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Taurine reduces cholesterol through CYP7A1 in a calcineurin-dependent manner

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Taurine (2-aminoethanesulfonic acid) could reduce serum and liver cholesterol concentrations via regulating expression and the activity of cholesterol 7α-hydroxylase (CYP7A1). To investigate the possible role of calcineurin in taurine upregulating CYP7A1 expression in HepG2 cells under high cholesterol conditions. High cellular cholesterol conditions were achieved using 0.2 mM cholesterol in HepG2 cells. Calcineurin was decreased by FK506 and was depleted in CnAβ-/- cells. HepG2 cells were cultured in a taurine-containing medium for 24 or 48 h. The levels of total intracellular cholesterol were determined by an enzymatic method, and the expression levels of calcineurin, CYP7A1, and key transcriptional regulatory molecules were detected by western blotting. High-cholesterol resulted in increased CYP7A1 and calcineurin expressions. Taurine exhibited cholesterol-lowering effects regardless of low/high cholesterol conditions or calcineurin status. Taurine induced the expression of CYP7A1, which was abolished by inhibiting or deleting calcineurin. Taurine suppressed MEK1/2, p-c-Jun, and SHP-1, key molecules in one inhibitory pathway of CYP7A1 transcription. In contrast, suppression of MEK1/2 but not p-c-Jun or SHP-1 was reversed after completely knocking down calcineurin. Calcineurin is required for taurine's upregulation of CYP7A1 expression through inhibiting MEK1/2, which was partly responsible for taurine's cholesterol-lowering effect.

Keywords: Calcineurin, Cholesterol, CYP7A1, MEK1/2, Taurine

Hypercholesterolemia is a disorder featuring elevated serum total cholesterol, low-density lipoprotein (LDL) cholesterol, non-high-density lipoprotein (HDL) cholesterol and apolipoprotein B (apo B)[1,2]. In addition, elevated non-HDL and LDL cholesterol amounts elevate the risk of cardiovascular disease and mortality[3,4]. The causes of hypercholesterolemia may include primary genetic disorders or may be secondary to obesity, diabetes, metabolic syndrome, chronic kidney disease, nephrotic syndrome, hypothyroidism, cholestatic liver disease, and selected medications such as anabolic steroids[1]. Taurine (2-aminoethanesulfonic acid) is broadly synthesised by mammals. Taurine has multiple beneficial functions in normal physiology, with antioxidative, detoxifying, osmoregulatory, cell membrane stabilising and hypolipidemic properties[5,6]. Clinical studies have shown that the levels of plasma taurine are lower in subjects with obesity and diabetes[5-8]. In contrast, taurine supplementation increased plasma taurine and adiponectin levels, and significantly decreased body weight, triglyceride (TG), total cholesterol (TC) and atherosclerotic index (AI) in obese patients[5,9]. In addition to clinical studies, numerous animal experiments with rats, mice, hamsters and rabbits also have confirmed that taurine supplementation has effective activity to reduce cholesterol[10]. Precisely, taurine lowers cholesterol amounts by increasing its liver biotransformation into bile acids, which are excreted in faeces[10,11]. Taurine elevates the mRNA and protein amounts of and activates cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the main signalling of cholesterol transformation into bile acids[10,12,13]. Still, how taurine controls CYP7A1 expression remains unclear.

Hepatocyte nuclear factor 4α (HNF4α) and liver receptor homolog-1 (LRH-1) represent critical players in CYP7A1 expression[14]. Additionally, two pathways downregulate CYP7A1 transcriptionally, including the farnesoid X receptor (FXR)-dependent pathway interacting with HNF4α and LRH-1 through FXR-induced small heterodimer partner-1 (SHP-1) and the FXR-independent pathway involving multiple players, including mitogen-activated protein kinase/c-Jun N-terminal kinase (MAPK/JNK) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK, MEK) signaling[15-18]. Taurine upregulates CYP7A1 by triggering HNF4α and decreasing MEK1/2 and phosphorylated-c-Jun (p-c-Jun) expression levels, promoting cholesterol

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transformation in HepG2 cells 19. Calcineurin represents an important calcium- and calmodulin-dependent serine/threonine protein phosphatase comprising calcineurin A (catalytic subunit, CnA) and B (regulatory subunit, CnB) and calmodulin (interacting with calcium)[20-22]. Somatic cells express two CnA (CnAα and β) and one CnB (CnB1) isoforms[20-22]. Calcineurin dephosphorylates specific substrates and binds to multiple protein phosphokinases to regulate the phosphorylation of associated effectors, including c-Jun, JNK, and MEK[22,23].

