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ORIGINAL ARTICLE

Cholesterol-lowering effects of taurine through the reduction of ileal FXR signaling due to the alteration of ileal bile acid composition

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Abstract

Studies using animal models of hypercholesterolemia have established that taurine reduces cholesterol levels; however, the precise mechanism underlying this cholesterol-lowering effect is unclear. This study addressed this issue by investigating whether bile acid/farnesoid X receptor (FXR) signaling is involved in taurine-mediated cholesterol-lowering effect. *Fxr*-null and wild-type mice were administered 2% (w/v) taurine in their drinking water and fed a control diet or control diet supplemented with 1% (w/w) cholesterol (cholesterol diet) for 10 days. Taurine intake did not significantly alter hepatic and serum total cholesterol (TC) levels and bile acid compositions of the liver and intestinal lumen in *Fxr*-null and wild-type mice fed the control diet. By changing to a cholesterol diet, taurine intake significantly decreased hepatic and serum cholesterol levels in wild-type mice. In contrast, it significantly decreased hepatic, not serum, cholesterol levels in *Fxr*-null mice. Taurine intake significantly altered the bile acid composition of the intestinal lumen in wild-type mice fed a cholesterol diet, but not in *Fxr*-null mice. An increase in FXR antagonistic bile acids was detected in the intestinal lumen of taurine-treated wild-type mice fed a cholesterol diet. Taurine intake reduced the ileal expression of FXR target genes fibroblast growth factor 15 (*Fgf15*) and small heterodimer partner (*Shp*). In contrast, it enhanced the hepatic expression of cholesterol 7α-hydroxylase (*Cyp7a1*) in wild-type mice fed a cholesterol diet, but not in *Fxr*-null mice. These results suggest that taurine is partially involved in cholesterol lowering by reducing the ileal FXR signaling due to the alteration of ileal bile acid composition.

Keywords Taurine · Cholesterol · FXR · Bile acid · CYP7A1

Introduction

Taurine (2-aminoethanesulfonic acid) is an abundant free amino acid found in mammalian tissues. It is synthesized de novo from cysteine and ingested in diets such as seafood to maintain its levels in the body. Several beverages containing high concentrations of taurine are commercially available. Taurine plays several critical roles in human and animal physiology, including antioxidant function, osmoregulation, membrane stabilization, and bile acid conjugation (HuxTable 1992; Wright et al. 1986). Notably, taurine prevents metabolic syndrome development (Chang et al. 2011; Chen et al. 2016; Imae et al. 2014; Militante and Lombardini 2002). Taurine is closely associated with bile acid and cholesterol metabolism. Several studies have shown that taurine efficiently reduces serum and liver cholesterol concentrations in animals with hypercholesterolemia induced by a high-cholesterol diet (Chen et al. 2012; Murakami et al. 1999; Yokogoshi et al. 1999).

As cholesterol levels in the body are primarily regulated by the balance between de novo cholesterol synthesis and the catabolism of cholesterol to bile acids, regulating the rate-limiting enzyme for bile acid synthesis cholesterol 7α-hydroxylase (CYP7A1) is an essential issue for elucidating the mechanism of cholesterol-lowering effects (Chiang 2009). Several studies have demonstrated that taurine elevates CYP7A1 expression in vivo and in vitro (Chen et al. 2005; Guo et al. 2017; Lam et al. 2006; Yokogoshi et al. 1999). Moreover, taurine decreases bile acid concentration in the portal vein and increases fecal bile acid excretion in rats with hypercholesterolemia (Chen et al. 2003; Nishimura

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et al. 2009). Taurine also alters the enterohepatic bile acid metabolism (Murakami et al. 2016).

Farnesoid X receptor (FXR) is a bile acid-activated nuclear receptor that regulates the metabolism of glucose and lipids, including triglycerides, cholesterol, and bile acids (Cariou and Staels 2007; Wang et al. 2008; Matsubara et al. 2013). Notably, FXR plays a crucial role in suppressing elevated bile acid levels in enterohepatic tissues through hepatic and intestinal pathways and the enterohepatic FGF15/19 pathway. The activation of intestinal FXR has a significant role, whereas the activation of hepatic FXR serves a minor role in suppressing CYP7A1 gene expression by inducing the ileal hormone fibroblast growth factor 15/19 (FGF15/19) and hepatic small heterodimer partner (SHP) (Goodwin et al. 2000; Holt et al. 2003; Kliewer and Mangelsdorf 2015). Intestinal FXR activation induces FGF15/19, which acts as an endocrine hormone to inhibit hepatic CYP7A1 expression by binding to fibroblast growth factor receptor 4 on hepatocytes (Shin and Osborne 2009). Furthermore, intestinal FXR affects intestinal bile acid absorption by expressing the apical sodium-dependent bile acid transporter (ASBT) that is negatively regulated by intestinal SHP (Neimark et al. 2004; Nguyen et al. 2021).

Although bile acids activate FXR signaling, certain bile acids can act as FXR antagonists. Typical FXR antagonists, such as tauro-α-muricholic acid (TαMCA) and tauro-β-muricholic acid (TβMCA), attenuate other bile acid-activated FXR signaling (Li et al. 2013; Sayin et al. 2013). The enterobacteria-mediated deconjugation of bile acids enhances ileal FXR signaling (Miyata et al. 2009; Kuribayashi et al. 2012). Alterations in bile acid composition are possibly reflected in the activation level of FXR signaling. Whether taurine acts as an FXR ligand remains unknown. However, it is possible that taurine indirectly affects FXR signaling by altering qualitative and quantitative enterohepatic bile acids.

