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Dietary taurine stimulates the hepatic biosynthesis of both bile acids and cholesterol in the marine teleost, tiger puffer (*Takifugu rubripes*)

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Abstract

Taurine (TAU) plays important roles in the metabolism of bile acids, cholesterol and lipids. However, little relevant information has been available in fish where TAU has been identified as a conditionally essential nutrient. The present study aimed to investigate the effects of dietary TAU on the metabolism of bile acids, cholesterol and lipids in tiger puffer, which is both an important aquaculture species and a good research model, having a unique lipid storage pattern. An 8-week feeding trial was conducted in a flow-through seawater system. Three experimental diets differed only in TAU level, that is, 1·7, 8·2 and 14·0 mg/kg. TAU supplementation increased the total bile acid content in liver but decreased the content in serum. TAU supplementation also increased the contents of total cholesterol and HDL-cholesterol in both liver and serum. The hepatic bile acid profile mainly includes taurocholic acid (94·48 %), taurochenodeoxycholic acid (4·17 %) and taurodeoxycholic acid (1·35 %), and the contents of all these conjugated bile acids were increased by dietary TAU. The hepatic lipidomics analysis showed that TAU tended to decrease the abundance of individual phospholipids and increase those of some individual TAG and ceramides. The hepatic mRNA expression study showed that TAU stimulated the biosynthesis of both bile acids and cholesterol, possibly via regulation of farnesoid X receptor and HDL metabolism. TAU also stimulated the hepatic expression of lipogenic genes. In conclusion, dietary TAU stimulated the hepatic biosynthesis of both bile acids and cholesterol and tended to regulate lipid metabolism in multiple ways.

Key words: ***Takifugu rubripes*: Taurine: Cholesterol: Bile acids: Lipid metabolism**

As a free amino acid which can conjugate with bile acids, taurine (TAU) has been demonstrated to have a strong hypocholesterolaemic effect in mammals by stimulating the conversion of cholesterol to bile acids in the liver[1]. The hypolipidaemic effect of TAU in terrestrial animals has also been widely reported[2], although lipid-increasing effects of TAU have been observed in some studies[3,4].

In fish, TAU plays an unique role in bile acid conjugation and has been identified as an conditionally essential nutrient[5,6]. In marine teleosts, especially, the concentration of TAU is high. However, precise functions of TAU in the metabolism of cholesterol and lipids in fish remain unclear. The limited studies reported with fish suggested that although the promoting effects on bile acid synthesis were consistently observed[7–9], the effects of TAU on cholesterol and lipid metabolism in fish were probably different from those observed in mammals and seemed more varied with fish species and fish tissue[10–14]. A recent study with juvenile totoaba showed that dietary TAU supplementation increased the concentrations of cholesterol and TAG in the plasma[13]. However, a study with yellow catfish showed that the contents of cholesterol and TAG in the serum decreased with increasing dietary TAU levels[12], while another study with white seabream showed that dietary TAU supplementation reduced the TAG levels in the plasma but did not affect the cholesterol level[14]. Different results have also been observed in studies with other fish species such as Japanese flounder and white grouper[10,11]. In Japanese flounder, neither the total cholesterol (TC) nor the TAG contents were affected by dietary TAU[10]. In white grouper, however, the response of lipid accumulation to dietary TAU differed among different

Abbreviations: CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesoid X receptor; LCAT, lecithin cholesterol acyl transferase; MAPK, mitogen-activated protein kinase; TAU, taurine; TBA, total bile acids; TC, total cholesterol.

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tissues[11]. With increasing dietary TAU levels, the total lipids and their constituent fatty acids in the liver decreased, but the total lipids and the fatty acids of all lipid classes in the eyes increased, while the lipid stores in the muscle were not significantly affected. Considering the large discrepancy among these results, it is worthwhile to investigate the interaction among TAU, cholesterol and lipids in fish.