The nuclear factor of activated T cell cytoplasmic (NFATc) undergoes dephosphorylation by calcineurin followed by nuclear translocation, which is important in immune activation[24,25]. Because of these immunomodulatory properties, calcineurin is targeted by an important group of immunosuppressants termed calcineurin inhibitors (CNIs), including cyclosporine A (CsA) and tacrolimus (FK506). Unfortunately, hypercholesterolemia is frequently encountered in patients treated with CsA and FK506, with a frequency reaching 40-50% or even as high as 60-70% in renal transplantation recipients[26-28]. Regulator of calcineurin 2 (RCAN2) inhibits calcineurin dependent transcriptional responses, and a clinical study has shown an increase in serum RCAN2 in overweight/obese subjects[29]. Taurine induces the metabolic pathways converting cholesterol into bile acids[10,12], which in turn activate calcineurin[30,31]. Taurine also downregulates MCIP1, a calcineurin suppressor, in C2C12 fibroblast myotubes and mouse fibroblasts[32]. In *C. elegans*, taurine positively affects calcineurin expression[33]. Based on the above evidence, the current work aimed to assess the possible function of calcineurin in taurine-related upregulation of CYP7A1 in HepG2 cells under high cholesterol conditions. The results could help understand the cholesterol-lowering effects of taurine.

Materials and Methods

Reagents

Taurine, FK506 and other major reagents for assessing cell cholesterol were from Sigma (USA). Cell culture constituents were provided by Hyclone (USA) apart from fetal bovine serum (FBS; Invitrogen, USA). The PowerOpti-ECL kit was provided by GenView (USA). Anti-MEK1/2 (#8727), anti-calcineurin (#2614), anti-c-Jun and anti-p-c-Jun (#9260) and anti-GAPDH (#2118) primary antibodies, as well as anti-rabbit IgG (#7074s) were provided by Cell Signaling Technology (USA). In addition, primary antibodies targeting CYP7A1 (sc-25536; Santa Cruz Biotechnology, USA), β-actin (GenView and SHP-1 (ab32559; Abcam, United Kingdom) were applied. Cell Lysis Buffer, PMSF and BCA Protein Assay Kit were provided by Beyotime (China). The remaining reagents were provided by Dingguo Changsheng Biotechnology (China).

Cells and treatments

HepG2 cells were provided by the Cell Resource Center of the School of Basic Medicine of Peking Union Medical College, Beijing, China. CnAβ-knockout HepG2 cells were provided by SyngenTech (China). DMEM containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin was sutilised for routine cell culture. Cells seeded in triplicate 6-well plates underwent incubation with 0 (control group) or 10 mM taurine in PBS for 24 and 48h, respectively.

Cell cholesterol detection

Intracellular cholesterol levels were determined as outlined in a previous report[19]. Briefly, after a 30 min treatment with 1 mL of hexane/isopropanol (2:1 v:v) at ambient, the cells underwent drying and resuspension in 130 μL of isopropanol containing 10% TritonX-100. Total cell cholesterol (TC) and free cholesterol (FC) were assessed by incubation of 50 μL of lipid-extracted specimens with TC working solutions or FC working solutions (Table 1) for 30 min at 37°C. Absorbance was determined at 500 nm on a μQuant microtiter plate reader (Bio-Tek, USA). Cell ester cholesterol (EC) was derived as TC minus FC.

Immunoblot

HepG2 cell lysis on ice was carried out with the lysis buffer. The lysates underwent a 10min centrifugation (12,000 rpm) at 4°C. The resulting

Table 1 — Composition of the working solutions for cholesterol detection

	TC (μL)	FC (μL)
Dipotassium hydrogen phosphate (0.1 M)	192	198
Cholesterol oxidase (5 U/mL)	6	6
Horse radish peroxidase (50 U/mL)	6	6
Sodium taurocholate hydrate (20 mM)	15	15
TritonX-100 (1%)	15	15
4-aminoantipyrine (5.5 mM)	45	45
Phenol (280 mM)	15	15
Cholesterol esterase (25 U/mL)	6	0
Total volume (μL)	300	300

supernatants were assessed for protein amounts with BCA Protein Assay Kit (BCA02; Dingguo Changsheng Biotech, China). Identical quantities of total protein underwent separation by SDS-PAGE, with subsequent electro-transfer onto PVDF membranes (Millipore, USA). Samples underwent incubation with primary antibodies in TBS with 0.05% Tween (TBS-T) and 5% skimmed milk overnight at 4°C. This was followed by a 1h incubation with secondary antibodies. A chemiluminescence (ECL) kit was sutilised for visualisation, and quantitation used a Gel imaging system (GE Healthcare, USA).

Statistical analysis

Data were represented by mean±standard deviation (SD). One-way ANOVA with post hoc LSD test was performed for comparisons, and $P<0.05$ indicated statistical significance.