Fxr-null mice exhibit elevated hepatic and serum cholesterol and bile acid levels due to the lack of FXR (Sinal et al. 2000); hence, they are considered endogenous models for hypercholesterolemia without FXR signaling. However, wild-type mice fed a cholesterol diet are exogenous models for hypercholesterolemia with FXR signaling. In this study, *Fxr*-null and wild-type mice fed a control or cholesterol diet were used to elucidate whether taurine is involved in cholesterol lowering through bile acid FXR signaling regulation. Taurine intake decreased hepatic cholesterol levels in wild-type and *Fxr*-null mice fed only a cholesterol diet. In serum, whereas wild-type mice showed the cholesterol-lowering effect of taurine, no changes were observed in *Fxr*-null mice. Furthermore, taurine attenuated ileal FXR signaling and increased FXR antagonistic bile acid levels only in the intestinal lumen of wild-type mice fed a cholesterol diet, whereas it increased Cyp7a1 expression levels. These results suggest that taurine is partially involved in cholesterol lowering by reducing the ileal FXR signaling due to the alteration of ileal bile acid composition.

Materials and methods

Materials

Fxr-null mice were kindly provided by Dr. Frank J. Gonzalez (National Institutes of Health, Bethesda, MD) (Sinal et al. 2000). The background of the *Fxr*-null mice is C57BL/6N. Eight-week-old C57BL/6N mice were purchased from CLEA Japan, Inc. (Tokyo, Japan), for use as wild-type mice. Tauro-murideoxycholic acid (TMDCA) was kindly provided by Dr. Alan F. Hofmann (University of California, San Diego, CA). 5β-Androstan-3α,17β-diol, β-muricholic acid (βMCA), TαMCA, and TβMCA were purchased from Steraroids Inc. (Newport, RI). The other bile acids were purchased from Sigma-Aldrich (St. Louis, MO).

Animal treatment and sample collection

Fxr-null and wild-type mice were housed under a standard 12-h light–dark cycle (7 a.m.–7 p.m.). Age-matched groups of 8- to 9-week-old male mice were used for all experiments. Mice were administered 2% (w/v) taurine in their drinking water for 10 d. They were fed an AIN-93M diet with or without 1% (w/w) cholesterol supplementation. After 10 days of drinking this water, the mice were euthanized between 9 and 11 a.m. All experiments were conducted in accordance with the guidelines for animal experiments of the National Fisheries University (Shimonoseki, Japan). The protocol was approved by the Institutional Animal Care and Use Committee at the National Fisheries University (Permission No. 2019-18-2).

Determination of hepatic and serum cholesterol levels

Hepatic samples were extracted using the Folch method (Folch et al. 1957). Hepatic and serum TC levels were determined using Cholesterol *E*-Test Wako Kits (FUJIFILM Wako Pure Chemical Co., Osaka, Japan).

Determination of mRNA levels

The lower end of the ileum (2 cm) was collected. Hepatic and intestinal total RNA samples were isolated using the acid guanidine–phenol–chloroform method. Single-stranded cDNA was synthesized using an oligo(dT) primer and the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The cDNA was subjected to

a polymerase chain reaction (PCR) using Gene *Taq* (Nippon Gene, Tokyo, Japan) in the TP350 TaKaRa PCR Thermal Cycler Dice Touch (Takara Bio, Otsu, Japan) and to a real-time quantitative polymerase chain reaction (qPCR) using SYBR Premix Ex Taq II (Tli RNaseH Plus) (Takara Bio) in the TP870 Thermal Cycler Dice Real-Time System (Takara Bio). The relative mRNA levels were calculated using the comparative threshold cycle method. The specific forward and reverse primers used in the PCR are described in Table 1.

Determination of bile acid composition

Bile acid samples in the small intestinal lumen were collected by washing out its contents with phosphate-buffered saline. The bile acid composition of the liver and small intestinal lumen was analyzed using high-performance liquid chromatography (HPLC) combined with immobilized 3α-hydroxysteroid dehydrogenase (3α-HSD) (Hasegawa et al. 1983). Analytical conditions followed a previously described method (Kitada et al. 2003). Briefly, the HPLC system consisted of a JASCO Pu-2080 Plus Intelligent HPLC Pump (JASCO, Tokyo, Japan) and an FP-2025 fluorescence detector (JASCO). For the separation of individual bile acids, an L-column 2 (3 μm, 2.1 × 150 mm) (Chemicals Evaluation and Research Institute, Tokyo, Japan) was employed at 35°C. The separation was started at 0.5 mL/min with a 60-min linear gradient of solution A [10 mM phosphate buffer, pH 7.2/acetonitrile (60:40)]/solution B [30 mM phosphate buffer, pH 7.2/acetonitrile (80:20) mixture (25:75)] to a solution A/solution B mixture (55:45) and then continued with a solution A/solution B mixture (55:45) for 25 min. The eluates from the L-column 2 were mixed with an NAD^+ solution (10 mM phosphate buffer, pH 7.2, 1 mM EDTA, 0.05% 2-mercaptoethanol, and 0.3 mM NAD^+) before introduction to 3α-HSD immobilized on an Enzymepak 3α-HSD column (JASCO) at 0.5 mL/min. The produced NADH level was measured by a fluorescence detector at an excitation wavelength of 365 nm and an emission wavelength of 470 nm. The retention times of βMCA, TαMCA, TβMCA, TMDCA, ursodeoxycholic acid (UDCA), cholic acid (CA), tauro-ursodeoxycholic acid (TUDCA), tauro-cholic acid (TCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), tauro-chenodeoxycholic acid (TCDCA), tauro-deoxycholic acid (TDCA), 5β-androstan-3α,17β-diol (internal standard), lithocholic acid (LCA), and tauro-lithocholic acid were 17.6, 26.5, 27.0, 31.3, 32.0, 35.6, 39.0, 42.0, 48.7, 51.9, 55.6, 58.4, 61.4, 67.9, and 75.2 min, respectively. Quantification of each bile acid was performed based on each of their calibration curves.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using Excel Statistics 2015 (Social Survey Research Information Co. Ltd., Tokyo, Japan). Statistical significance was determined by Student's *t* test and two-way analysis of variance (ANOVA), followed by post hoc analysis using Tukey's test.