Regarding the mechanisms involved in TAU actions on bile acids, cholesterol and lipids, the mammal studies have well revealed that TAU stimulated the expression of cholesterol 7α-hydroxylase (CYP7A1)[4,15–18], the rate-limiting enzyme for bile acid synthesis, and farnesoid X receptor (FXR (nuclear receptor subfamily 1, group H, member 4, NR1H4)) activation might be involved in this process[19]. Besides, regulation of lipoproteins and the LDL receptor contributes to the effects of TAU on cholesterol metabolism[3,4,20]. At the level of cellular signalling transduction, recent studies indicated that mitogen-activated protein kinase (MAPK) signalling pathways may play important roles in actions of TAU on the metabolism of bile acids and cholesterol[21,22]. However, in fish, no information has been available regarding the mechanisms involved in TAU actions on bile acids, cholesterol and lipids. Studies with fish are evidently needed to investigate whether teleosts share the same mechanisms with mammals, especially considering that inconsistent apparent TAU effects have been observed between fish and mammals.

The present study was conducted with a marine teleost, tiger puffer. Tiger puffer is not only an important aquaculture species in Asia but also a good model marine fish due to the detailed genome information[23–26]. What is more interesting about tiger puffer is that this fish has a unique lipid storage pattern. They have no intraperitoneal adipose tissue and a very low lipid content in the muscle and thus store lipids predominantly in the liver[27]. Additionally, we are curious about whether this special lipid storage pattern would influence the TAU–lipid interaction. With a feeding trial on tiger puffer, the present study aimed to comprehensively assess the effects of dietary TAU supplementation on the metabolism of bile acids, cholesterol and lipids. The possible mechanisms indicated by mammal studies were also investigated in the present study at the gene transcription level. Results of the present study will not only be beneficial to better understanding the TAU–lipid interaction but also provide useful information about the regulation of lipid metabolism in fish that store lipids in the liver.

Methods

Experimental diets

Three experimental diets differing only in TAU content were used in the present study (Table 1). The control diet used a low-fishmeal formulation, containing a basic TAU level of 1·7 g/kg DM[5]. Crystalline TAU (purity > 99 %; Shanghai Macklin Biochemical Co. Ltd) was supplemented into the control diet, replacing wheat meal, to obtain two treatment diets with a medium (8·2 g/kg, M-TAU) or high-TAU level (14·0 g/kg, H-TAU) (Table 2). The experimental diets were prepared following the routine procedures in our laboratory[28].

Table 1. Formulation and proximate composition of the experimental diets (g/kg DM basis)

Ingredients	Control	M-TAU	H-TAU
Formulation			
Fishmeal	250·0	250·0	250·0
Soya protein concentrate	220·0	220·0	220·0
Maize protein concentrate	180·0	180·0	180·0
Wheat meal	200·1	194·1	188·1
Taurine*	0·0	6·0	12·0
L-Lysine*	10·3	10·3	10·3
L-Methionine*	3·9	3·9	3·9
L-Arginine*	0·7	0·7	0·7
Fish oil	40·0	40·0	40·0
Soyabean oil	35·0	35·0	35·0
Vitamin premix†	10·0	10·0	10·0
Mineral premix‡	5·0	5·0	5·0
L-Ascorbyl-2-polyphosphate	5·0	5·0	5·0
Choline chloride§	10·0	10·0	10·0
Monocalcium phosphate	15·0	15·0	15·0
Soya lecithin	15·0	15·0	15·0
Proximate composition			
DM	974·0	977·8	976·8
Crude protein	514·2	511·3	511·4
Crude lipids	89·2	86·8	89·0
Ash	82·2	82·0	80·0

M-TAU, medium-taurine group; H-TAU, high-taurine group.
* Crystalline L-amino acids were purchased from Shanghai Macklin Biochemical Co. Ltd. Purity > 99 %.
† Vitamin premix (mg/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0·1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin, 1·2 mg; retinyl acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 120 mg; wheat middlings, 8·66 g.
‡ Mineral premix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1 %), 50 mg; $NaSeSO_3 \cdot 5H_2O$ (1 %), 20 mg; $Ca(IO_3)_2 \cdot 6H_2O$ (1 %), 60 mg; zoelite, 3·49 g.
§ Choline chloride, 35 % purity (with corncob as a carrier).

