DOI 10.1002/mnfr.201100611 *Mol. Nutr. Food Res.* 2012, *56*, 900–911

RESEARCH ARTICLE

Taurine is a liver X receptor-α ligand and activates transcription of key genes in the reverse cholesterol transport without inducing hepatic lipogenesis

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Received: September 8, 2011
Revised: February 10, 2012
Accepted: February 20, 2012

Scope: Taurine, which is abundant in seafood, has antiatherogenic activities in both animals and humans; however, its molecular target has been elusive. We examined whether taurine could activate liver X receptor-α (LXR-α), a critical transcription factor in the regulation of reverse cholesterol transport in macrophages.

Methods and results: Taurine bound directly to LXR-α in a reporter gene assay, time-resolved fluorescence resonance energy transfer analysis, and limited protease digestion experiment. Macrophage cells incubated with taurine showed reduced cellular cholesterol and induced medium cholesterol in a dose-dependent manner with the induction of ATP-binding cassette transporter A1 and G gene and protein expression. In hepatocytes, taurine significantly induced Insig-2a levels and delayed nuclear translocation of the sterol regulatory element-binding protein 1 (SREBP-1) protein, resulting in a dose-dependent reduction in the cellular lipid levels without inducing the expression of fatty acid synthesis genes.

Conclusion: Taurine is a direct LXR-α ligand, represses cholesterol accumulation, and modulates the expression of genes involved in reverse cholesterol transport in macrophages, without inducing hepatic lipogenesis. The induction of Insig-2a suppressed the nuclear translocation of SREBP-1c.

Keywords:
Cholesterol / Insig-2a / LXR-α / SREBP-1c / Taurine

1 Introduction

Taurine, 2-aminoethanesulfonic acid, is present at high concentrations (2–30 mM) in mammalian plasma and cells. It is not incorporated into proteins and is in fact the most abundant free in many tissues. Taurine is taken in via the diet in carnivores and omnivores, but small amounts of taurine are also synthesized endogenously in the liver from cysteine or methionine present in the diet [1]. Taurine has

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Abbreviations: **ABC**, ATP-binding cassette transporter; **APOE**, apolipoprotein E; **CETP**, cholesteryl ester transfer protein; **ChREBP**, carbohydrate responsive element-binding protein; **CYP7A1**, cholesterol 7α-hydroxylase; **FAS**, fatty acid synthase; **FXR**, farnesoid X receptor; **GCK**, glucokinase; **G6Pase**, glucose 6-phosphatase; **LBD**, ligand-binding domain; **L-CAD**, long-chain acyl CoA dehydrogenase; **LXR**, liver X receptor; **NPC1L1**, Niemann-Pick C1-Like 1; **PEPCK-1**, phosphoenolpyruvate carboxykinase 1; **PK**, protease K; **PMA**, phorbol 12-myristate 13-acetate; **RCT**, reverse cholesterol transport; **SCAP**, SREBP-cleavage-activating protein; **SCD-1**, stearoyl-CoA desaturase 1; **SREBP**, sterol regulatory element-binding protein; **TG**, triglyceride; **TR-FRET**, time-resolved fluorescence resonance energy transfer

been reported to play critical roles in several essential biological and physiological functions including immune and antioxidative activity, and hepatic detoxification [1–3]. Taurine shows hypocholesterolemic activities that may reduce the risk of coronary heart disease as well. A worldwide cross-sectional WHO-CARDIAC study revealed a strong inverse association of urinary taurine excretion with ischemic heart disease mortality, suggesting the role of taurine intake in the prevention of atherosclerosis and cardiovascular disease [4]. In addition, numerous in vitro and in vivo experiments have suggested that taurine could reduce cellular lipid levels and modulate plasma lipoprotein metabolism, e.g. the reduction of cellular cholesterol [5] and induction of plasma high-density lipoprotein (HDL) cholesterol levels [6, 7]. In these reports, the primary mechanism suggested for the lipid metabolism of taurine was increased bile acid synthesis and subsequent excretion via transcriptional activation of cholesterol 7α-hydroxylase (CYP7A1) [7] through unidentified mechanisms.

Macrophage liver X receptor-α (LXR-α) has been demonstrated to play a critical role in protection against atherosclerosis [8]. LXR activation, by upregulating the expression of adenosine triphosphate-binding cassette (ABC) proteins A1, ABCG1, and apolipoprotein E (APOE), increases cholesterol efflux and stimulates reverse cholesterol transport (RCT) from peripheral tissues and elevates HDL cholesterol levels, thereby providing antiatherogenic potential by inhibiting the progression and even promoting the regression of atherosclerosis [9–11]. LXR-α also impacts systemic cholesterol levels by reducing intestinal cholesterol absorption and increasing biliary cholesterol excretion through regulation of the transporters ABCG5 and ABCG8 [12]. Mutations in either human ABCG5 or ABCG8 lead to sitosterolemia (abnormal absorption of sitosterols and hyperabsorption of cholesterol) and the development of premature cardiovascular disease [13]. Moreover, LXR-α 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 [14]. CYP7A1 encodes cholesterol 7-α hydroxylase, which is a rate-limiting enzyme in the classic pathway of hepatic bile acid synthesis. The gene expression of CYP7A1 is regulated by multiple transcription factors, and LXR-α has been identified as one of the major transcription factors, particularly in rodent livers [15], as revealed in a series of experiments [16, 17]. In reporter gene assays, the cotransfection of vectors containing LXR-α and RXR-α in HepG2 cells potently stimulated rat CYP7A1 promoter activity [18] and in subsequent transactivation studies, taurohyodeoxycholic acid, a ligand of LXR-α, confirmed the stimulation of CYP7A1 transcription through the binding of LXR-α to the LXR element (LXRE) in the promoter [19]. According to these previous findings, we investigated whether taurine is a ligand for LXRs and stimulates transactivation, thereby altering lipid metabolism in macrophage, hepatocyte, and intestinal cells.

2 Methods

2.1 Reagents

Cell culture reagents and supplies were obtained from Hyclone (Logan, UT). Taurine, T0901317, β-mercaptoethanol, and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma (St. Louis, MO). Proteinase K (PK) was from Invitrogen (Carlsbad, CA). Total RNA extraction reagent (RNAiso Plus) and real-time PCR premix (SYBR® Premix Ex Taq™) were obtained from Takara (Otsu, Japan). Oligo $(dT)_{15}$ primer was purchased from Promega (Madison, WI). PowerOpti-ECL Western blotting detection reagent was purchased from Amersham-Pharmacia (Seoul, Korea). Primary (anti-LXR-α, -ABCA1, -ABCG1, -sterol regulatory element-binding protein 1 (SREBP-1), and α-tubulin) and secondary (antirabbit, mouse, and goat immunoglobulin G) antibodies were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Primary antiphospho-Akt (Ser473) was obtained from Cell Signaling Technology (Beverly, MA). MiScript reverse transcription kit, miScript SYBR® PCR kit, and miR-33b PCR primers were obtained from Qiagen (Valencia, CA). All other chemicals were purchased from Sigma.