Results

Control diet and drinking water with taurine

Taurine reduces cholesterol levels, but the precise mechanism underlying this remains unclear. We investigated whether FXR signaling is involved in taurine-mediated cholesterol regulation using *Fxr*-null and wild-type mice. Wild-type and *Fxr*-null mice were fed a control diet (AIN-93M) and provided water with or without 2% taurine for 10 d. Significant increases in the levels ($p < 0.001$) of hepatic TC were detected in *Fxr*-null mice of the control (3.60 ± 0.15 vs. 3.06 ± 0.23 mg/g liver) and taurine (3.70 ± 0.15 vs. 2.90 ± 0.21 mg/g liver) groups, compared with those in the corresponding wild-type mice. In contrast, no significant alterations in these levels were detected between the control and taurine groups among the wild-type and *Fxr*-null mice (Fig. 1a). In addition, serum TC and hepatic levels changed. Significant increases in the levels ($p < 0.001$) of serum TC were detected in *Fxr*-null mice of the control (1.35 ± 0.32 vs. 0.79 ± 0.10 mg/mL) and taurine (1.22 ± 0.28

Table 1 Primer sequences

Gene	Sequence (5′–3′)	Sequence (5′–3′)
β-Actin	GCCAACACAGTGCTGTCTG	CCTGCTTGCTGATCCACATC
Hmgcr	GAATGCCTTGTGATTGGAGTTG	ACACAGGCCGGGAAGAATG
Shp	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Fgf15	GAGGACCAAAACGAACGAAATT	ACGTCCTTGATGGCAATCG
Cyp7a1	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
Fxr	CAAACAGAGAATGCCTCAGG	CATCCCAGATCTCACAGAGG

Hmgcr: hydroxymethylglutaryl-CoA reductase, *Shp:* small heterodimer partner, *Fgf15:* fibroblast growth factor 15, *Cyp7a1:* cholesterol 7α-hydroxylase, *Fxr:* farnesoid X receptor

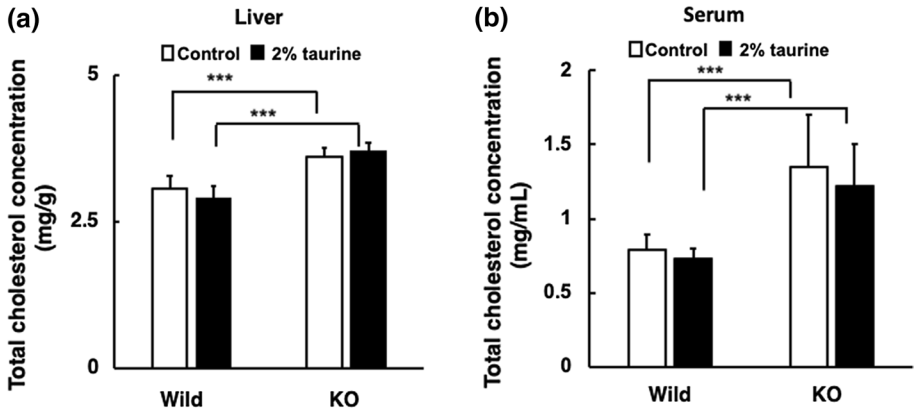

Fig. 1 Cholesterol levels in mice fed a control diet. **a** Liver. **b** Serum. *Fxr*-null and wild-type mice were administered 2% (w/v) taurine in their drinking water for 10 d. Values are presented as mean ± SD ($n = 8$) analyzed using the Cholesterol E-Test Wako Kits. The significance of differences (***$p < 0.001$) was evaluated using two-way ANOVA followed by post hoc analysis using Tukey's test. Wild: wild-type mice, KO: *Fxr*-null mice

vs. 0.71 ± 0.07 mg/mL) groups, compared with those in the corresponding wild-type mice. In contrast, no significant differences in these levels were detected between the control and taurine groups among both mouse types (Fig. 1b). Because serum and hepatic TC levels in *Fxr*-null mice were significantly higher than those in wild-type mice, *Fxr*-null mice were considered to have hypercholesterolemia. However, taurine treatment did not decrease hepatic and serum TC levels in *Fxr*-null mice. The bile acid composition of the liver and intestinal lumen was analyzed to determine whether taurine treatment affected mouse bile acid metabolism. TCA and TβMCA were identified as the major bile acid components of mouse liver and intestinal lumen (Fig. 2a–d). Hepatic bile acid concentrations in *Fxr*-null mice were higher than those in wild-type mice. In both mice, no significant alterations in the bile acid composition of the liver and intestinal lumen were found between the control and taurine groups.