Experimental fish and feeding procedure

Juvenile tiger puffer with an average initial body weight of 20·05 (SD 0·06) g was purchased from Tangshan Haidu Seafood Co. Ltd. The feeding trial was conducted in a flow-through seawater system in a local farm, Huanghai Aquaculture Co. Ltd. Before the start of the feeding trial, experimental fish were reared in polyethylene tanks and fed the control diet for 7 d to acclimate to the experimental conditions. At the beginning of the feeding trial, experimental fish were distributed into nine polyethylene tanks (200 litres) and each diet was randomly assigned to triplicate tanks (thirty fish in each tank). Fish were hand-fed to apparent satiation three times daily (08.00, 14.00 and 20.00 hours). The feeding trial lasted for 8 weeks. During the experiment, the water temperature ranged from 22 to 27 °C; salinity, 24–26 and dissolved O_2, >6 mg/l. The tanks were cleaned by syphoning out residual feed and faeces 2 h after each feeding.

At the end of the feeding trial, after being fasted for 12 h, fish were anaesthetised with eugenol (1:10 000) and then the number and weight of fish in all tanks were recorded. After that, three randomly selected whole fish were collected from each tank for the analysis of proximate composition. Ten more randomly selected fish per tank were dissected to collect the samples of serum, liver and muscle for other assays. Gallbladder samples were not collected. Blood was collected from the caudal vein and allowed to clot first at room temperature for 2 h and then

Table 2. Amino acid composition of experimental diets (g/kg DM)

Amino acid	Control	M-TAU	H-TAU
Valine	23·0	23·3	23·7
Methionine	10·5	10·2	10·9
Isoleucine	21·0	21·3	21·2
Leucine	44·3	44·9	44·0
Threonine	18·9	18·4	18·6
Phenylalanine	24·2	24·8	24·9
Lysine	34·8	34·7	34·6
Histidine	10·5	11·0	11·1
Arginine	29·3	29·4	29·6
Cysteine	5·6	5·2	5·7
Aspartic acid	46·4	45·2	45·3
Tyrosine	18·2	18·0	18·2
Serine	22·9	22·1	22·5
Glutamic acid	99·7	98·3	97·3
Alanine	28·1	27·9	28·3
Glycine	22·2	21·8	21·9
Taurine	1·7	8·2	14·0

M-TAU, medium-taurine group; H-TAU, high-taurine group.

at 4 °C for 6 h. After that, centrifugation (836 ***g***, 10 min, 4 °C) was conducted and the straw-coloured supernatants were collected as serum samples. After dissection, four small pieces of liver tissue (the small tip part) and two pieces of muscle tissue (dorsal muscle, about 3 cm × 1·5 cm) were collected for subsequent potential use. Faeces samples were not successfully collected because the faeces were not shaped, but easily diffused into water. All tissue samples were frozen with liquid N_2 immediately and then stored at −86 °C before use. All sampling protocols, as well as fish rearing practices, were reviewed and approved by the Animal Care and Use Committee of Yellow Sea Fisheries Research Institute.

Analysis of proximate composition, lipid content, amino acids and fatty acids

The proximate composition analysis of experimental diets (triplicate assays for each diet) and whole fish (three individual fish per tank) was performed according to the standard methods of Association of Official Analytical Chemists. Briefly, samples of diets and fish were oven-dried at 105 °C to constant weight for moisture assay. Protein was determined by measuring N (N × 6·25) using the Kjeldahl method, lipids by diethyl ether extraction using the Soxhlet method and ash by combustion at 550°C. The lipid content of fish liver was assayed with the Soxhlet method, but the lipids in muscle were extracted and analysed with the chloroform–methanol method according to Folch *et al.*[29].

Compositions of amino acids and TAU in the diets, as well as the TAU content in the liver and serum, were determined using an automatic amino acid analyser (L-8900; Hitachi High-Technologies Corp.). The fatty acid compositions of fish liver (a pooled sample for each tank with three samples from different fish) were analysed with GC (HP6890; Agilent Technologies Inc.) according to the methods described in our previous studies[30].

Analysis of TAG, cholesterol, NEFA, and total bile acids, and lecithin cholesterol acyl transferase concentration

All these parameters were assayed using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. An ELISA kit was used in the assay of hepatic lecithin cholesterol acyl transferase (LCAT) concentration. Tissue samples were homogenised in normal saline (v/w = 9:1) and centrifuged (694 ***g***, for 20 min), and then the supernatant was collected for following assays. Competition method was used in the ELISA assay. Briefly, antigen in samples interacts with antibodies pre-coated on the well in competition with recognition antigen labelled with horse radish peroxidase. Tetramethylbenzidine was used to react with horse radish peroxidase to generate coloured solution for spectrophotometry. Zebrafish (*Danio rerio*) anti-rabbit polyclonal antibodies were used in the assays. Standard curves were plotted based on optical density of standard assays (ELISAcalc software; logistic model), and experimental samples were assayed at the same time. The inter- and intra-assay CV for the assays were <10 and <13 %, respectively. Pooled serum or liver samples with three samples from different fish of each tank were used for these assays.