2.2 Cell culture and treatments

Chinese hamster ovary (CHO-K1), human monocytic THP-1, H4IIE, HepG2, and Caco2 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). CHO-K1 cells were maintained in Dulbecco's modified Eagle's medium mixed 1:1 with Ham's F-12 (DMEM/F12) medium containing 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (PES), and used for luciferase reporter assays. Human monocytic THP-1 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, 0.05 mM β-mercaptoethanol, and 1% PES. The cells were differentiated in the presence of 50 ng/mL PMA for 72 h prior to treatments and RNA or protein extraction. HepG2 and H4IIE cell lines were cultured in DMEM and MEM, respectively, supplemented with 10% FBS and 1% PES before treatment. Caco2 cells were maintained in MEM medium supplemented with 20% of FBS, 1% of nonessential amino acids, 2.5 mM of hydroxyethyl piperazineethanesulfonic acid, and 1% of PES. All cell lines were grown in 5% CO_2 at 37°C. For the experiment, THP-1-derived macrophage, HepG2, and H4IIE, and Caco2 cells were preincubated in RPMI-1640, 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 media containing 1 μM T0901317 or taurine (10, 50, and 100 μM). Ten millimolar taurine and 1 mM T0901317 stock solutions were prepared in water. Vehicle control cells were given the corresponding water amounts (1%) as used for the highest substance concentration. Each treatment was carried out at least in triplicate.

2.3 Transfection and luciferase assay

The transfection and reporter gene assay were performed with CHO-K1 cells, as described previously [20].

2.4 Time-resolved fluorescence resonance energy transfer (TR-FRET) assay for LXR-α ligand-binding activity

The potential LXR-α-activating capacity of taurine was investigated using LanthaScreen™ TR-FRET LXR-α coactivator assays (Invitrogen) according to the manufacturer's instructions. To determine the concentration required to produce a 50% effect (EC_{50}), the data were fit to a sigmoidal dose–response curve (varying slope) using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA).

2.5 Limited protease digestion assay

The ligand-binding domain (LBD) of human LXR-α was purified in-house, as described previously [20]. Purified human LXR-α-LBD, then, was preincubated with 100 μM taurine or 1 μM T0901317 for 15 min at room temperature. Protease K (0, 0.2, and 2 μg/mL) was added and incubated for 15 min at room temperature, then the reaction was terminated by the addition of SDS sample loading buffer and boiling for 5 min. Digestion products were analyzed by SDS-PAGE and stained with Coomassie blue.

2.6 Modeling method

The binding properties of taurine were analyzed as described previously [21]. Briefly, the crystal structure of the LXR-α:RXR-β complex bound with T0901317 (PDB 1UHL) was used as the target for docking calculations [22]. The structure of taurine was initially built using the program Maestro v7.0 (Schrodinger, Portland, OR). Taurine was energy minimized with the MM3* force field prior to docking in MacroModel v8.1 [23]. Docking calculations were carried out with the Glide software (Schrodinger, Portland, OR) [24]. The best-docked pose was chosen and scored from the calculated binding affinity: the receptor–ligand molecular mechanics interaction energy and ligand strain energy. Furthermore, the poses were subjected to another final optimization procedure in which the receptor–ligand complex underwent a full molecular mechanics energy minimization (OPLS–AA force field) to optimize flexible LXR side-chain residues that may interact with the ligands. The lowest energy poses obtained in this fashion were subjected to a Monte Carlo procedure to obtain the final docking solution.

2.7 Cellular lipid measurements

Cellular and medium lipids were extracted as described previously [25, 26]. The cellular and medium contents of triglyceride (TG) were quantified using an enzymatic method with a Cobas C111 automatic analyzer (Roche, Basel, Switzerland). The cholesterol levels were measured using an Amplex Red Cholesterol Assay Kit (Invitrogen) according to the manufacturer's instructions.

2.8 Oil Red O staining

Cells were washed with ice-cold PBS and fixed 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, microscopic images were collected. Lipid accumulation was quantitated by isopropanol extraction of Oil Red O from stained cells and optical density determinations at 500 nm using a Bio-Rad model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA).

2.9 Quantitative (q)PCR

Total RNA was extracted from THP-1-derived macrophages and HepG2 cells using an RNAiso Plus kit according to the manufacturer's protocol after 2 days of treatment with T0901317 and taurine or vehicle control (1% H_2O). Real-time qPCR was performed with Bio-Rad iQ SYBR® Green Supermix reagent and the Bio-Rad iQ5 Cycler System. The primers were described in Supporting Information Table S1. Expression levels were normalized to that of glyceraldehyde 3-phosphate dehydrogenase or cyclophilin with the normalized expression (CT) method according to the manufacturer's guidelines.

For miR-33b expression, real-time PCR was performed using the miScript SYBR® PCR kit (Qiagen) with miR-33b specific primer, according to the manufacturer's protocol.

2.10 Immunoblotting analysis

THP-1-derived macrophage and HepG2 cells were lysed in ice-cold lysis buffer containing 10 mM Tris–HCl (pH 7.4), 0.1 M EDTA, 10 mM NaCl, 0.5% Triton X-100, and protease inhibitor cocktail (Roche, Mannheim, Germany). The 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 manufacturer's protocol. Protein concentration was determined using a Bio-Rad protein kit with bovine serum albumin (Sigma) as the standard. SDS-PAGE and immunoblotting were performed as described previously [27].

2.11 Statistical analysis

All data are expressed as the mean ± standard error. Two groups were compared using Student's *t*-test. Differences were considered to be statistically significant at $p < 0.05$.

3 Results

3.1 Taurine binds directly to LXR-α and stimulates transactivation

The effect of taurine on LXR transactivation was assessed by luciferase reporter assays. CHO-K1 cells were transfected with the LXRE-luciferase reporter vector and the expression vector encoding human LXR-α or human LXR-β, and subsequently incubated with various concentrations (0–100 μM) of taurine for 24 h. Treatment with taurine significantly stimulated the transcriptional activity of LXR-α (+90% at 100 μM; $p < 0.05$), but not LXR-β (Fig. 1A).