Cholesterol diet and drinking water with taurine

Both wild-type and *Fxr*-null mice were fed a cholesterol (1%)-containing diet (cholesterol diet) and provided water with or without 2% taurine for 10 d. Consistent with previous reports, hepatic (3.20 ± 0.09 vs. 3.69 ± 0.29 mg/g liver; $p < 0.001$) and serum (0.98 ± 0.07 vs. 1.16 ± 0.10 mg/mL; $p < 0.05$) TC levels were significantly decreased in the taurine-treated wild-type (C57BL/6N) mice fed the cholesterol diet compared with those in control wild-type mice fed the cholesterol diet (Fig. 3a, b). However, hepatic (3.89 ± 0.25 vs. 4.38 ± 0.20 mg/g liver; $p < 0.001$; not serum) TC levels were significantly decreased in the taurine-treated *Fxr*-null mice compared with the levels in the control *Fxr*-null mice. Significant alterations ($p < 0.001$) in hepatic and serum TC levels were detected between the wild-type and *Fxr*-null mice among the control and taurine groups. Significant increases in the levels ($p < 0.001$) of hepatic and serum TC were detected in *Fxr*-null mice of the control and taurine groups, compared with those in the corresponding wild-type mice.

There were no significant alterations in the hepatic bile acid composition of both mice between the control and taurine groups (Fig. 4a, b). In the intestinal lumen, TβMCA (3.14 ± 0.62 vs. 1.82 ± 0.71 μmol), TMDCA (0.70 ± 0.27 vs. 0.36 ± 0.12 μmol), and UDCA (0.13 ± 0.07 vs. 0.05 ± 0.03 μmol) levels were significantly higher ($p < 0.05$) in the taurine-treated wild-type mice than those in the control wild-type mice, as determined using Student's *t* test. In contrast, no significant alterations were found in the bile acid composition of the intestinal lumen in the *Fxr*-null mice between the control and taurine groups (Fig. 4c, d). Two-way ANOVA, followed by a post hoc analysis using Tukey's test was performed on TβMCA, TMDCA, and UDCA, which were significantly increased (Student's *t* test) in the taurine-treated wild-type mice, compared with the levels in the control wild-type mice. TβMCA ($p < 0.01$), TMDCA ($p < 0.05$), and UDCA ($p < 0.05$) of the taurine group were significantly higher than those of the control group in wild-type mice, but not in *Fxr*-null mice (Fig. 4e–g).

To confirm the status of the *Fxr*-null mice, hepatic *Fxr* mRNA levels in the control wild-type and *Fxr*-null mice were analyzed by reverse-transcription PCR. Clear bands corresponding to *Fxr* were found in the wild-type mouse sample, but not in the *Fxr*-null mouse sample, by 40-cycle PCR amplification, whereas clear bands corresponding to *β-actin* were found in both mouse samples by 30-cycle PCR amplification (Fig. 5). No bands were found in the *Fxr*-null mouse sample even by 45-cycle PCR amplification (data not shown).

To elucidate the mechanism underlying the taurine-mediated cholesterol-lowering effect, we analyzed the expression levels of cholesterol and bile acid synthesis genes. The mRNA levels of the rate-limiting enzyme for cholesterol synthesis, *Hmgcr*, in the liver were not altered in both mouse types between the control and taurine groups (Fig. 6a). In contrast, the mRNA levels of the rate-limiting enzyme for bile acid synthesis, *Cyp7a1*, in the liver were significantly