Quantitative analysis of hepatic lipidomics and bile acid omics

The quantitative analysis of hepatic lipidomics and bile acid omics was analysed in collaboration with Shanghai Biotree Biotech Co. Ltd. Ultra-high-performance liquid tandem chromatography quadrupole time-of-flight MS was used in the lipidomics analysis according to Tu *et al.*[31]. High-throughput target-based ultra-high-performance liquid tandem chromatography-MS/MS was used in the bile acid omics analysis according to Han *et al.*[32]. A pooled liver sample for each tank with three samples from different fish was used for these analyses (three replicates for each dietary treatment). The detailed procedures of these analyses can be found in the online Supplementary material.

Quantitative real-time PCR analysis

Total RNA in the liver samples (pools of five samples from different fish of each tank) was extracted using RNAiso Plus (TaKaRa) and reverse-transcribed with a PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa) according to the user's manual.

Specific primers for target genes and the reference genes (18SRNA and β-actin) were designed based on the sequences available in the GenBank database (Table 3). The amplification efficiency for all primers, which was estimated by standard curves based on a six-step 4-fold dilution series of target template, was within 95–105 %, and the coefficients of linear regression (R^2) were more than 0·99. SYBR Green Real-time PCR Master Mix (TaKaRa) and a quantitative thermal cycler (Roche LightCycler 96) were used for the real-time quantitative PCR. The detailed programme was similar to those described by Xu *et al.*[33]. The mRNA expression levels were calculated with the quantitative real-time-PCR method: $2^{-\Delta\Delta CT}$[34].