Next, we conducted two independent experiments to determine whether taurine activates LXR-α through direct interaction with the LXR-LBD proteins. First, the LanthaScreen™ TR-FRET assay showed that T0901317, a synthetic ligand for LXRs, strongly enhanced the recruitment of Trap 220/Drip-2 coactivator peptide to LXR-α-LBD, with an EC_{50} values of 143 nM (Fig. 1B). Taurine induced the recruitment of Trap 220/Drip-2 coactivator peptide to LXR-α-LBD in a dose-dependent manner ($EC_{50} = 10$ μM; Fig. 1C).

In the second experiment, a limited protease digestion analysis was performed to demonstrate the taurine ligand for the LXR protein. Ligand binding alters the conformation of LXR-α-LBD, and thus shows distinct peptides resistant to partial digestion with protease K. The partial treatment of LXR-α-LBD with protease K (up to 0.2 μg/mL) led to complete digestion (Fig. 1D). In contrast, incubation of LXR-α-LBD in the presence of T0901317 showed 22- and 30-kDa peptide fragments after partial digestion with protease K treatment. Similarly, the digestion of LXR-α-LBD incubated with taurine displayed 22- and 30-kDa peptide fragments as well, indicating the direct binding of taurine to LXR-α-LBD.

To gain insight into the determinant for the binding affinity of taurine, taurine was docked into the LXR-α receptor LBD. In virtual modeling, taurine is bound to LXR-α through

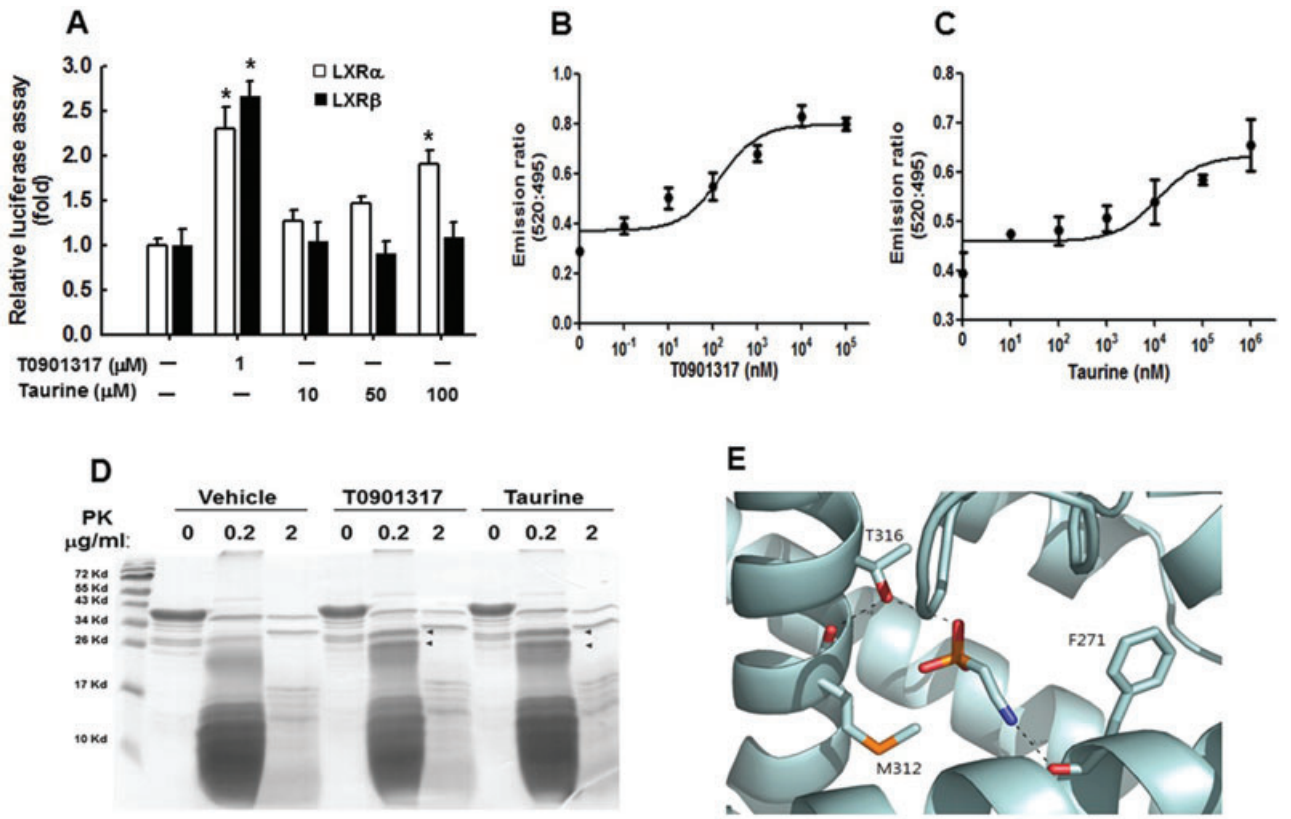

Figure 1. Taurine induces LXR-α activation and interacts directly with the ligand-binding domain of LXR-α. Taurine activates LXR-α but not LXR-β transactivation activity (A). The LanthaScreen™ TR-FRET LXR-α coactivator assay was used to evaluate the ability of T0901317 (B) or taurine (C) to interact with LXR-α. Taurine is significantly more susceptible than the control to proteolytic digestion in a limited protease digestion assay with LXR-α-LBD protein (D). Arrows indicate the most prominent proteolytic fragments protected by the presence of taurine and T0901317. The proposed complex of taurine with the ligand-binding pocket of LXR-α (E). Tau, taurine; F271, Phe-271; T316, Tyr-316; M312, Met-312.