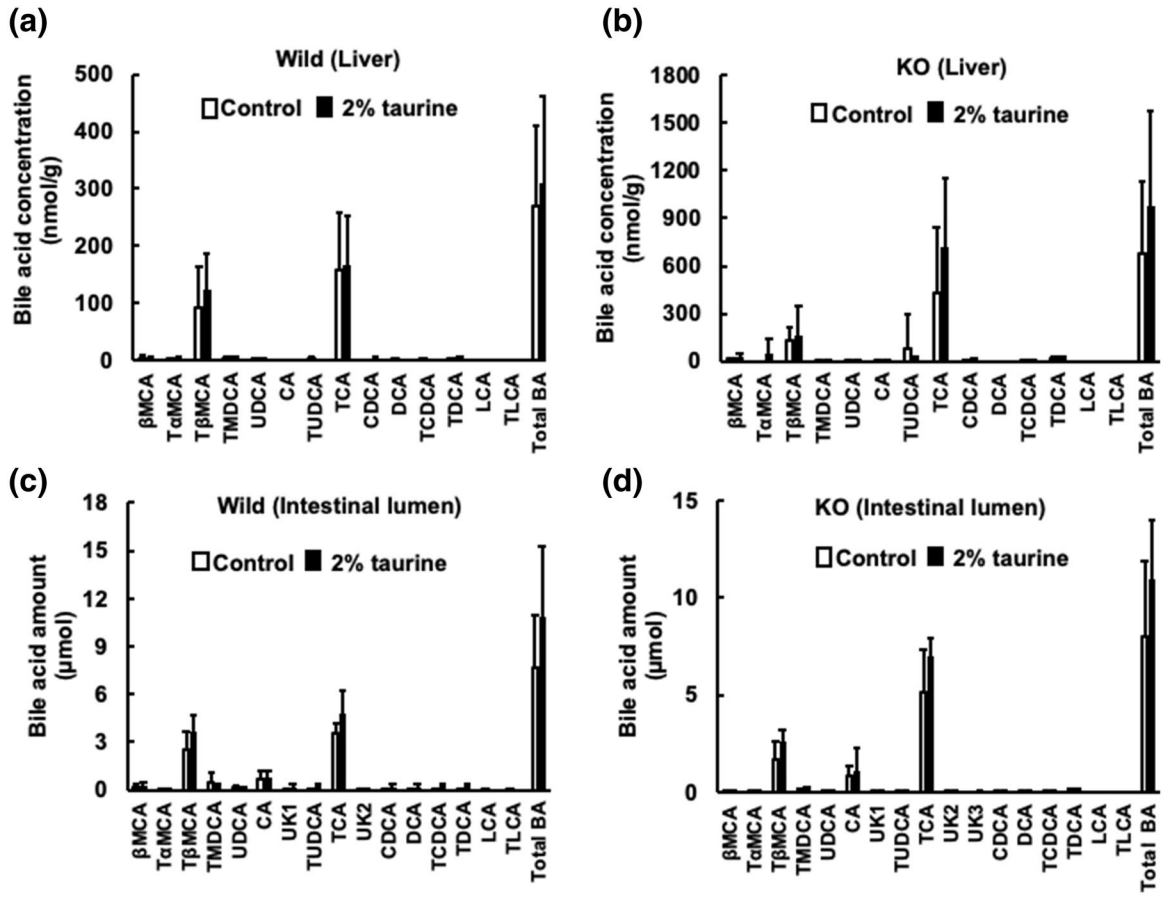

Fig. 2 Bile acid composition in mice fed a control diet. **a** Liver in wild-type mice. **b** Liver in *Fxr*-null mice. **c** Intestinal lumen in wild-type mice. **d** Intestinal lumen in *Fxr*-null mice. *Fxr*-null and wild-type mice were administered 2% (w/v) taurine in their drinking water for 10 days. Values are expressed as mean ± SD ($n = 8$) analyzed by fluorophotometry using HPLC. The significance of differences was evaluated using Student's t test. Unidentified bile acids were designated as UK1, UK2, and UK3. Wild: wild-type mice, KO: *Fxr*-null mice

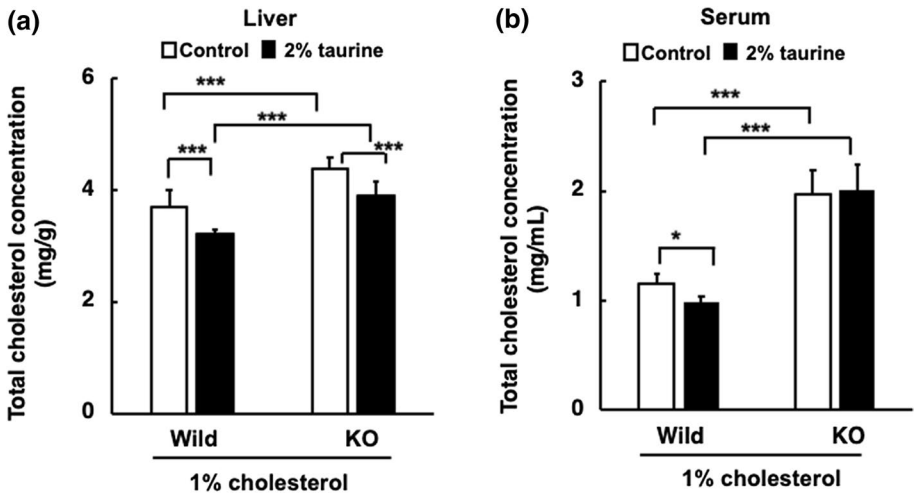

Fig. 3 Cholesterol levels in mice fed a diet supplemented with 1% cholesterol. **a** Liver. **b** Serum. *Fxr*-null and wild-type mice were administered 2% (w/v) taurine in their drinking water for 10 days. Values are presented as mean ± SD ($n = 8$) analyzed using the Cholesterol E-Test Wako Kits. The significance of differences (*$p < 0.05$; ***$p < 0.001$) was evaluated using two-way ANOVA followed by post hoc analysis using Tukey's test. Wild: wild-type mice, KO: *Fxr*-null mice

increased in the wild-type mice (2.99 ± 1.94 vs. 1.00 ± 0.52; $p < 0.01$), but not in the *Fxr*-null mice (Fig. 6b). As bile acid composition was significantly altered between the control and taurine groups, we analyzed the mRNA levels of FXR-targeted genes in the liver and intestine. Despite being a typical FXR-targeted gene and negative regulator of