Results

Calcineurin is involved in the occurrence of high liver cholesterol

To clarify the hepatic molecular mechanism of cholesterol-lowering effect by taurine, the experimental design is shown in Fig. 1A.We build a highcholesterol model by treating HepG2 cells with 0.02 mM cholesterol. As depicted in Fig. 1B, intracellular cholesterol amounts in HepG2 cells were starkly elevated after administration of 0.02 mM cholesterol for 24 or 48h in comparison with control cells. Calcineurin expression was increased in HepG2 cells under high-cholesterol conditions for 24 and 48h, respectively (Fig. 1C). Next, HepG2 cells were treated with the calcineurin inhibitor FK506 to detect the role of calcineurin on cholesterol accumulation. The results showed that after calcineurin inhibition, cholesterol amounts were increased at 24h (Fig. 1D) and 48h (Fig. 1E). In CnAβ-knockout HepG2 cells, elevated intracellular cholesterol amounts were detected in comparison with wild-type control cells (Fig. 1F). The above findings suggested calcineurin participated in the occurrence of high cholesterol levels in the liver.

Calcineurin contributes to taurine's cholesterol-lowering property

In order to assess whether calcineurin is important in decreasing cell cholesterol by taurine, its cholesterol-lowering activity was confirmed in the HepG2 cell line. Corroborating mouse and rat data[10,12,19], taurine reduced cell cholesterol amounts at 10 mM following 24h of treatment in both normal and high-cholesterol HepG2 cells (Fig. 2A). Upon calcineurin suppression using FK506, the cholesterol-lowering activity of taurine was still observed but control cells tended to show reduced amounts (12.5% vs. 16.6%, Fig. 2B). After complete CnAβ deletion, taurine still had cholesterol-lowering effects, but the reduction in CnAβ$^{-/-}$ cells was less pronounced in comparison with control cells administered taurine (13.2% vs. 25.3%), resulting in higher cholesterol levels in the CnAβ$^{-/-}$+Tau group compared with the Tau group (Fig. 2C). Hence, calcineurin knockout resulted in suppressed inhibitory effect of taurine on cholesterol. Moreover, after taurine treatment, TC level decrease in wild-type cells mostly resulted from FC reduction, while in CnAβ$^{-/-}$ cells, it was caused by

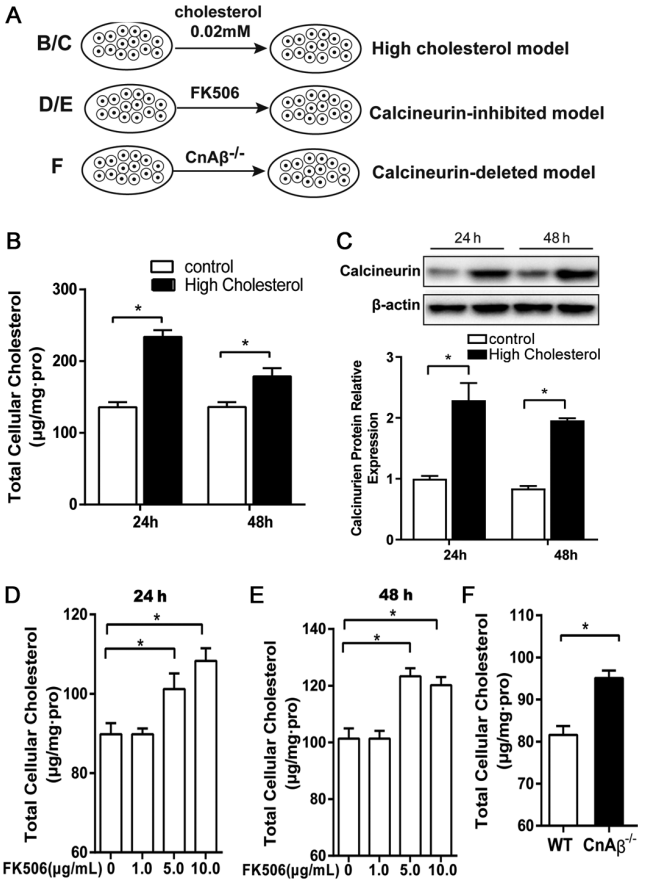

Fig. 1 — Effect of calcineurin on total intracellular cholesterol levels. HepG2 cells were cultured under normal or high (0.02 mM) cholesterol conditions, and with FK506 at 0, 1.0, 5.0, or 10.0 μg/mL. CnAβ$^{-/-}$ cells were also examined. Cellular cholesterol amounts (B) were starkly increased while calcineurin was upregulated (C) by high cholesterol in HepG2 cells. Cellular cholesterol amounts were increased after inhibiting calcineurin activity by FK506 for 24 (D) or 48h incubation (E) or deleting CnAβ (F) in HepG2 cells. Data are mean ± standard deviation, and were compared by ANOVA with post hoc LSD test. *$P<0.05$.

CE level reduction (Fig. 2D). These findings suggested calcineurin was involved, at least in part, in taurine's cholesterol-lowering activity.