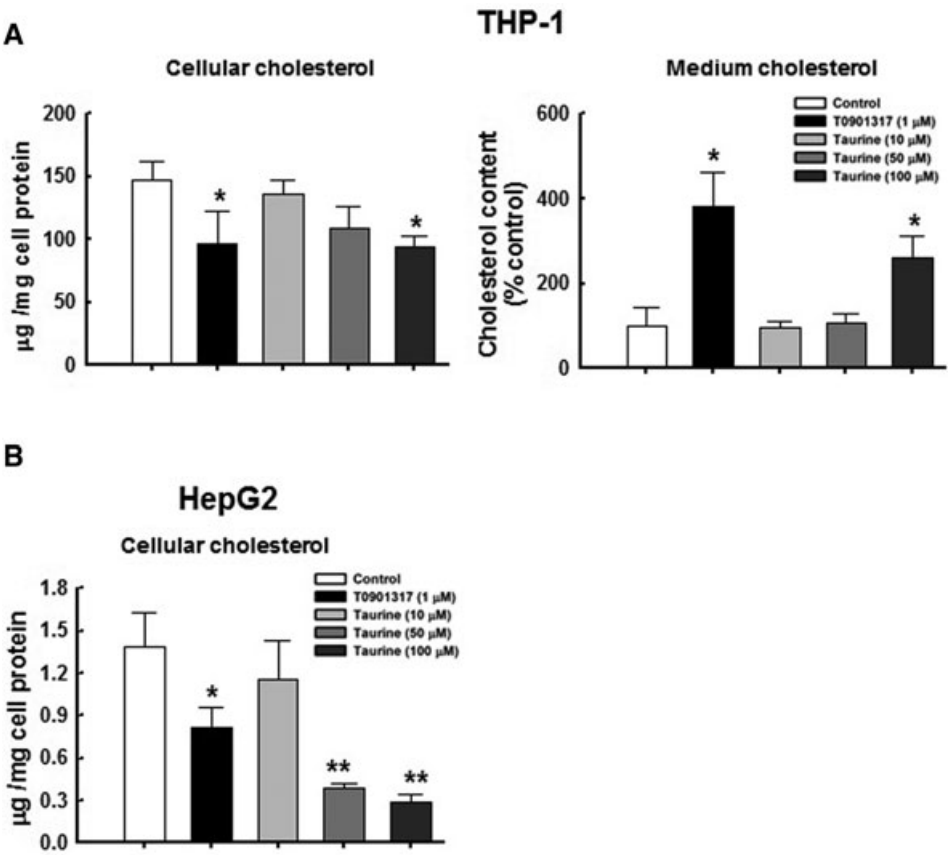

Figure 2. Effects of taurine on the cellular and medium lipid levels in THP-1-derived macrophages (A) and hepatocyte cells (B). THP-1 monocytes were incubated with PMA (50 ng/mL) for 3 days to differentiate them into adherent macrophages. The macrophages and HepG2 cells were then treated with 10, 50, and 100 μM taurine, 1 μM T0901317, or vehicle control (1% water) for 48 h. Cellular and medium cholesterol in THP-1-derived macrophages and cellular cholesterol in HepG2 cells were measured by the enzymatic method. Data are represented as the mean ± SEM ($n = 3$). *, **Significantly different from the control group, $^{*}p < 0.05$, $^{**}p < 0.001$.

hydrogen bonding via the hydroxyl and amino groups present on the molecule (Fig. 1E). The hydroxyl group of taurine interacts with Tyr-316, while the amine group makes contact with Phe-271.

3.2 Taurine reduces cellular cholesterol and stimulates medium cholesterol by regulating the expression of LXR-α and its responsive genes

The activation of LXR-α promotes cholesterol efflux, stimulates RCT in macrophages, and inhibits the accumulation of cholesterol in hepatocytes in vitro and in vivo [10, 14]. To examine the effect of taurine on the cellular cholesterol contents in macrophages and hepatocytes, lipid fractions were extracted from THP-1-derived macrophages and HepG2 cells after incubation with taurine, T0901317 or vehicle for 48 h. Taurine significantly reduced cellular total cholesterol, free cholesterol, and cholesteryl esters levels in macrophages (Fig. 2A and Table 1) and correspondingly increased cholesterol concentration in the culture medium in a dose-dependent manner as compared to that in the controls (Fig. 2A). In hepatocytes, taurine reduced the cholesterol levels in

Table 1. Effects of taurine on the cellular mass in THP-1-derived macrophages. THP-1 monocytes were incubated with PMA (50 ng/mL) for 3 days to differentiate them into adherent macrophages. The macrophages cells were then treated with 10, 50, and 100 μM taurine, 1 μM T0901317, or vehicle control (1% water) for 48 h. Cellular cholesterol and cholesteryl ester mass content were measured by an Amplex Red Cholesterol Assay Kit (Invitrogen) according to the manufacturer's instructions. Data are represented as the mean ± SEM ($n = 3$). *Significantly different from the control group, $^{*}P < 0.05$.

	Vehicle	T0901317 (1 μM)	10 μM	Taurine 50 μM	100 μM
TC	161.1 ± 6.1	145.1 ± 3.3*	161.5 ± 4.7	157.6 ± 2.9	147.5 ± 2.6*
FC	150.6 ± 4.2	140.5 ± 5.4*	151.0 ± 3.2	147.8 ± 3.9	139.5 ± 2.7*
CE	10.5 ± 2.2	4.7 ± 1.5*	10.6 ± 2.4	9.8 ± 2.4	8.1 ± 1.2
CE/TC (%)	6.5 ± 0.5	3.2 ± 0.2*	6.5 ± 0.4	6.2 ± 0.4	5.5 ± 0.1*

TC, total cholesterol; FC, free cholesterol; CE, cholesteryl esters.

a dose-dependent manner and the reduction was significant relative to the control at 50 and 100 μM taurine (Fig. 2B).

LXR-α mRNA levels increased significantly and dose-dependently in macrophages incubated with taurine (Fig. 3A). Thus, LXR-α mRNA expression was induced by 2.0-, 2.7-, and 3.4-fold ($p < 0.05$) at 10, 50, and 100 μM, respectively, as compared to the control group. The expression levels of several LXR-α responsive genes were altered significantly by incubation with taurine. In macrophages, ABCA1 mRNA levels increased by 2.1-, 2.3-, and 3.0-fold with taurine concentrations of 10, 50, and 100 μM, respectively. ABCG1 expression also increased significantly by 1.3-, 2.9-, and 3.3-fold with 10, 50, and 100 μM taurine, respectively. Similar trends were observed in cells stimulated with T0901317, but to a greater degree. A small but significant increase in APOE gene expression was observed in taurine-incubated macrophages at 100 μM. Immunoblot analysis showed results similar to those of the qRT-PCR analysis (Fig. 3B).

In HepG2 cells, taurine (100 μM) also significantly induced ABCA1, ABCG5, and ABCG8 mRNA expression levels by 1.4-, 2.0-, and 1.7-fold ($p < 0.05$), respectively (Fig. 3B), whereas no change in the expression of ABCG1 or cholesteryl ester transfer protein (CETP) was observed. CYP7A1 gene expression is regulated by LXR in rodents, but not in humans because the human promoter lacks the LXR-responsive element [28]. Thus, we confirmed the taurine effect in rat hepatocytes (the H4IIE cell line), of which the CYP7A1 gene contains LXRE promoter. As expected, incubation with taurine increased CYP7A1 mRNA levels in a dose-dependent manner in H4IIE rat hepatocytes, and the induction was significant at 100 μM relative to the control (Fig. 3C). These results suggest that taurine may stimulate LXR-α-mediated cholesterol efflux in macrophage cells and suppressed cholesterol accumulation in hepatocytes by activating CYP7A1.