Table 3. Sequences of the primers used in this work

Primer	Sequence (5′–e′)	GenBank reference	Tm (°C)	PL (bp)
cyp7a1-F	CCTACCTGCTACCTTCTGGAGT	XM_003975521.2	57·3	237
cyp7a1-R	TCCTCTTTGGCAACACGAA		57·1	
hmgcr-F	GCTGCTGGCAATCAAGTACAT	XM_003974466.2	58·0	143
hmgcr-R	AAACATACAACTCCTTCCTACAGC		57·0	
abcg5-F	CAGGGTGTTCAGTAGAATCGC	XM_003963888.2	57·3	134
abcg5-R	CATAGATGTCGAAAGGGTTGC		57·4	
abcg8-F	CAAGACAGACTTCCTGGCAAAC	XM_003963887.2	59·1	224
abcg8-R	ACAGCGAATGGAGTGAGAGC		57·5	
fxr-F	GTGAACGACCACAAGTTTACCC	XM_003967283.2	58·7	166
fxr-R	AGACCAACAGATTACACCGGAT		57·8	
lxra-F	GTGACGCACCACTAACAGCA	XM_011609917.1	57·5	191
lxra-R	CTGACAACACCGAGCAAGACT		57·6	
hnf4a-F	GAGCCACGGGCAAACACTA	XM_011619034.1	59·2	199
hnf4a-R	AGGGTCCTACCTTCTTTCTTCAT		57·7	
lrh-1-F	CGCTGACATGCTGCCTAAA	XM_003974281.2	58·0	140
lrh-1-R	TCTCGTCCAAGTCTTCGTCAT		57·0	
apoa1-F	CGATGACGCCGAGTACAAA	AB183289.1	57·7	104
apoa1-R	CGGTTATGGGAGAAACGCTA		58·1	
apoa4-F	TGCTTTCTGGGACTATGTTGC	NM_001078591.1	57·9	124
apoa4-R	GTTGACTTTGTCGGCACTCTC		57·1	
apob100-F	AGGGACATAGTCAAACCAAGGA	XM_011619944.1	58·0	127
apob100-R	AGAACACGAAGGCTGGACAC		57·2	
mttp-F	ATGCTAAGGGTCTGGTTCTGC	XM_011612378.1	58·6	124
mttp-R	ATGTCAGTGCTGCCGATCTT		57·8	
apoe1-F	TATTCAGACCCGCACCTCA	NM_001078592.1	56·8	201
apoe1-R	ATTTCCTCCATCTTGTCCTCC		57·2	
ldlr-F	TCCATTGGGTGGTTTGTTTC	XM_011613084.1	57·9	175
ldlr-R	TTCAGTTTCCGACCTGCTTC		57·7	
scarb1-F	CTCAGTGTAACATGATTAACGGC	XM_003965441.2	57·1	170
scarb1-R	TTTAGGAGCAACAAAGCGGT		57·9	
hdlbp-F	ACGACCTGACGACCTTAGCA	XM_003973786.2	58·0	179
hdlbp-R	CTCCAACCAACTTATTATAGGCAC		57·6	
fas-F	CTTTGCCGCTGTCATTCG	XM_011619859.1	58·1	78
fas-R	TGTCTCAACCCATTTGTAGTCG		57·8	
cpt-1-F	GGGGTTTGTGGTCAAGTTAGG	XM_011607269.1	58·6	186
cpt-1-R	ATAGATCCGTGGCGCTCAT		57·8	
srebf1-F	TTTCAGCATCCCACCTTCC	XM_011603881.1	57·9	158
srebf1-R	GGTGAACCGTGAGGACAACTA		57·3	
pparα1-F	TCAGTAGTTTATGGGTTGGTGG	NM_001097630.1	57·0	119
pparα1-R	GCGTGGACTCCGTAGTGGTA		58·3	
pparα2-F	CCAGAAGAAGAACCGCAACA	NM_001097629.1	58·6	149
pparα2-R	CCTCTTTCTCCACCATCTTGTT		57·7	
pparγ-F	CGCTGTCCCGACATCTGTAT	NM_001097627.1	58·2	146
pparγ-R	GAACTGCTCGCCTTCCATT		57·5	
bsal-F	TTGAAGATGACTGACCCCGA	XM_003978375.2	58·3	162
bsal-R	GATGTCTGCTGCGTTGTGAA		57·5	
mek1-F	CTGGCATGTCGTATGGTTCTG	XM_003967448.2	58·8	162
mek1-R	CTGCTCTATCTGCTGGGTTCTT		58·2	
mek2-F	ATGGAGGAGTGGTCAACAAGG	XM_003973998.2	58·7	160
mek2-R	CCGTAGAAGCCCACGATGTA		58·6	
erk2-F	ACAAACGGATTGAAGTGGAAGA	XM_003975069.2	58·9	197
erk2-R	TGTGAGACGTTAGGACCTGAATC		58·5	
jnk1-F	CTGTCCAAGATGCTGGTGATT	XM_011603371.1	57·4	203
jnk1-R	TCCTCCCATTCGCTCACTT		57·6	
jnk2-F	TCACTGTCCTCAAACGCTACC	XM_003970391.2	57·7	205
jnk2-R	AGACGGATGATGTTCTTGTGG		57·2	
c-jun-Fn	CAAGAACGTCACGGAAGAGC	AJ511781.1	58·1	245
c-jun-Rn	TGATGGCCGGGTTGTAAGT		58·4	
18sRNA-F	ATCAGATACCGTCGTAGTTCC	KT718779.1	55·6	158
18sRNA-R	CCCTTCCGTCAATTCCTT		52·6	
β-actin-F	CCAGAAAGACAGCTACGTTGG	U37499.1	58·6	147
β-actin-R	GCAACTCTCAGCTCGTTGTAG		59·3	

Tm, melting temperature; PL, product length; *cyp7a1*, cholesterol 7α-hydroxylase; F, forward; R, reverse; *hmgcr*, 3-hydroxy-3-methylglutaryl-CoA reductase; *abcg*, ATP-binding cassette subfamily G; *fxr*, farnesoid X receptor (nuclear receptor subfamily 1, group H, member 4, *nr1h4*); *lxra*, liver X receptor alpha (nuclear receptor subfamily 1, group H, member 3, *nr1h3*); *hnf4a*, hepatocyte nuclear factor 4, alpha; *lrh-1*, liver receptor homolog-1 (nuclear receptor subfamily 5, group A, member 2, *nr5a2*); *mttp*, microsomal TAG transfer protein; *ldlr*, LDL receptor; *scarb1*, scavenger receptor class B, member 1; *hdlbp*, HDL-binding protein; *fas*, fatty acid synthase; *cpt-1*, carnitine palmitoyltransferase-1; *srebf1*, sterol regulatory element-binding factor 1; *bsal*, bile salt-activated lipase-like; *mek1*, mitogen-activated protein kinase kinase 1 (*map2k1*); *mek2*, mitogen-activated protein kinase kinase 2 (*map2k2*); *erk2*, mitogen-activated protein kinase 1 (*mapk1*); *jnk1*, mitogen-activated protein kinase 8 (*mapk8*); *jnk2*, mitogen-activated protein kinase 9 (*mapk9*); *c-jun*, c-Jun protein.

