *in vitro* cell-based reporter gene assays. We suggest that fucosterol may not easily penetrate the cell membrane. Vahouny et al.[34] reported that only 3−4% of the phytosterol treatment dose is absorbed in the lymph of rats. Thus, we used two concentrations (100 and 200 $\mu$M) in further experiments. These concentrations are rather high but not unusual for *in vitro* experiments using phytosterols and their derivatives.[23,33]

LXRs act as critical transcription factors in the regulation of RCT, a process by which excess cholesterol is transferred from peripheral tissues to the liver via HDL particles. Indeed, subsequent studies have revealed that LXRs stimulate almost every aspect of this process. Cholesterol efflux from the cells is the first step in RCT and is primarily mediated by ABCA1 and ABCG1 transporters. ABCA1 transfers both cholesterol and phospholipids from the plasma membrane to lipid-free apolipoprotein A-I (apoA-I). This transporter is also crucial for the formation of nascent HDL particles in the liver. On the other hand, the function of ABCG1 is to transfer cholesterol to HDLs. It was demonstrated that LXR-$\alpha$ and -$\beta$ upregulate the expression of rodent as well as human ABCA1 and ABCG1 via functional LXREs found in their genes.[9] Fucosterol and T0901317 were shown to induce ABCA1, ABCG1, and ApoE mRNA levels and to enhance cholesterol efflux in THP-1-derived macrophages. The LXR antagonist $As_2O_3$ abated the effect of fucosterol on ABCA1 expression in THP-1-derived macrophages, suggesting that fucosterol upregulates ABCA1 at least in part by activating LXRs.

LXR regulates systemic cholesterol homeostasis by decreasing intestinal cholesterol absorption and increasing biliary cholesterol excretion through regulation of membrane transporters, including NPC1L1 and ABCA1. NPC1L1 mediates apical cholesterol uptake from the gut lumen, whereas ABCA1 facilitates basolateral efflux of cholesterol for HDL formation. T0901317 increased intestinal ABCA1 mRNA levels and repressed NPC1L1 expression in Caco2 cells, in agreement with the results by Yoon et al.[35] Similarly, fucosterol induced intestinal ABCA1 mRNA levels and suppressed intestinal NPC1L1 expression in a dose-dependent manner. The altered cholesterol transporter gene expression in the intestinal epithelium following fucosterol stimulation may provide additional metabolic benefits ameliorating hypercholesterolemia and atherosclerosis.

Induction of hypertriglyceridemia by LXR agonists is controversial. Increases in plasma TG levels caused by LXR agonists have been reported.[10] However, other reports showed no change in the plasma TG level[36] or only a transient increase.[37] In the current study, fucosterol increased the expression of ABCA1, ABCG1, ABCG5, ABCG8, and CETP in HepG2 cells and CYP7A1 in H4IIE cells; no change in the TG concentration or the expression of FAS or SCD-1 was observed. Fucosterol induced SREBP-1c mRNA and pSREBP-1 protein expression, whereas the nSREBP-1 protein level was unaltered. In contrast, T0901317 increased SREBP-1c mRNA and pSREBP-1 protein levels and led to an increase in nSREBP-1 levels and, consequently, FAS and SCD-1 mRNA levels. Previously, the endogenous LXR agonist 22*R*-HC was shown to inhibit the nuclear translocation of SREBP-1c by activating Insig2a gene expression and, thus, did not stimulate FAS or SCD-1 gene expression in macrophages or the mouse liver.[38,39] We therefore further probed the molecular mechanism responsible for the inhibitory effect of fucosterol on SREBP processing.