In Caco2 cells, taurine significantly induced intestinal ABCA1, ABCG5, and ABCG8 mRNA expression levels in a dose-dependent manner (Fig. 3E), whereas no change in the expression of Niemann-Pick C1-Like 1 (NPC1L1) was observed (Fig. 3F). Compared with controls, the expression of NPC1L1 mRNA was significantly reduced 49.2% in Caco2 cells stimulated with T0901317.

3.3 Taurine induces glucokinase gene expression in hepatocytes

The effects of taurine incubation on the expression of LXR-α responsive genes in glucose metabolism, such as carbohydrate responsive element-binding protein (ChREBP), glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase 1 (PEPCK-1), and glucokinase (GCK), were assessed in HepG2 cells (Supporting Information Fig. S1). Taurine induced ChREBP and GCK mRNA levels in a dose-dependent manner, and the inductions were significant relative to the control at 100 μM. Taurine did not affect G6Pase or PEPCK-1 expression, whereas the expression of these genes was repressed in the T0901317 group as compared to the control.

3.4 Taurine reduces cellular TG concentrations by suppressing the nuclear translocation of SREBP-1c in hepatocytes

The activation of LXR-α has been demonstrated to frequently promote hepatic lipogenesis and hyperlipidemia in vitro and in vivo through the induction of SREBP-1c, which is a critical transcription factor that promotes hepatic lipogenesis [14]. Notably, the cellular TG concentrations decreased significantly with taurine in hepatocytes while inducing LXR-α activation (Fig. 4A). Compared to the control, taurine at 10, 50, and 100 μM significantly reduced the TG level by 22.6%, 28.4%, and 45.8%, respectively, whereas T0901317 increased the cellular TG concentration as compared to the control (Fig. 4A). Oil Red O staining in cells treated with taurine showed marked reductions in cellular lipid accumulation, whereas T0901317-treated cells showed a significant accumulation of cellular lipid droplets (Fig. 4B).

Taurine (100 μM) significantly induced the gene expression of SREBP-1c by 1.6-fold compared to the control; however, it did not affect the expression of fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD-1), or long-chain acyl CoA dehydrogenase (L-CAD) genes, which are known LXR-α responsive genes whose expression levels were significantly induced by T0901317. SREBP-1c acts as a key transcription factor in hepatic lipogenesis by the upregulation of FAS and SCD-1 (Fig. 5). We further assessed SREBP-1 protein expression in hepatocytes to investigate the hypolipidemic mechanism of taurine in hepatocytes. Taurine induced precursor SREBP-1 (pSREBP-1) in HepG2 cells in a dose-dependent manner in accordance with its transcriptional activation. Taurine at 10, 50, and 100 μM increased pSREBP-1 significantly to 135%, 201%, and 227%, respectively ($p < 0.05$). However, the level of nuclear SREBP-1 (nSREBP-1), an active form of SREBP-1 as a transcription factor, was repressed by taurine in a dose-dependent manner (Fig. 5). Incubation with taurine at 50 μM and 100 μM showed marked reductions of nSREBP-1c to 73% and 46%, respectively, as compared to the control ($p < 0.05$). These findings suggest that taurine inhibited the nuclear translocation of SREBP-1c, and thus, did not stimulate FAS and SCD-1 gene expressions in hepatocytes. Insig-2a and Akt regulate the posttranscriptional regulation of SREBP-1c [29, 30], and miR-33b for ABCA1 and ABCG1 [31]. We therefore next assessed the expression of these three proteins in hepatocytes. Taurine did not alter the phospho-Akt levels, a negative regulator of SREBP-1c transcription, or miR-33b, a negative regulator of ABCA1 and ABCG1, respectively, whereas T0901317 reduced protein expression of phosphorylated Akt and induced the expression of miR-33b. However, incubation with taurine significantly induced the gene expression of Insig-2a, which could retain pSREBP-1c in endoplasmic reticulum (ER),