Calcineurin is important in CYP7A1 upregulation by taurine

Next, the function of calcineurin in taurine cholesterol-lowering effect via CYP7A1 was examined. Under normal circumstances, taurine could upregulate calcineurin and CYP7A1, while under high cholesterol levels, taurine upregulated CYP7A1 but not calcineurin (Fig. 3A-B). When calcineurin was inhibited or knocked out, taurine could not promote the expression of CYP7A1, suggesting a role for calcineurin in CYP7A1 upregulation (Fig. 3B). Taurine could not control CYP7A1 expression in the presence of FK506 (Fig. 3C) or after CnAβ suppression (Fig. 3D). The above findings suggested calcineurin might be an important factor in CYP7A1 upregulation associated with taurine.

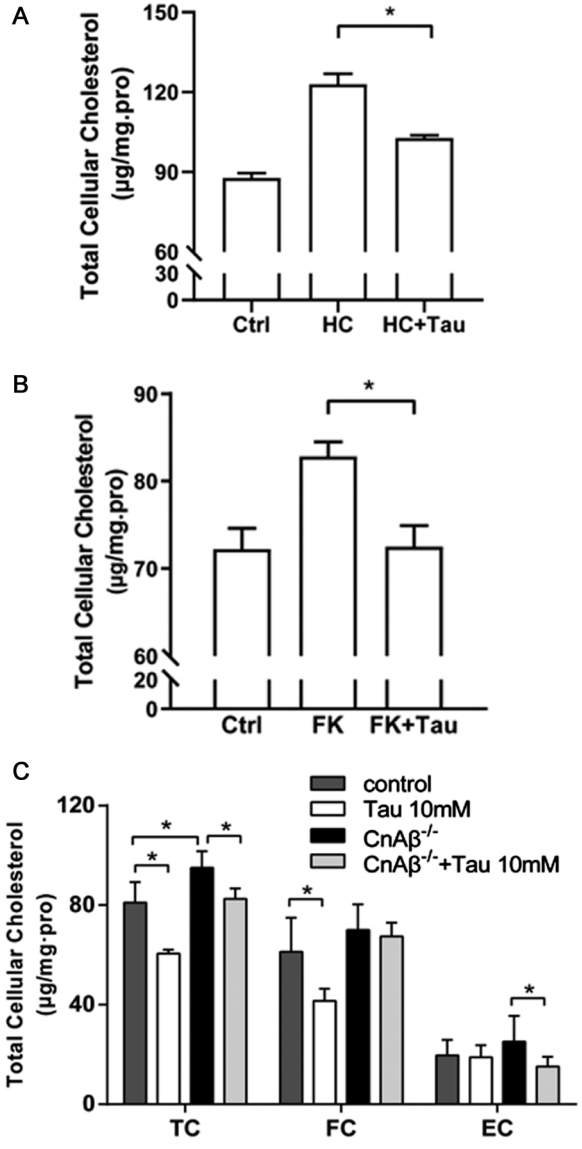

Fig. 2 — Role of calcineurin on the cellular cholesterol-lowering effect by taurine in HepG2 cells. HepG2 cells were cultured under normal or high (0.02 mM) cholesterol conditions in presence of FK506 at 10.0 μg/mL. CnAβ$^{-/-}$ cells were also analysed. Cells were treated or not with taurine at 10 mM. Comparative analysis of cellular cholesterol levels after treatment with taurine are shown in HepG2 cells after administration of cholesterol (A), inhibiting CnA activity by FK506 (B) and deleting CnAβ (C). Data are mean ± standard deviation, and were compared by ANOVA with post hoc LSD test. *P<0.05.

Calcineurin has an important function in MEK1/2 downregulation by taurine

In order to verify the above data, multiple major effectors of the FXR-independent pathway of CYP7A1 downregulation were assessed. The latter pathway involves multiple factors, including MAPK/JNK, MAPK/ERK and MEK signal transduction[15,16]. CnAβ knockout in HepG2 cells resulted in increased MEK1/2 and p-c-Jun protein amounts, while c-Jun and SHP-1