Statistical methods

All percentage data were arcsine-transformed before analysis. All data were subjected to one-way ANOVA in SPSS 16.0 for Windows. Tukey's multiple range test was used to detect the significant differences between the means. The significance level was $P < 0{\cdot}05$. The results are presented as mean values of triplicate tanks with their pooled standard errors.

Results

Lipids, taurine and fatty acid compositions in fish

No significant ($P > 0{\cdot}05$) difference was observed either in the lipid content of liver and muscle or in the proximate composition of whole fish (Table 4). TAU accumulation in the liver and serum significantly ($P < 0{\cdot}05$) increased with increasing dietary TAU levels. No significant ($P > 0{\cdot}05$) difference was observed in the liver fatty acid composition among dietary groups (online Supplementary Table S1).

In addition, fish eat and grew normally during the feeding trial. The average final body weight and average feed efficiency ratio were 55·9 g and 0·7, respectively. Group H-TAU showed the best growth performance, and the control group showed the worst.

TAG, cholesterol and total bile acid contents in the liver and serum

For the liver, the TC content in group H-TAU was significantly ($P < 0{\cdot}05$) higher compared with groups Control and M-TAU (Table 5). The HDL-cholesterol content in group H-TAU was significantly ($P < 0{\cdot}05$) higher than that in the control group, and group M-TAU showed an intermediate value. The hepatic total bile acid (TBA) content showed a similar trend to HDL-cholesterol in response to dietary TAU.

The TC and HDL-cholesterol contents in the serum showed similar trends to their counterparts in the liver in response to dietary TAU, that is, significantly ($P < 0{\cdot}05$) higher in group H-TAU compared with the control group. However, the TBA content in serum showed an opposite trend to liver TBA content in response to dietary TAU. The TBA content in the serum significantly ($P < 0{\cdot}05$) decreased with increasing dietary TAU levels.

Table 4. Proximate composition of whole fish, lipid contents in fish tissues and taurine accumulation in fish tissues (Mean values with their standard errors, *n* 3)

Parameter	Control	M-TAU	H-TAU	Pooled SEM	*P*
Proximate composition of whole fish					
Crude protein (% wet matter)	13·97	15·12	14·71	0·27	0·231
Crude lipids (% wet matter)	4·03	3·99	4·20	0·25	0·343
Ash (% wet matter)	2·48	2·68	2·44	0·06	0·222
Moisture (% wet matter)	78·75	76·38	77·71	0·65	0·376
Lipid content in fish tissues					
In liver (% wet matter)	40·33	45·60	43·45	3·95	0·888
In muscle (% wet matter)	0·36	0·35	0·39	0·03	0·543
Taurine concentrations in fish tissues					
Liver (mg/g DM)	1·04^{b}	1·41^{b}	1·96^{a}	0·15	0·006
Serum (μg/ml)	100^{b}	158^{a,b}	206^{a}	16·9	0·005

M-TAU, medium-taurine group; H-TAU, high-taurine group.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

Table 5. TAG, cholesterol and total bile acid (TBA) contents in the liver and serum of tiger puffer fed experimental diets (Mean values with their standard errors, *n* 3)