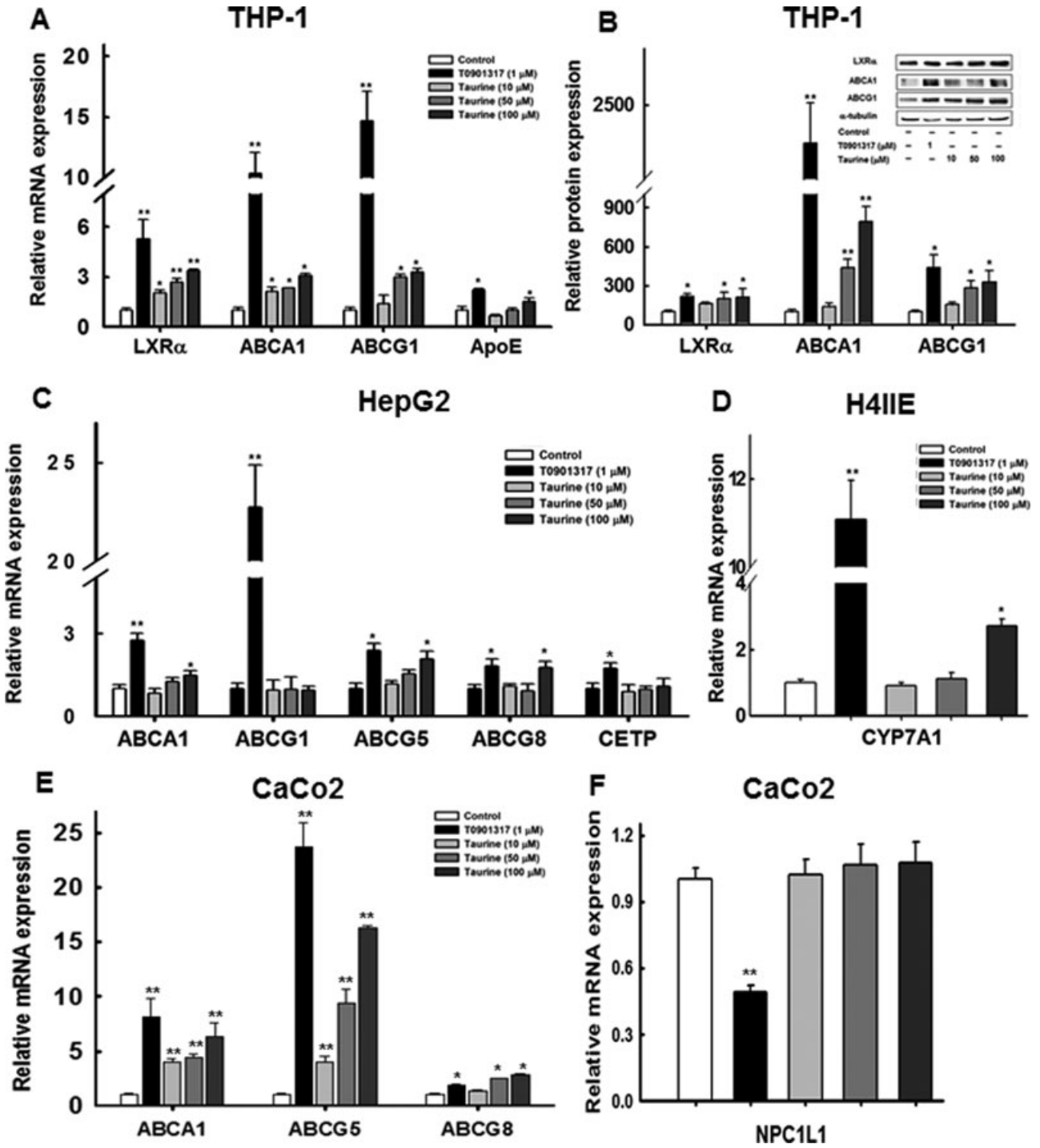

Figure 3. Induction of LXR-α and its responsive genes by taurine in multiple cell types. THP-1 monocytes were incubated with PMA (50 ng/mL) for 3 days to differentiate them into adherent macrophages. The macrophages, HepG2, H4IIE, and Caco2 cells were then treated with 10, 50, and 100 μM taurine, 1 μM T0901317, or vehicle control (1% water) for 48 h. Total RNA was extracted and mRNA expression levels of LXR-α, ABCA1, ABCG1, and APOE in macrophages (A); ABCA1, ABCG1, ABCG5, ABCG8, and CETP in HepG2 (C), CYP7A1 in H4IIE (D), ABCA1, ABCG5, ABCG8, and NPC1L1 in Caco2 (E, F) were measured by qPCR. The protein levels were determined by immunoblotting (B). Data are given as the mean ± SEM ($n = 3$). *, **Significantly different gene expression from the control group, *$p < 0.05$, **$p < 0.001$.

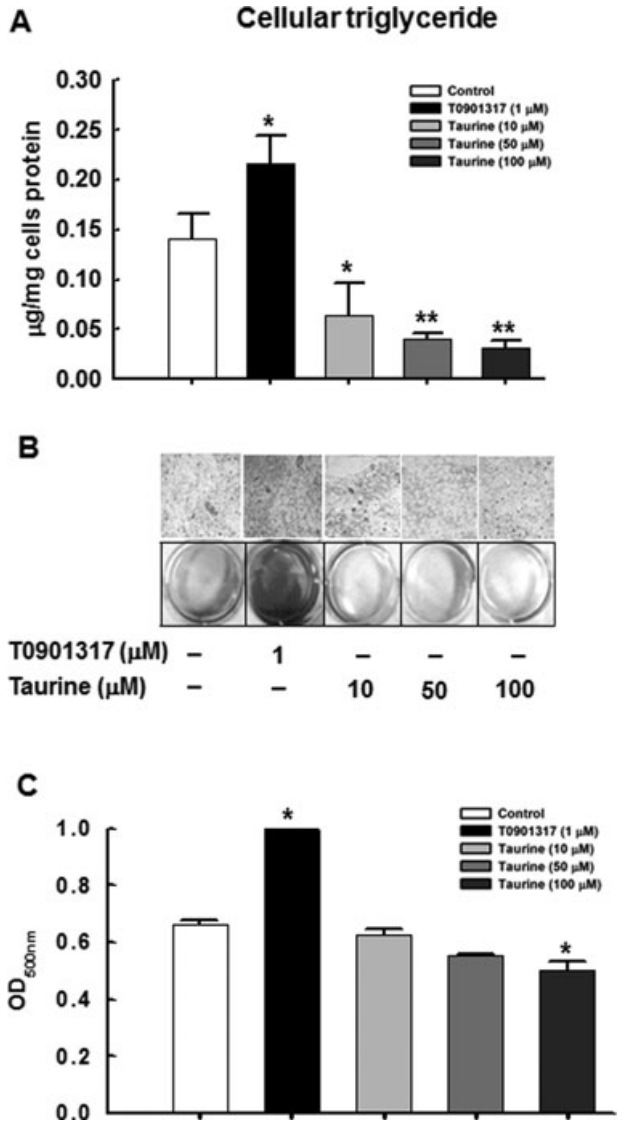

Figure 4. Effects of taurine on the lipid levels in HepG2 cells. HepG2 cells were treated with 10, 50, and 100 μM taurine, 1 μM T0901317, or vehicle control (1% water) for 48 h. Cellular TG (A) was measured using an enzymatic method. (B) Images of cells were captured by microscope at 100× original magnification, showing fat accumulation in the cells stained by Oil Red O and (C) quantitative analysis of lipid deposition in cells by Oil Red O staining. Data are given as the mean ± SEM ($n = 3$). *, **Significantly different from the control group, *$p < 0.05$, **$p < 0.001$.

by threefold ($p < 0.05$) compared to the control (Fig. 5).

4 Discussion

The hypocholesterolemic and antiatherogenic activity of taurine has been consistently reported. Several in vivo studies have shown that taurine elevates HDL in rodent models [6, 7] and induces hepatic CYP7A1 activity, thereby increasing bile acid synthesis [7]. Several LXR agonists including taurohyodeoxycholic acid, an endogenous LXR-α ligand, activate LXRE in the CYP7A1 promoter via LXR-α [19]. Therefore, we hypothesized that taurine could activate LXR-α to stimulate RCT and antiatherosclerosis effects.

The ligand-binding pocket of LXR isoforms can adopt a diverse array of ligands of various shapes, structure, and volume [22]. This adaptability has encouraged the search for novel LXR agonists. Through virtual modeling, we showed that taurine could dock into LXR-α in a manner different from the binding of a known LXR agonist, T0901317, to LXR-α [22]. We used a limited protease digestion assay to demonstrate that taurine activates the nuclear receptor LXR-α by directly binding to the ligand domain of the receptor, thereby stimulating target gene transcription in macrophages and hepatocytes in a TR-FRET.

The ligand of LXR-α acts as a critical transcriptional factor in the regulation of RCT by which excess cholesterol is transferred from peripheral tissues to the liver via HDL particles. Consistent with the LXR activation of RCT, a significant decrease in cellular cholesterol mass content and a corresponding increase in cholesterol content in the cell culture medium occurred after macrophages were stimulated with taurine and T0901317, in agreement with previous study by Aravindhan et al. [32]. The upregulation of ABCA1- and ABCG1-mediated efflux to the regulatory proteins apoA-I and HDL, respectively [33], could increase the plasma HDL concentration and contribute to the prevention of atherosclerosis, which has been reported previously [6, 7].