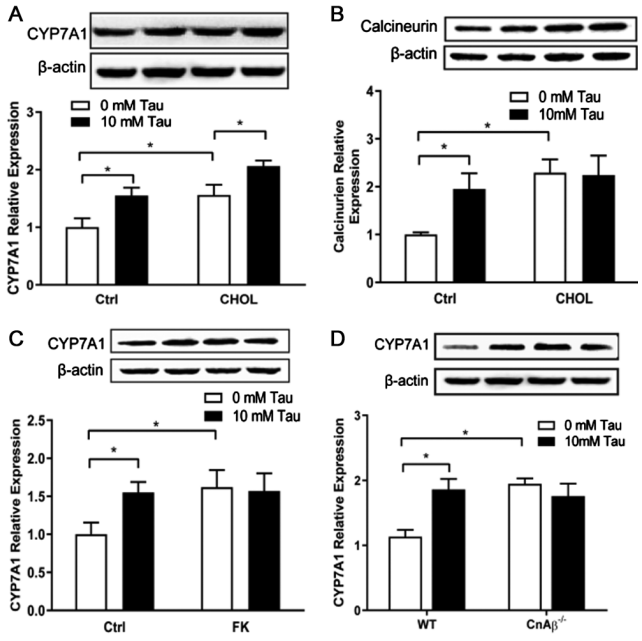

Fig. 3 — Effect of calcineurin on CYP7A1 expression induced by taurine. HepG2 cells were cultured under normal (Ctrl) or high (0.02 mM) cholesterol conditions (CHOL) in presence of FK506 at 10.0 μg/mL(FK). CnAβ$^{-/-}$ cells were also analysed. Cells were treated or not with taurine at 10 mM. Taurine upregulated CYP7A1 under normal cholesterol and high-cholesterol conditions (A). Taurine increased the protein amounts of calcineurin in cells under normal cholesterol but not under high-cholesterol conditions (B). Taurine did not increase the protein amounts of CYP7A1 in the presence of FK506 (C) or in CnAβ$^{-/-}$ cells (D). Data are mean ± standard deviation, and were compared by ANOVA with post hoc LSD test. *P<0.05.

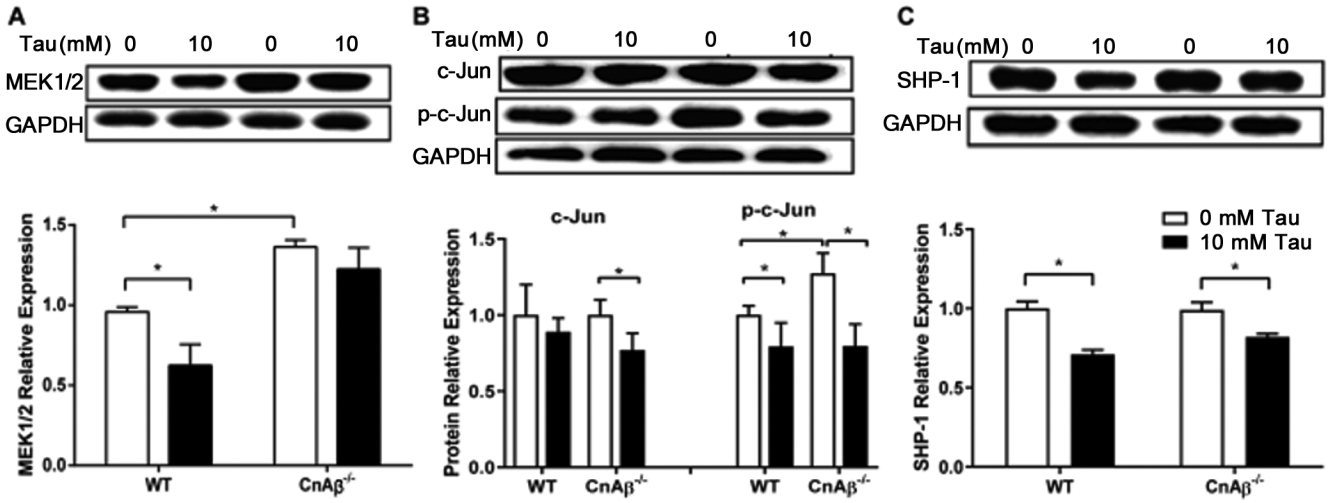

Fig. 4 — Effects of calcineurin on key signalling molecules regulated by taurine in CYP7A1 expression. Wild-type and CnAβ$^{-/-}$ HepG2 cells were cultured without or with taurine at 10 mM. Effects of taurine on MEK1/2 (A), c-Jun/p-c-Jun (B) and SHR-1 (C) protein amounts in WT and CnAβ$^{-/-}$ HepG2 cells. Data are mean± standard deviation, and were compared by ANOVA with post hoc LSD test. *P<0.05.

amounts were unaltered, in comparison with wild-type cells (Fig. 4). Taurine markedly downregulated MEK1/2, p-c-Jun and SHP-1 in HepG2 cells, and MEK1/2 repression was rescued upon calcineurin suppression (Fig. 4A). In contrast, p-c-Jun and SHP-1 downregulation was unaffected by calcineurin suppression (Figure 4B-C). The above findings suggested calcineurin was important in the suppressive activity on MEK1/2 expression associated with taurine.