Inhibition of SREBP processing by cholesterol and oxysterols is the major homeostatic mechanism that balances cellular cholesterol and fatty acid metabolism. When the cellular sterol concentration becomes low, the precursor SREBP is chaperoned by sterol cleavage-activating protein (SCAP) from the endoplasmic reticulum (ER) to the Golgi, where proteolytic cleavage occurs. However, when cholesterol high, cholesterol binding to the sterol-sensing domain of SCAP, recruits insulin signaling protein (INSIG) retaining the INSIG−SCAP−SREBP complex in the ER, the expressions of SREBP-regulated gene were downregulated.[40,41] In fact, Insig-2 is required for sterols to retain the SREBP−SCAP complex in the ER.[42,43] Similar to 22*R*-HC, fucosterol delayed nuclear translocation of SREBP-1c by inducing Insig-2a gene expression. Fucosterol stimulation may retain the INSIG2−SCAP−SREBP-1c complex in the ER and, consequently, reduces the expression of SREBP-1c-responsive hepatic lipogenic genes, such as FAS and SCD-1. This may be due to the structure of fucosterol being similar to that of 22*R*-HC, suggesting that fucosterol may bind to the SCAP−INSIG−SREBP-1 complex. Adams et al.[44] reported that a 3$\beta$-hydroxyl group was required for sterol binding to SCAP or a SCAP−INSIG-binding protein. Fucosterol is a sterol with a 3$\beta$-hydroxyl group in its structure; we thus suggest that fucosterol may bind to the SCAP−INSIG−SREBP-1 complex via its 3$\beta$-hydroxyl group.

Various studies have shown that LXRs regulate FAS and SCD-1 expression through direct interaction with their promoters and by activating SREBP-1c expression.[37,45] In the present study, fucosterol did not alter the expression of FAS or SCD-1, suggesting that the regulation of these genes by SREBP-1c may be the dominant mechanism. Recently, Miao et al.[46] and Albers et al.[47] reported that the selective LXR modulators GW3965 and 22*R*-HC differ from T0901317 in terms of induction of FAS and SCD-1 in the liver because of differences in the extent of co-activator recruitment. This possibility should be examined in the future.

Fucosterol isolated from *Pelvetia siliquosa* was found to exhibit potent anti-diabetic potential in animal experiments.[48] It was suggested that the hypoglycemic effect of fucosterol is due to the inhibition of glycogen breakdown and gluconeogenesis in the liver, enhanced peripheral glucose consumption, or direct inhibition of insulin release in the liver. Laffitte et al.[49] reported that activation of LXR led to the suppression of the liver gluconeogenic program, including downregulation of PEPCK-1 and G6 Pase expression. Inhibition of gluconeogenic genes was accompanied by an induction in expression of glucokinase, which promotes hepatic glucose use. In this study, one of the mechanisms behind the hypoglycemic effects of fucosterol was increased activation of LXR and subsequent expression of LXR target genes involved in gluconeogenesis and glucose storage in hepatocytes.

In summary, we have demonstrated that fucosterol is a direct ligand of LXRs and that it may have nutritional implications in hypercholesterolemia and atherosclerosis. Fucosterol did not induce the expression of fatty acid synthesis genes, including FAS and SCD-1, because it inhibited the nuclear translocation of pSREBP-1 by inducing Insig-2a gene expression. These interactions resulted in unaltered TG in hepatocytes.

## AUTHOR INFORMATION

### Corresponding Author

*E-mail: junelee@korea.ac.kr.

### Funding

This study was supported by the Technology Development Program for Fisheries of the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (iPET, F20926409H220000110), the Korean Forest Service (Forest Science and Technology Project S120909L130110), and the Globalization of Korean Foods Research and Development Program, funded by the Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea.

### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

LXR, liver X receptor; ABC, ATP-binding cassette transporter; ApoE, apolipoprotein E; SREBP, sterol regulatory element-binding protein; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase 1; LDL, low-density lipoprotein; CYP7A1, cholesterol 7$\alpha$-hydroxylase; HDL, high-density lipoprotein; PMA, phorbol 12-myristate 13-acetate; TR-FRET, time-resolved fluorescence resonance energy transfer; LBD, ligand-binding domain; TG, triglyceride; RCT, reverse cholesterol transport; CETP, cholesteryl ester transfer protein; G6 Pase, glucose-6-phosphatase; PEPCK-1, phosphoenolpyruvate carboxykinase

## REFERENCES

(1) Murray, C. J.; Lopez, A. D. Evidence-based health policy—Lessons from the Global Burden of Disease Study. *Science* **1996**, *274*, 740−743.

(2) Lusis, A. J. Atherosclerosis. *Nature* **2000**, *407*, 233−241.

(3) Whitney, K. D.; Watson, M. A.; Goodwin, B.; Galardi, C. M.; et al. Liver X receptor (LXR) regulation of the LXR$\alpha$ gene in human macrophages. *J. Biol. Chem.* **2001**, *276*, 43509−43515.