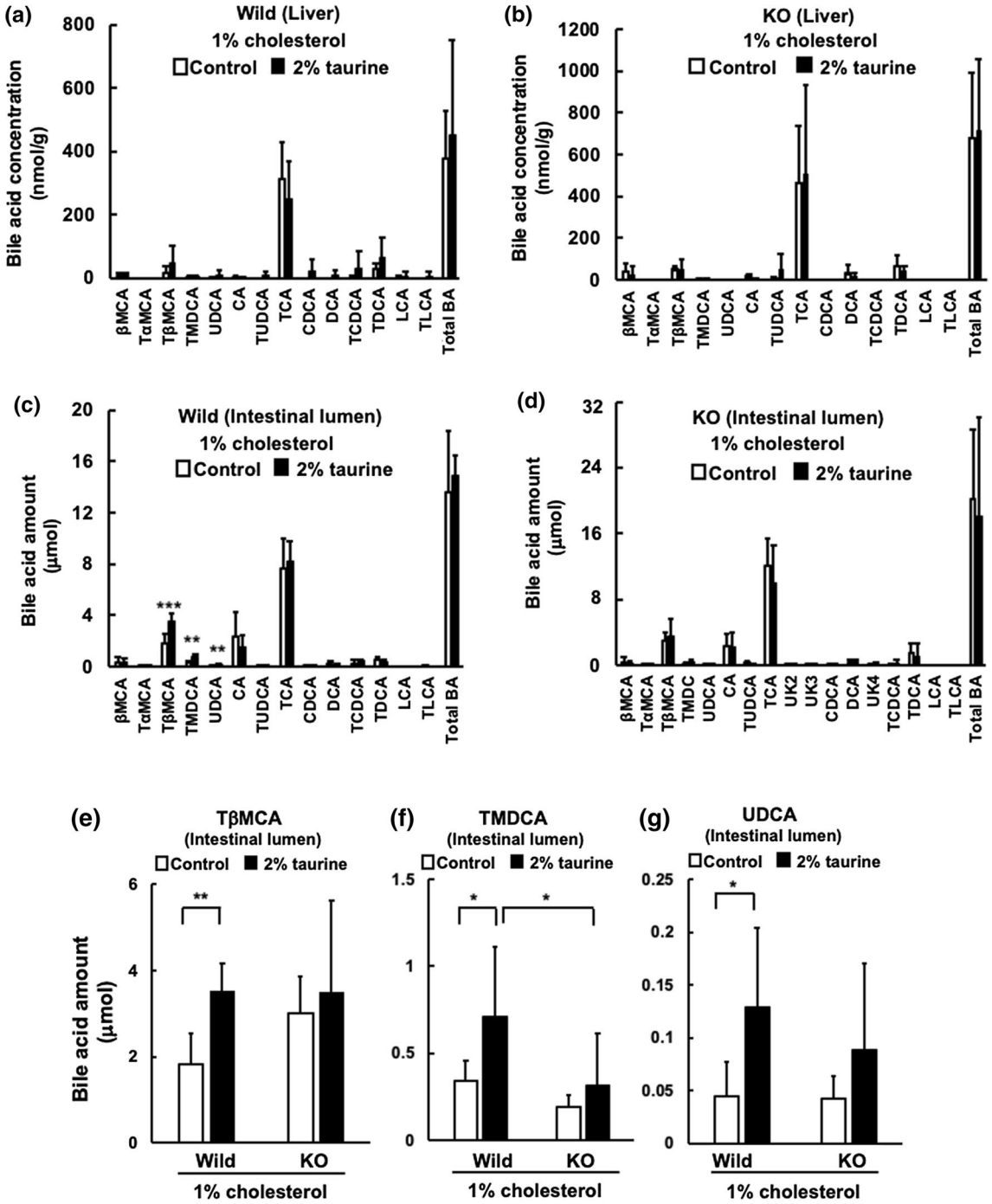

Fig. 4 Bile acid composition in mice fed a diet supplemented with 1% cholesterol. *Fxr*-null and wild-type mice were administered 2% (w/v) taurine in their drinking water for 10 days. **a** Liver in wild-type mice. **b** Liver in *Fxr*-null mice. **c** Intestinal lumen in wild-type mice. **d** Intestinal lumen in *Fxr*-null mice. Values are expressed as mean ± SD ($n = 8$) analyzed by fluorophotometry using HPLC. The significance of differences (**$p < 0.01$; ***$p < 0.001$) was evaluated using Student's t test. Unidentified bile acids were designated as UK2, UK3, and UK4. Wild: wild-type mice, KO: *Fxr*-null mice. **e** TβMCA in the intestinal lumen. **f** TMDCA in the intestinal lumen. g) UDCA in the intestinal lumen. The significance of differences (*$p < 0.05$; **$p < 0.01$) was evaluated using two-way ANOVA followed by post hoc analysis using Tukey's test

CYP7A1, hepatic *Shp* mRNA levels were not significantly altered in taurine-treated mice compared with the levels in control mice (Fig. 6c). However, intestinal *Shp* mRNA levels were significantly decreased in the taurine-treated wild-type mice (0.18 ± 0.13 vs. 1.00 ± 0.69; $p < 0.001$), but not in the *Fxr*-null mice (Fig. 6d). The intestinal *Fgf15* mRNA levels

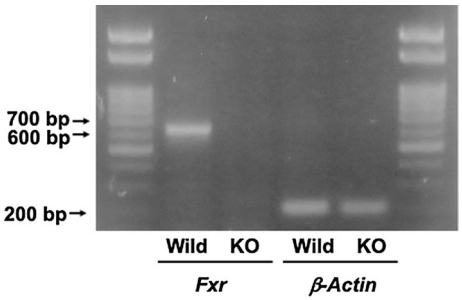

Fig. 5 Reverse-transcription PCR for the detection of hepatic *Fxr* mRNA. Hepatic *Fxr* and *β-actin* mRNA levels were analyzed in the wild-type and *Fxr*-null mice fed a diet supplemented with 1% cholesterol. Products of 30-cycle PCR amplification (*β-actin)* and 40-cycle PCR amplification *(Fxr*) were electrophoresed in 2% agarose gels and detected by ethidium bromide staining

were also significantly decreased in the taurine-treated wild-type mice compared with those in the control wild-type mice (0.42 ± 0.30 vs. 1.00 ± 0.52; $p < 0.01$).