Discussion

The present work showed calcineurin affected MEK1/2 and p-c-Jun pathways, which suppress CYP7A1 transcription, demonstrating calcineurin is involved in cell cholesterol homeostasis via CYP7A1 modulation. In addition, taurine decreased intracellular cholesterol amounts by upregulating CYP7A1, but the cholesterol decrease and CYP7A1 upregulation disappeared with calcineurin suppression or deficiency in comparison with controls, suggesting calcineurin requirement for taurine's cholesterol-lowering effect mediated by CYP7A1 upregulation. Furthermore, calcineurin was found to represent a key factor for taurine to upregulate CYP7A1 via MEK1/2 but not p-c-Jun or SHP-1. This study revealed high-cholesterol conditions upregulated CYP7A1 and calcineurin, and calcineurin inhibition or suppression resulted in elevated cell cholesterol amounts and CYP7A1 expression. Calcineurin suppressors, including CsA and FK506, are clinically applied but may induce hypercholesterolemia[28,34] and serum RCAN2, an endogenous calcineurin inhibitor, has been found an increase in overweight/obese subjects[29]. In addition, mice lacking calcineurin Aβ show hyperlipidemia, with elevated cholesterol, triglycerides and free fatty acids[35]. The present study corroborates these reports. CYP7A1 plays a critical regulatory role in cholesterol homeostasis, serving as a rate limiting enzyme for converting cholesterol into bile acids and a potential target for improving hypercholesterolemia. *In vitro* data demonstrated CYP7A1 was starkly upregulated in CnAβ$^{-/-}$ and FK506-treated cells, indicating cholesterol elevation resulting from calcineurin deficiency might also upregulate CYP7A1 to compensate for such cholesterol stress. Still, a rat study suggested CsA treatment for three weeks reduced liver CYP7A1 expression, with stark downregulation of skeletal muscle and fat tissue lipoprotein lipase[36]. These intriguing findings may explain the different compensatory mechanisms observed between systemic regulation in the whole animal and direct liver cell killing occurring in cultured cells. Furthermore, calcineurin deficiency was accompanied by upregulated MEK1/2 and p-c-Jun but not SHP-1, which are major factors regulating the FXR-independent inhibitory pathway of CYP7A1 transcription. Calcinuerin is a Ca^{2+} dependent dephosphorylase that dephosphorylates substrates and interacts with numerous protein phosphokinases to control the phosphorylation status of related signaling molecules[22,23]. It is suggest calcineurin may dephosphate p-c-Jun and depress MEK1/2, releasing the inhibitory pathway of CYP7A1 transcription.

Jointly, the latter findings indicate calcineurin is critical for cell cholesterol homeostasis, regulating CYP7A1.

Many feeding studies in guinea pig, rat, mouse and hamster models revealed taurine has cholesterol-lowering effects in hypercholesterolemia associated with cholesterol-rich diets, due to its promotion of CYP7A1 expression[37] and through CYP7A1 pathway, taurine accelerates the biological conversion of cholesterol to bile acids and exerts its cholesterol-lowering effect[38]. This work further revealed taurine reduced intracellular cholesterol levels, although the degree of reduction was less pronounced in HepG2 cells with calcineurin suppression or deficiency compared with untreated or wild-type cells. Taurine induced CYP7A1 expression, but inhibition or deletion of calcineurin abolished this induction. These results suggest that calcineurin is necessary for taurine-associated CYP7A1 upregulation, but CYP7A1 is partly responsible for taurine-related decrease of hepatocellular cholesterol. This study also found that taurine directly increased the expression of calcineurin, but did not induce further elevations under high-cholesterol conditions. Such failure is possible because calcineurin amounts were already very high. Ko *et al*.[33] reported taurine upregulates calcineurins in *C. elegans*. Miyazaki *et al*.[32] revealed the calcineurin suppressor MCIP1 was specifically downregulated transcriptionally by taurine. There is evidence that taurine increases cell Ca^{2+} amounts and reduces phosphorylation in associated signaling molecules[39]. The above data indicate calcineurin is indispensable for taurine's cholesterol-lowering process through upregulating CYP7A1.

CYP7A1 is regulated by many signalling pathways, including one enhancing and two feedback-repressing pathways[16,40]. A previous study by our group demonstrated taurine promotes cholesterol biotransformation in HepG2 cells by upregulating CYP7A1 via HNF4α induction in the enhancing pathway and downregulating MEK1/2 and phosphorylated-c-Jun in the feedback repressing pathways[19]. It is shown that taurine promotes the expression of CYP7A1by inhibiting the MEK1/2 pathway associated with c-Jun dephosphorylation. This study showed that taurine downregulated MEK1/2 and SHP-1 in wild type HepG2 cells, and only downregulation of MEK1/2 but not SHP-1 was blunted by complete knockdown of calcineurin. While taurine had no effect on c-Jun expression in wild type HepG2 cells, but showed a significant reduction after complete knockdown of calcineurin. As mentioned before, knockdown calcineurin induced an increase in p-c-Jun, but the dephosphorylation of p-c-Jun by taurine still does not weaken compared with wild type HepG2. It is suggested that taurine not only activates calcineurin, but may also activates other dephosphatases to dephosphorize c-Jun. Jointly, these data indicate calcineurin is required for taurine's CYP7A1 upregulation by suppressing MEK1/2. This study had limitations. Among all genes and proteins involved in cholesterol metabolism and homeostasis, only five proteins (CYP7A1, calcineurin, MEK1/2, c-Jun and SHP-1) were examined. In addition, their mRNA expression levels were not quantitated. Finally, the experiments were only performed *in vitro*, which cannot recapitulate the fine regulation found in actual living organisms. Therefore, future studies should examine the multiple pathways involved in cholesterol homeostasis, in addition to animal studies.