(4) Levin, N.; Bischoff, E. D.; Daige, C. L.; Thomas, D.; et al. Macrophage liver X receptor is required for antiatherogenic activity of LXR agonists. *Arterioscler., Thromb., Vasc. Biol.* **2005**, *25*, 135−142.

(5) Naik, S. U.; Wang, X.; Da Silva, J. S.; Jaye, M.; et al. Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. *Circulation* **2006**, *113*, 90−97.

(6) Joseph, S. B.; McKilligin, E.; Pei, L.; Watson, M. A.; et al. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7604−7609.

(7) Repa, J. J.; Berge, K. E.; Pomajzl, C.; Richardson, J. A.; et al. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors $\alpha$ and $\beta$. *J. Biol. Chem.* **2002**, *277*, 18793−18800.

(8) Berge, K. E.; Tian, H.; Graf, G. A.; Yu, L.; et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* **2000**, *290*, 1771−1775.

(9) Geyeregger, R.; Zeyda, M..; Stulnig, T. M. Liver X receptors in cardiovascular and metabolic disease. *Cell. Mol. Life Sci.* **2006**, *63*, 524−539.

(10) Schultz, J. R.; Tu, H.; Luk, A.; Repa, J. J.; Medina, J. C.; Li, L.; Schwendner, S.; Wang, S.; Thoolen, M.; Mangelsdorf, D. J.; Lustig, K. D.; Shan, B. Role of LXRs in control of lipogenesis. *Genes Dev.* **2000**, *14*, 2831−2838.

(11) Hoang, M. H.; Jia, Y.; Jun, H. J.; Lee, J. H.; Hwang, K. Y.; et al. Taurine is a liver X receptor-$\alpha$ ligand and activates transcription of key genes in the reverse cholesterol transport without inducing hepatic lipogenesis. *Mol. Nutr. Food Res.* **2012**, *56*, 900−911.

(12) Hoang, M. H.; Jia, Y.; Jun, H. J.; Lee, J. H.; Lee, D. H.; et al. Ethyl 2,4,6-trihydroxybenzoate is an agonistic ligand for liver X receptor that induces cholesterol efflux from macrophages without affecting lipid accumulation in HepG2 cells. *Bioorg. Med. Chem. Lett.* **2012**, *15*, 4094−4099.

(13) Quinet, E. M.; Basso, M. D.; Halpern, A. R.; Yates, D. W.; Steffan, R. J.; et al. LXR ligand lowers LDL cholesterol in primates, is