In contrast, in the *Fxr*-null mice, the intestinal *Fgf15* mRNA levels remained unchanged compared with those in the control and taurine groups (Fig. 6e). The intestinal *Asbt* mRNA levels in wild-type mice were not significantly altered between the control and taurine groups (data not shown). The decreases in ileal *Fgf15* and *Shp* mRNA levels suggested the attenuation of ileal FXR signaling in the taurine-treated wild-type mice. Consistent with previous reports (Jiang et al. 2015; Miyata et al. 2009; Sinal et al. 2000), hepatic and intestinal *Shp* and *Fgf15* mRNA levels were significantly decreased in *Fxr*-null mice without taurine supplementation compared with those in the wild-type mice.

Discussion

This study determined whether bile acid/FXR signaling is involved in the taurine-mediated cholesterol-lowering effect. Our findings showed that taurine decreased serum

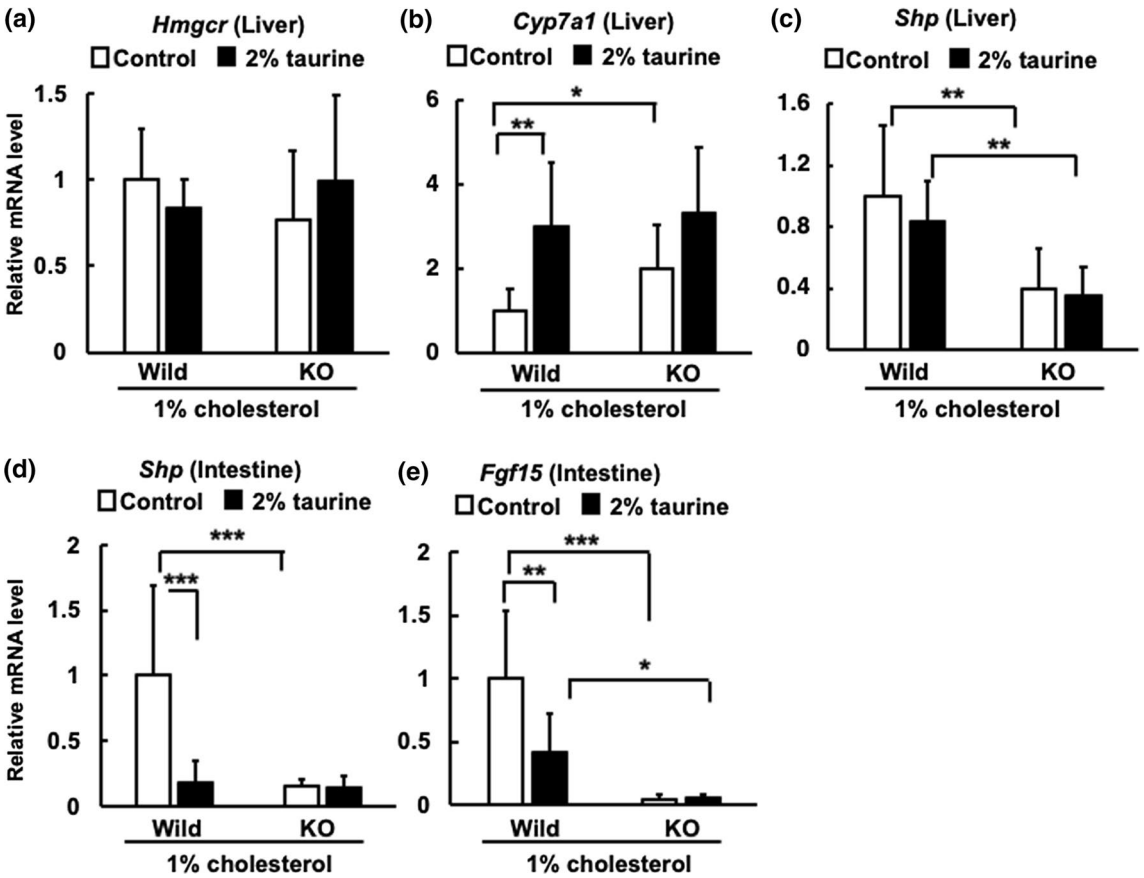

Fig. 6 Hepatic and intestinal mRNA levels in mice fed a diet supplemented with 1% cholesterol. **a** Hepatic *Hmgcr*. **b** Hepatic *Cyp7a1*. **c** Hepatic *Shp*. **d** Intestinal *Fgf15*. **e** Intestinal *Shp*. *Fxr*-null and wild-type mice were administered 2% (w/v) taurine in their drinking water for 10 days. Values are presented as mean ± SD ($n = 8$) analyzed by real-time qPCR. The significance of differences (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) was evaluated using two-way ANOVA followed by post hoc analysis using Tukey's test. Wild: wild-type mice, KO: *Fxr*-null mice