Conclusion

Overall, this work reveals calcineurin plays an important role in cell cholesterol homeostasis through CYP7A1 regulation. In addition, calcineurin was shown to be indispensable in the process of taurine-related upregulation of CYP7A1 by inhibiting MEK1/2, which was partly responsible for taurine's cholesterol-lowering effects. Thus, the results may help understand the cholesterol-lowering features of taurine.

Author contribution

JXG and OT carried out most of the experiments, interpreted the data, and drafted the manuscript. YG and YXZh participated in the detection of protein expression. JZh and YZHZh participated in the interpretation of the data and statistical analysis. WCh participated in experimental design and coordination, corrected the manuscript, and supervised the study. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no known conflict of interests.

References

1 Mach F, Baigent C & Catapano AL, 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J*, 41 (2020) 111.

2 Grundy SM, Stone NJ, Guideline Writing Committee for the Cholesterol G, 2018 Cholesterol Clinical Practice Guidelines: Synopsis of the 2018 American Heart Association/American College of Cardiology/Multisociety Cholesterol Guideline. *Ann Intern Med*, 170 (2019) 779.

3 Johannesen CDL, Langsted A, Mortensen MB & Nordestgaard BG, Association between low density lipoprotein and all cause and cause specific mortality in Denmark: prospective cohort study. *BMJ*, 371 (2020) m4266.

4 Mortensen MB, Nordestgaard BG, Elevated LDL cholesterol and increased risk of myocardial infarction and atherosclerotic cardiovascular disease in individuals aged 70-100 years: a contemporary primary prevention cohort. *Lancet*, 396 (2020) 1644.

5 Lambert IH, Kristensen DM, Holm JB, Mortensen OH, Physiological role of taurine--from organism to organelle. *Acta Physiol (Oxf)*, 213 (2015) 191.

6 Schaffer SW, Jong CJ, Ramila KC, Azuma J, Physiological roles of taurine in heart and muscle. *J Biomed Sci*, 17 Suppl 1 (2010) S2.

7 Calvani R, Rodriguez-Mañas L, Picca A, *et al*., Identification of a Circulating Amino Acid Signature in Frail Older Persons with Type 2 Diabetes Mellitus: Results from the Metabofrail Study. *Nutrients*, 12 (2020)199.

8 Santulli G, Kansakar U, Varzideh F, Mone P, Jankauskas SS, Lombardi A, Functional Role of Taurine in Aging and Cardiovascular Health: An Updated Overview. *Nutrients*, 15 (2023) 4236.

9 Guan L, Miao P, The effects of taurine supplementation on obesity, blood pressure and lipid profile: A meta-analysis of randomized controlled trials. *Eur J Pharmacol*, 885 (2020) 173533.

10 Murakami S, Fujita M, Nakamura M, *et al*., Taurine ameliorates cholesterol metabolism by stimulating bile acid production in high-cholesterol-fed rats. *Clin Exp Pharmacol Physiol*, 43 (2016) 372.

11 Tagawa R, Kobayashi M, Sakurai M, *et al.*, Long-Term Dietary Taurine Lowers Plasma Levels of Cholesterol and Bile Acids. *Int J Mol Sci*, 23 (2022) 1793.

12 Chang YY, Chou CH, Chiu CH, *et al*., Preventive effects of taurine on development of hepatic steatosis induced by a high-fat/cholesterol dietary habit. *J Agric Food Chem*, 59 (2011) 450.

13 Dong Y, Li X, Liu Y, Gao J, Tao J, The molecular targets of taurine confer anti-hyperlipidemic effects. *Life Sci*, 278 (2021) 119579.

14 Kir S, Zhang Y, Gerard RD, Kliewer SA, Mangelsdorf DJ, Nuclear receptors HNF4alpha and LRH-1 cooperate in regulating Cyp7a1 in vivo. *J Biol Chem*, 287 (2012) 41334.

15 Kong B, Wang L, Chiang JY, Zhang Y, Klaassen CD, Guo GL, Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology*, 56 (2012) 1034.

16 Li C, Yang J, Wang Y, Qi Y, Yang W, Li Y, Farnesoid X Receptor Agonists as Therapeutic Target for Cardiometabolic Diseases. *Front Pharmacol*, 11 (2020) 1247.

17 Katafuchi T, & Makishima, M., Molecular Basis of Bile Acid-FXR-FGF15/19 Signaling Axis. *Int J Mol Sci*, 23 (2022) 6046.

18 Ghosh S, Devereaux, M. W., Orlicky, D. J., & Sokol, R. J., Pharmacologic inhibition of HNF4α prevents parenteral nutrition associated cholestasis in mice. *Scientific reports*, 13 (2023) 7752.