LXR also impacts systemic cholesterol levels by decreasing intestinal cholesterol absorption and increasing biliary cholesterol excretion through the regulation of membrane transporters, including NPC1L1, ABCA1, ABCG5, and ABCG8. NPC1L1 mediates apical cholesterol uptake from the gut lumen in the intestine, whereas ABCA1 facilitates basolateral cholesterol efflux for HDL formation. The ABCG5/G8 heterodimer transporter effluxes cholesterol and phytosterol from the intestinal epithelium to the gut lumen. Intestinal ABCA1, ABCG5, and ABCG8 mRNA levels increased markedly in Caco2 cells after stimulation by T0901317, in agreement with previous study by Yoon et al. [34]. Similarly, taurine induced intestinal ABCA1, ABCG5, and ABCG8 mRNA levels in a dose-dependent manner. Taurine did not affect NPC1L1 expression, whereas expression of this gene was repressed in the T0901317 group as compared to that in the control. Although not all intestinal gene expression was regulated the same by the synthetic ligand and taurine, the expression patterns were similar. The altered cholesterol transporter genes in the intestinal epithelium following taurine stimulation may provide additional metabolic benefits during cholesterol homeostasis.

Taurine also induced the expression levels of ABCA1, ABCG5, and ABCG8 in HepG2 cells and CYP7A1 in H4IIE cells, leading to reduced cholesterol concentrations, whereas

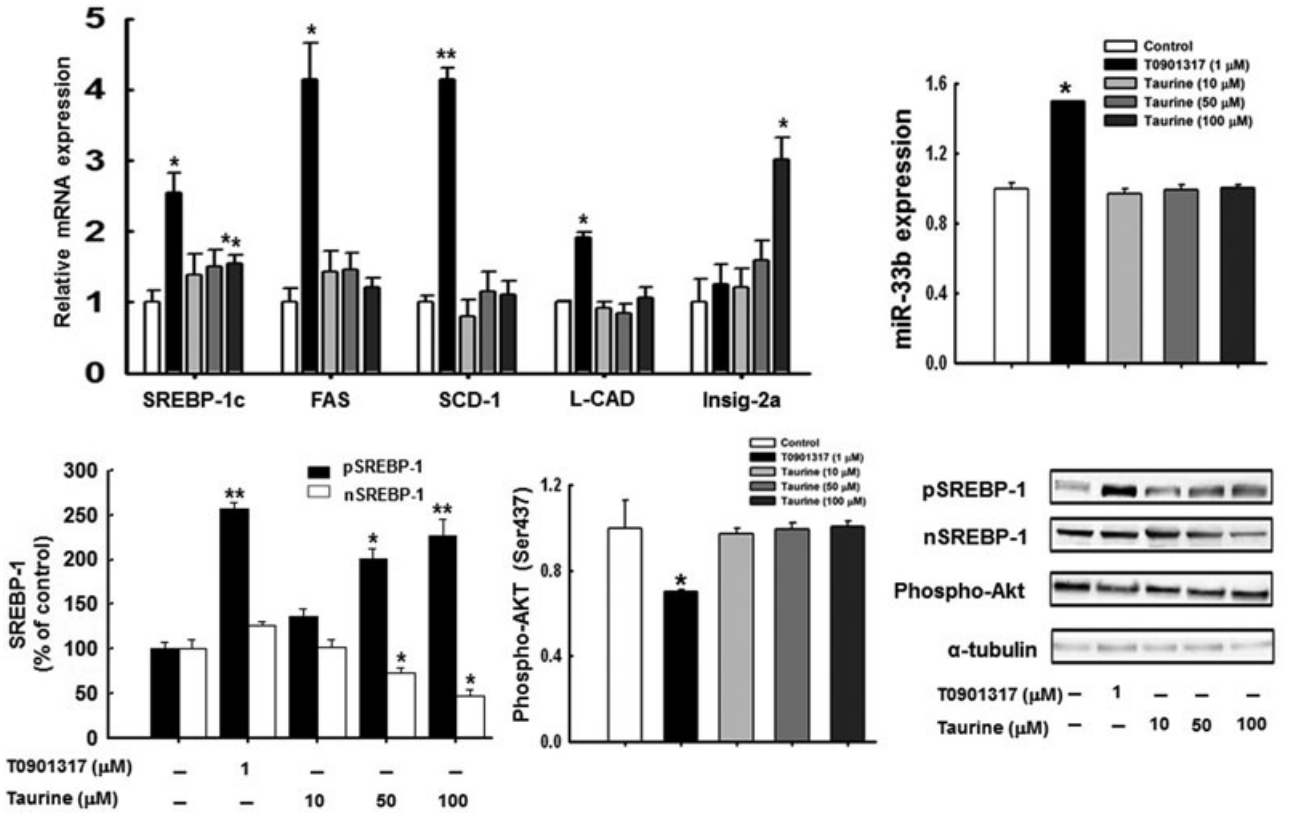

Figure 5. Effects of taurine on the mRNA of LXR-α target genes in fatty acid synthesis and the protein expression of SREBP-1 in hepatocyte cells. HepG2 cells were treated with 10, 50, and 100 μM taurine, 1 μM T0901317, or control (1% water) for 48 h. Total RNA was extracted from the cells and gene expression levels were measured by qPCR analysis. Protein levels were determined by immunoblotting with anti-SREBP-1 and antiphospho-Akt. Data are given as the mean ± SEM ($n = 3$). *Significantly different from the control group, *$p < 0.05$.

no change in the expression of FAS, SCD-1, or L-CAD was observed in the study. SREBP-1c encodes miR-33b in humans and shows translational inhibition or destabilization of ABCA1 and ABCG1 mRNA [31]. The reduction or inhibition of miR-33b expression upregulates ABCA1 and ABCG expression and increases plasma HDL cholesterol concentrations in vivo [31]. However, taurine stimulation did not alter the miR-33b expression in hepatocytes, indicating that induction of ABCA1 and ABCG1 transcription was not altered by miR-33b expression but by direct ligand-dependent activation of LXR-α.

Induction of hypertriglyceridemia by LXR agonists is controversial. Increases in plasma TGs by LXR agonists have been reported [14]. However, other reports showed no change in plasma TGs [35] or only a transient increase [36]. The reason for this inconsistency is unclear at present. In the current study, taurine did not induce lipogenesis while activating LXR-α in hepatocytes; therefore, we further investigated the molecular mechanism behind these unexpected findings. Taurine induced SREBP-1c mRNA and pSREBP-1 protein expression, whereas nSREBP-1 protein level was downregulated. In contrast, T0901317 increased SREBP-1c mRNA and the pSREBP-1 protein and led to an increase in nSREBP-1, and consequently, FAS and SCD-1 mRNA abundance.