cholesterol levels in wild-type mice fed a cholesterol-containing diet, but not in *Fxr*-null mice. This suggests that FXR signals are partially involved in the taurine-mediated cholesterol-lowering effect. *Fxr*-null mice are endogenous hypercholesterolemia models (Sinal et al. 2000). In the *Fxr*-null mice, the cholesterol levels did not decrease for 4 weeks in addition to 10 days of taurine treatment (Miyata et al. 2020). Moreover, in the mice fed the cholesterol diet, taurine treatment did not decrease serum cholesterol levels. These results suggest that most taurine-mediated cholesterol-lowering effects modulate FXR signaling and not the compensation for FXR signaling. The FXR antagonist guggulsterone, a phytosteroid found in the resin of the guggul plant, decreased cholesterol levels in wild-type mice fed a high-cholesterol diet, but it was ineffective in *Fxr*-null mice, suggesting that the inhibition of FXR activation is the basis for the cholesterol-lowering activity of guggulsterone (Urizar et al. 2002). In this study, the mRNA levels of ileal *Shp* and *Fgf15*, but not hepatic *shp*, were significantly reduced in the taurine-treated wild-type mice fed the cholesterol diet, suggesting that taurine attenuates ileal, not hepatic, FXR signaling. Theabrownin, an abundant pigment in Pu-erh tea, attenuates hypercholesterolemia due to the reduction of intestinal FXR–FGF15 signaling (Huang et al. 2019). Similar to theabrownin, taurine attenuated intestinal FXR–FGF15 signaling in the wild-type mice fed the cholesterol diet. These results support the idea that the taurine-mediated attenuation of FXR–FGF15 signaling is involved in the decrease in hepatic and serum cholesterol levels in wild-type mice.

Cyp7a1 mRNA levels were significantly increased in the wild-type mice of the taurine group fed the cholesterol diet, but not in the *Fxr*-null mice. These results raise the possibility that enhanced bile acid synthesis due to the significant elevation of *Cyp7a1* expression is involved in the cholesterol-lowering effect of taurine in mice fed the cholesterol diet. However, these mice did not show increases in the levels of enterohepatic bile acid. Further study on bile acid metabolism is needed to elucidate the mechanism behind the taurine-mediated cholesterol lowering. In vitro experiments demonstrated that taurine could enhance CYP7A1 expression by inducing HNF4α and p-c-Jun expression in HepG2 cells (Guo et al. 2017) and activating LXRα, a positive regulator of CYP7A1 transcription, in H4IIE and HepG2 cells (Hoang et al. 2012). However, taurine did not significantly increase *Cyp7a1* mRNA levels in the *Fxr*-null mice. Nevertheless, taurine significantly decreased ileal *Fgf15* mRNA levels in the wild-type mice fed the cholesterol diet, but not in the *Fx*r-null mice. These results suggest that taurine significantly increases *Cyp7a1* mRNA levels due to the reduction of ileal FXR/FGF15 signaling rather than the alteration of other nuclear receptor and transcriptional signaling in mice fed the cholesterol diet.

Increased levels of ileal FXR antagonistic bile acids or decreased levels of agonistic bile acids attenuate ileal FXR signaling, resulting in the reduction of Fgf15 and Shp expression. In mice, TCA and TMCAs are the predominant bile acids in the liver and intestine. TCA is a weak FXR agonist (EC_{50} = ~590 μM), whereas TαMCA (IC_{50} = 28 μM) and TβMCA ((IC_{50} = 40 μM) are potent FXR antagonists (Sayin et al. 2013). Significant increases in TβMCA levels in the intestinal lumen are probably partly involved in attenuating ileal FXR signaling in the taurine-treated wild-type mice fed the cholesterol diet.

Theabrownin attenuates intestinal FXR signaling by altering intestinal bile acid composition due to a dominant decrease in the number of microbes associated with bile salt hydrolase activity (Huang et al. 2019). It remains unclear whether taurine alters intestinal bile acid composition due to modification of the intestinal microbiota. Yu et al. demonstrated that taurine could regulate the gut microecology, which might benefit health (Yu et al. 2016). Taurine may alter intestinal bile acid composition by modifying intestinal microbiota. Taurine may also have altered the ileal bile acid composition in an FXR-dependent manner in wild-type mice fed the cholesterol diet based on the finding that there was no significant alteration in the ileal bile acid composition of *Fxr*-null mice. There are two bile acid synthesis pathways: classical and alternative (Chiang and Ferrell 2019). The classical pathway produces CA and CDCA, whereas the alternative pathway produces only CDCA. In rodents, unlike in humans, CDCA is further converted into αMCA, βMCA, and MDCA. Taurine might alter the balance between the classical and alternative pathways, elevating TβMCA and TMDCA. Although the mechanism underlying the taurine-mediated alteration of bile acid composition remains unclear, it appears to require the presence of FXR.

Previous studies showed that taurine inhibits bile acid absorption from the ileum in rats fed a high-cholesterol diet (Nishimura et al. 2009) and upregulates the expression of low-density-lipoprotein receptor in hamsters (Murakami et al. 2002). Thus, we cannot rule out the possibility that taurine-mediated FXR-independent signaling regulates intestinal bile acid and hepatic cholesterol transport in addition to hepatic bile acid synthesis.

This study suggests that taurine decreases cholesterol levels by attenuating ileal FXR signaling due to the alteration of bile acid composition. The relationship among taurine, bile acids, and FXR signaling remains poorly understood. It is necessary to clarify the mechanism by which taurine alters bile acid composition in wild-type mice fed a cholesterol diet. Taurine could affect the alternative pathway of bile acid synthesis in synthesizing MCAs or affecting gut microbiota involved in bile acid metabolism. Furthermore, it is crucial to identify why taurine's action is not observed in *Fxr*-null mice.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human participants and/or animals This article does not describe any studies with human participants. All experiments were conducted in accordance with the guidelines for animal experiments of the National Fisheries University (Shimonoseki, Japan). The protocol was approved by the Institutional Animal Care and Use Committee at the National Fisheries University (Permission No. 2019-18-3).

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