19 Guo J, Gao Y, Cao X, Zhang J, Chen W, Cholesterol-lowing effect of taurine in HepG2 cell. *Lipids Health Dis*, 16 (2017) 56.

20 Connolly S, Quasi-Woode D, Waldron L, *et al*., Calcineurin Regulatory Subunit Calcium-Binding Domains Differentially Contribute to Calcineurin Signaling in Saccharomyces cerevisiae. *Genetics*, 209 (2018) 801.

21 Li SJ, Wang J, Ma L, *et al.*, Cooperative autoinhibition and multi-level activation mechanisms of calcineurin. *Cell Res*, 26 (2016) 336.

22 Creamer TP, Calcineurin. *Cell Commun Signal*, 18 (2020) 137.

23 Liang Q, Bueno OF, Wilkins BJ, Kuan CY, Xia Y, Molkentin JD, c-Jun N-terminal kinases (JNK) antagonize cardiac growth through cross-talk with calcineurin-NFAT signaling. *EMBO J*, 22 (2003) 5079.

24 Murakami N, Riella LV, Funakoshi T, Risk of metabolic complications in kidney transplantation after conversion to mTOR inhibitor: a systematic review and meta-analysis. *Am J Transplant*, 14 (2014) 2317.

25 Danowska M, Straczkowski M, The Ca2+/Calmodulin-dependent Calcineurin/NFAT Signaling Pathway in the Pathogenesis of Insulin Resistance in Skeletal Muscle. *Exp Clin Endocrinol Diabetes*, 131 (2023) 589.

26 Kockx M, Jessup W, Kritharides L, Cyclosporin A and atherosclerosis—cellular pathways in atherogenesis. *Pharmacol Ther*, 128 (2010) 106.

27 Riella LV, Gabardi S, Chandraker A, Dyslipidemia and its therapeutic challenges in renal transplantation. *Am J Transplant*, 12 (2012) 1975.

28 Parlakpinar H, Gunata M, Transplantation and immunosuppression: a review of novel transplant-related immunosuppressant drugs. *Immunopharmacol Immunotoxicol*, 43 (2021) 651.

29 Wang H, Fang X, Ren Q, *et al*., Association Between Circulating Regulator of Calcineurin 2 Concentrations With Overweight and Obesity. *Front Endocrinol (Lausanne)*, 13 (2022) 857841.

30 Muili KA, Wang D, Orabi AI, *et al*., Bile acids induce pancreatic acinar cell injury and pancreatitis by activating calcineurin. *J Biol Chem*, 288 (2013) 570.

31 Liu S, Chen X, He J, *et al.*, Oleanolic acid promotes skeletal muscle fiber type transformation by activating TGR5-mediated CaN signaling pathway. *J Nutr Biochem*, 123 (2024) 109507.

32 Miyazaki T, Honda A, Ikegami T, Matsuzaki Y, The role of taurine on skeletal muscle cell differentiation. *Adv Exp Med Biol*, 776 (2013) 321.

33 Ko YJ, Chang H, Lee DH, Analysis of Taurine's Anti-Down Syndrome Potential in Caenorhabditis elegans. *Adv Exp Med Biol*, 975 Pt 2 (2017) 1113.

34 Engwenyu LR, Anderson AS, A Comprehensive Review of Calcineurin Inhibitors Used for Immunosuppression in Cardiac Transplantation. *Handb Exp Pharmacol*, 272 (2022) 27.

35 Suk HY, Zhou C, Yang TT, *et al*., Ablation of calcineurin Abeta reveals hyperlipidemia and signaling cross-talks with phosphodiesterases. *J Biol Chem*, 288 (2013) 3477.

36 Vaziri ND, Liang K, Azad H, Effect of cyclosporine on HMG-CoA reductase, cholesterol 7alpha-hydroxylase, LDL

receptor, HDL receptor, VLDL receptor, and lipoprotein lipase expressions. *J Pharmacol Exp Ther*, 294 (2000) 778.

37 Chen W, Guo JX, Chang P, The effect of taurine on cholesterol metabolism. *Mol Nutr Food Res*, 56 (2012) 681.

38 Bae M, Ahmed K, Yim JE, Beneficial Effects of Taurine on Metabolic Parameters in Animals and Humans. *J Obes Metab Syndr*, 31 (2022) 134.

39 Wei S, Huang Q, Li J, *et al.*, Taurine attenuates liver injury by downregulating phosphorylated p38 MAPK of Kupffer cells in rats with severe acute pancreatitis. *Inflammation*, 35 (2012) 690.

40 Wang S, Sheng F, Zou L, Xiao J, Li P, Hyperoside attenuates non-alcoholic fatty liver disease in rats via cholesterol metabolism and bile acid metabolism. *J Adv Res*, 34 (2021) 109.