To produce active nSREBP, the precursor protein must be chaperoned by SREBP-cleavage-activating protein (SCAP) from the ER to the Golgi in order to access the specific proteases S1P and S2P [37]. Yellaturu et al. [30] reported that stimulating ER-to-Golgi transport of the SREBP-1c–SCAP complex via SREBP-1c phosphorylation is enhanced by activated Akt enzyme. However, neither T0901317 nor taurine altered Akt phosphorylation. This implies that LXR activation by either a synthetic ligand or taurine does not induce nuclear translocation of SREBP-1c via Akt-dependent phosphorylation.

Insig-2 is required for sterols to retain the SREBP–SCAP complex in the ER [38, 39]. Under conditions of sterol depletion, SREBP–SCAP associates preferentially with the Insig-2a isoform in the liver and is retained in the ER membrane, thus preventing the formation of nSREBP-1c and decreasing the expression of SREBP-1c-regulated genes [29]. In our study, stimulation with taurine induced Insig-2a mRNA expression in HepG2 cells. This suggests that delayed nuclear

translocation of SREBP-1c by taurine stimulation may be mediated by Insig-2a gene induction. Thus, taurine stimulation retains the INSIG2–SCAP–SREBP-1c triple complex in the ER and consequently reduces the expression of SREBP-1c-responsive hepatic lipogenic genes such as FAS and SCD-1.

Previous studies have demonstrated that the ligand of farnesoid X receptor (FXR) is capable of inhibiting pSREBP processing to nSREBP in hepatocytes by a mechanism that induces Insig-2a [40, 41]. Taurine-conjugated bile acids may be able to induce Insig-2a expression by activating FXR [42]. Additionally, taurine-conjugated bile acids may be able to bind to the SCAP–INSIG–SREBP-1 triple complex, thus delaying SREBP-1 processing because the chemical structure of taurine-conjugated bile acids resembles cholesterol and oxysterol [43]. The essential structural motif of the sterol for the SCAP-INSIG complex is the 3β-hydroxyl group, which is present in taurine-conjugated bile acids. This possibility should be examined in the future.

In humans, dietary intake is the main source of plasma taurine, although a minor amount can be endogenously synthesized in the liver from the amino acid cysteine (7–50 nM/min/g wet tissue) [44]. Some studies have investigated the hypolipidemic effects of dietary cysteine, but the data were mixed, with large variations [45, 46]. A study by Seidel et al. [46] in 1960 suggested that dietary supplements of cysteine were more effective than dietary taurine intake in reducing serum cholesterol concentration, although the mechanism has never been studied and the data need to be confirmed through additional investigations. The data also suggest that dietary taurine, but not endogenous taurine, may lead to hypocholesterolemic effects.

Additionally, various studies have shown that LXRs regulate FAS and SCD-1 expression through direct interaction with their promoter and by activating SREBP-1c expression [36, 47]. In our study, taurine did not alter the expression of FAS and SCD-1, suggesting that the regulation of these genes by SREBP-1c may be the dominant mechanism. Recently, Miao et al. [48] and Albers et al. [49] reported that the selective LXR modulators GW3965 and 22R-HC differ from T0901317 in the induction of FAS or SCD-1 in the liver because of differences in the extent of coactivator recruitment.

Several mechanisms have been proposed for the hypotriglyceridemic effects of taurine in vivo. Cantafora et al. [50] reported that the decrease in hepatic TG concentration is likely due to taurine inhibition of diacylglycerol:acyl-CoA acyl transferase, the key enzyme in hepatic TG synthesis. Other studies have suggested that the TG-lowering effects of taurine result from a combination of the effects of taurine on ion channels, transporters, and enzymes, leading to the modulation of intracellular Ca2+ levels, or from the antioxidant properties of taurine [1, 51]. These mechanisms, in combination with delayed SREBP-1c nuclear translocation, may contribute to the reduction of cellular TG concentrations through taurine stimulation in hepatocytes.

Finally, taurine has reasonable bioavailability, as demonstrated in a human pharmacokinetic study [52]. Taurine intake (4 g/person/day) showed a maximum plasma taurine concentration (C_{max}) of 86.1 ± 19.0 mg/L (0.69 ± 0.15 mM). In addition, the half-life in plasma (T1/2) and the ratios of clearance/bioavailability (Cl/F) were 1.0 ± 0.3 h and 21.1 ± 7.8 L/h, respectively, of which the data were comparable with typical nutrients [52]. Because taurine is not metabolized intensively in humans, the findings from the current study may be physiologically relevant.

In summary, we demonstrated that taurine is a direct ligand of LXR-α and that it may have nutritional implications in hypercholesterolemia and atherosclerosis. Note that taurine did not induce fatty acid synthesis genes including FAS and SCD-1 via inhibition of the nuclear translocation of pSREBP-1 into nSREBP-1 by including the gene expression of Insig-2a. These interactions resulted in the net reduction of cellular cholesterol and TG levels in hepatocytes (Fig. 6).

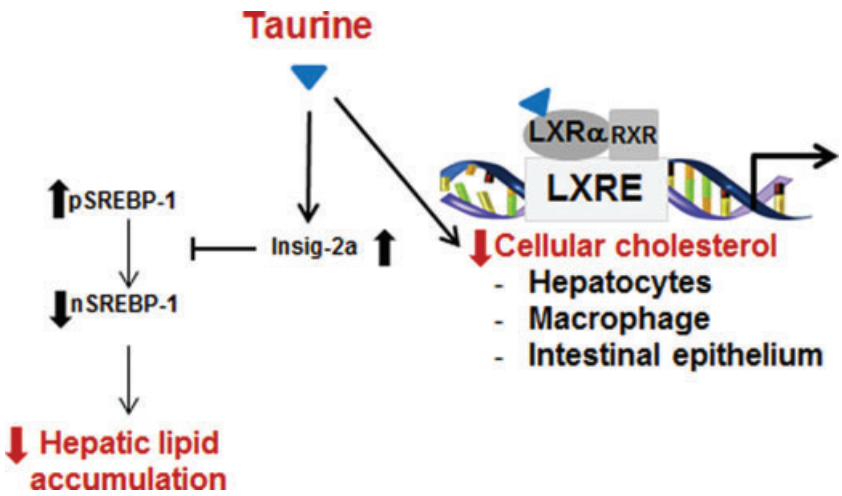

Figure 6. Mechanism of LXR-α-dependent hypocholesterolemic action of taurine in THP-1-derived macrophages, hepatocyte, and intestinal cells.

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), by the Korean Forest Service (Forest Science & Technology Project No. S120909L130110) and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20100028180).

The authors have declared no conflict of interest.

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