lipid neutral in hamster, and reduces atherosclerosis in mouse. *J. Lipid Res.* **2009**, *50*, 2358−2370.
(14) Kratzer, A.; Buchebner, M.; Pfeifer, T.; Becker, T. M.; Uray, G.; et al. Synthetic LXR agonist attenuates plaque formation in ApoE−/− mice ithout inducing liver steatosis and hypertriglyceridemia. *J. Lipid Res.* **2009**, *50*, 312−326.
(15) Moghadasian, M. H.; Frohlich, J. J. Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: Clinical and experimental evidence. *Am. J. Med.* **1999**, *107*, 588−594.
(16) Ostlund, R. E., Jr. Phytosterols and cholesterol metabolism. *Curr. Opin. Lipidol.* **2004**, *15*, 37−41.
(17) Plat, J.; Kerckhoffs, D. A. J. M.; Mensink, R. P. Therapeutic potential of plant sterols and stanols. *Curr. Opin. Lipidol.* **2000**, *11*, 571−576.
(18) Calpe-Berdiel, L.; Escola-Gil, J. C.; Ribas, V.; Navarro-Sastre, A.; Garces-Garces, J.; Blanco-Vaca, F. Changes in intestinal and liver global gene expression in response to a phytosterol-enriched diet. *Atherosclerosis* **2005**, *181*, 75−85.
(19) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA, J. Am. Med. Assoc.* **2001**, *285*, 2486−2497.
(20) Miettinen, T. A.; Puska, P.; Gylling, H.; Vanhanen, H.; Vartiainen, E. Reduction of serum-cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *N. Engl. J. Med.* **1995**, *333*, 1308−1312.
(21) Kaneko, E.; Matsuda, M.; Yamada, Y.; Tachibana, Y.; Shimomura, I.; Makishima, M. Induction of intestinal ATP-binding cassette transporters by a phytosterol-derived liver X receptor agonist. *J. Biol. Chem.* **2003**, *278*, 36091−36098.
(22) Plat, J.; Mensink, R. P. Increased intestinal ABCA1 expression contributes to the decrease in cholesterol absorption after plant stanol consumption. *FASEB J.* **2002**, *16*, 1248−1253.
(23) Plat, J.; Nichols, J. A.; Mensink, R. P. Plant sterols and stanols: effects on mixed micellar composition and LXR (target gene) activation. *J. Lipid Res.* **2005**, *46*, 2468−2476.
(24) Tapiero, H.; Townsend, D. M.; Tew, K. D. Phytosterols in the prevention of human pathologies. *Biomed. Pharmacother.* **2003**, *57*, 321−325.
(25) Kritchevsky, D.; Tepper, S. A.; Czarnecki, S. K.; Kyle, D. J. Effects of 4-methylsterols from algae and of beta sitosterol on cholesterol metabolism in rats. *Nutr. Res. (N. Y., NY, U. S.)* **1999**, *19*, 1649−1654.
(26) Padovani, A. M.; Molina, M. F.; Mann, K. K. Inhibition of liver X receptor/retinoid X receptor-mediated transcription contributes to the proatherogenic effects of arsenic in macrophages in vitro. *Arterioscler., Thromb., Vasc. Biol.* **2010**, *30*, 1228−1236.
(27) Atshaves, B. P.; Starodub, O.; McIntosh, A.; Petrescu, A.; Roths, J. B.; Kier, A. B.; Schroeder, F. Sterol carrier protein-2 alters high-density lipoprotein-mediated cholesterol efflux. *J. Biol. Chem.* **2000**, *275*, 36852−36861.
(28) Hozumi, Y.; Kawano, M.; Jordan, V. C. In vitro study of the effect of raloxifene on lipid metabolism compared with tamoxifen. *Eur. J. Endocrinol.* **2000**, *143*, 427−430.
(29) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.
(30) Hoang, M. H.; Houng, S. J.; Jun, H. J.; Lee, J. H.; Choi, J. W.; Kim, S. H.; Kim, Y. R.; Lee, S. J. Barley intake induces bile acid excretion by reduced expression of intestinal ASBT and NPC1L1 in C57BL/6J mice. *J. Agric. Food Chem.* **2011**, *59*, 6798−6805.
(31) Naik, S. U.; Wang, X.; Da Silva, J. S.; Jaye, M.; Macphee, C. H.; Reilly, M. P.; Billheimer, J. T.; Rothblat, G. H.; Rader, D. J. Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. *Circulation* **2006**, *113*, 90−97.
(32) Goodwin, B.; Watson, M. A.; Kim, H.; Miao, J.; et al. Differential regulation of rat and human CYP7A1 by the nuclear oxysterol receptor liver X receptor-$\alpha$. *Mol. Endocrinol.* **2003**, *17*, 386−394.
(33) Yang, C. D.; McDonald, J. G.; Patel, A.; Zhang, Y.; Umetani, M.; Xu, F.; Westover, E. J.; Covey, D. F.; Mangelsdorf, D. J.; Cohen, J. C.; Hobbs, H. H. Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. *J. Chem. Biol.* **2006**, *281*, 27816−27826.
(34) Vahouny, G. V.; Connor, W. E.; Subramaniam, S.; Lin, D. S.; Gallo, L. L. Comparative lymphatic absorption of sitosterol, stigmasterol, and fucosterol and differential inhibition of cholesterol absorption. *Am. J. Clin. Nutr.* **1983**, *37*, 805−809.
(35) Yoon, H. S.; Ju, J. H.; Kim, H.; Lee, J.; Park, H. J.; Ji, Y.; Shin, H. K.; Do, M. S.; Lee, J. M.; Holzapfel, W. *Lactobacillus rhamnosus* BFE 5264 and *Lactobacillus plantarum* NR74 promote cholesterol excretion through the up-regulation of ABCG5/8 in Caco-2 cells. *Probiotics Antimicrob. Proteins* **2011**, *3*, 194−203.
(36) Grefhorst, A.; Elzinga, B. M.; Voshol, P. J.; Kok, T.; Bloks, V. W.; van der Sluijs, F. H.; Havekes, L. M.; Romijn, J. A.; Verkade, H. J.; Kuipers, F. Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. *J. Chem. Biol.* **2002**, *277*, 34182−34190.
(37) Joseph, S. B.; Laffitte, B. A.; Patel, P. H.; Watson, M. A.; Matsukuma, K. E.; Walczak, R.; Collins, J. L.; Osborne, T. F.; Tontonoz, P. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J. Chem. Biol.* **2002**, *277*, 11019−11025.
(38) Beyea, M. M.; Heslop, C. L.; Sawyez, C. G.; Edwards, J. Y.; Markle, J. G.; Hegele, R. A.; Huff, M. W. Selective up-regulation of LXR-regulated genes ABCA1, ABCG1, and ApoE in macrophages through increased endogenous synthesis of 24(*S*),25-epoxycholesterol. *J. Biol. Chem.* **2007**, *282*, 5207−5216.
(39) Dang, H. X.; Liu, Y.; Pang, W.; Li, C. H.; Wang, N. P.; Shyy, J. Y. J.; Zhu, Y. Suppression of 2,3-oxidosqualene cyclase by high fat diet contributes to liver X receptor-$\alpha$-mediated improvement of hepatic lipid profile. *J. Chem. Biol.* **2009**, *284*, 6218−6226.
(40) Peng, Y.; Schwarz, E. J.; Lazar, M. A.; Genin, A.; Spinner, N. B.; Taub, R. Cloning, human chromosomal assignment, and adipose and hepatic expression of the CL-6/INSIG1 gene. *Genomics* **1997**, *43*, 278−284.
(41) Brown, A. J.; Sun, L.; Feramisco, J. D.; Brown, M. S.; Goldstein, J. L. Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. *Mol. Cell* **2002**, *10*, 237−245.
(42) Yabe, D.; Brown, M. S.; Goldstein, J. L. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12753−12758.
(43) Yellaturu, C. R.; Deng, X.; Park, E. A.; Raghow, R.; Elam, M. B. Insulin enhances the biogenesis of nuclear sterol regulatory element-binding protein (SREBP)-1c by posttranscriptional down-regulation of Insig-2A and its dissociation from SREBP cleavage-activating protein (SCAP).SREBP-1c complex. *J. Biol. Chem.* **2009**, *284*, 31726−31734.
(44) Adams, C. M.; Reitz, J.; De Brabander, J. K.; Feramisco, J. D.; Li, L.; Brown, M. S.; Goldstein, J. L. Cholesterol and 25-hydroxycholesterol inhibit activation of SREBPs by different mechanisms, both involving SCAP and Insigs. *J. Biol. Chem.* **2004**, *279*, 52772−52780.
(45) Miyazaki, M.; Dobrzyn, A.; Man, W. C.; Chu, K. K.; Sampath, H.; Kim, H. J.; Ntambi, J. N. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. *J. Biol. Chem.* **2004**, *279*, 25164−25171.
(46) Miao, B.; Zondlo, S.; Gibbs, S.; Cromley, D.; Hosagrahara, V. P.; Kirchgessner, T. G.; Billheimer, J.; Mukherjee, R. Raising HDL cholesterol without inducing hepatic steatosis and hypertriglyceridemia by a selective LXR modulator. *J. Lipid Res.* **2004**, *45*, 1410−1417.
(47) Albers, M.; Blume, B.; Schlueter, T.; Wright, M. B.; Kober, I.; Kremoser, C.; Deuschle, U.; Koegl, M. A novel principle for partial agonism of liver X receptor ligands—Competitive recruitment of activators and repressors. *J. Biol. Chem.* **2006**, *281*, 4920−4930.

(48) Lee, Y. S.; Shin, K. H.; Kim, B. K.; Lee, S. Anti-diabetic activities of fucosterol from *Pelvetia siliquosa*. *Arch. Pharm. Res.* **2004**, *27*, 1120–1122.

(49) Laffitte, B. A.; Chao, L. C.; Li, J.; Walczak, R.; Hummasti, S.; Joseph, S. B.; Castrillo, A.; Wilpitz, D. C.; Mangelsdorf, D. J.; Collins, J. L.; Saez, E.; Tontonoz, P. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5419–5424.