Parameters	Control	M-TAU	H-TAU	Pooled SEM	*P*
Liver					
TAG (mmol/gprot)	0·64	0·48	0·53	0·04	0·201
TC (μmol/gprot)	175·07^{b}	172·45^{b}	205·55^{a}	8·66	0·002
HDL-cholesterol (μmol/gprot)	65·13^{b}	76·28^{a,b}	84·82^{a}	3·41	0·027
LDL-cholesterol (μmol/gprot)	19·28	19·87	20·30	0·70	0·873
TBA (μmol/gprot)	2·77^{b}	3·07^{a,b}	3·71^{a}	0·16	0·012
Serum					
TAG (mmol/l)	1·48	1·45	1·60	0·11	0·880
TC (mmol/l)	2·81^{b}	2·93^{a,b}	3·51^{a}	0·13	0·026
HDL-cholesterol (mmol/l)	2·51^{b}	2·64^{a,b}	3·25^{a}	0·14	0·032
LDL-cholesterol (mmol/l)	0·38	0·27	0·40	0·04	0·369
NEFA (mmol/l)	0·22	0·31	0·21	0·03	0·278
TBA (μmol/l)	0·98^{a}	0·82^{a,b}	0·47^{b}	0·09	0·045

M-TAU, medium-taurine group; H-TAU, high-taurine group; TC, total cholesterol.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

Hepatic lipidomics

Raw data were first normalised with respect to total ion current[35]. The results from positive and negative ion modes were combined. After management of raw data, a total of 690 peaks were extracted, successfully identified and quantified. However, only ten, six and twelve individual lipids showed significant difference in concentration between M-TAU and control, H-TAU and control, and H-TAU and M-TAU, respectively (online Supplementary Table S2, Fig. 1). Generally, dietary supplementation of TAU decreased the contents of a series of phospholipids but increased those of several TAG. Group H-TAU had higher contents of ceramides than groups control and M-TAU.

Hepatic bile acid profiles

While forty-one bile acids (see the list in online Supplementary Table S3) can be quantitatively analysed with the method used in the present study, only three conjugated bile acids, taurocholic acid, taurochenodeoxycholic acid and taurodeoxycholic acid, had concentrations above the lowest limit of quantitation in the present analysis. Trace amount of cholic acid can be detected in some samples, but not in all samples. The bile acid profile of the liver was averagely included 94·48 % taurocholic acid, 4·17 % taurochenodeoxycholic acid and 1·35 % taurodeoxycholic acid. The concentration of these three conjugated bile acids was significantly ($P < 0·05$) increased by dietary TAU supplementation (Table 6).

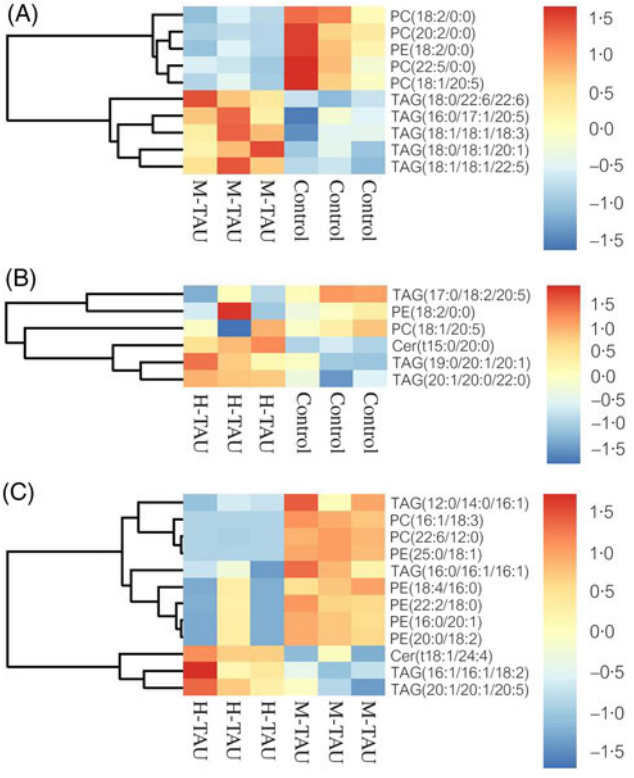

Fig. 1. Heatmap of lipids with significantly different concentrations between the control and medium-taurine (M-TAU) groups (A), the control and high-taurine (H-TAU) groups (B), and the M-TAU and H-TAU groups (C). PC, phosphatidylcholine; PE, phosphatidylethanolamine; Cer, ceramide.

Table 6. Hepatic bile acid profile of tiger puffer fed experimental diets (nmol/g wet liver tissue)
(Mean values with their standard errors, *n* 3)

Bile acid	Control	M-TAU	H-TAU	Pooled SEM	*P*
Taurocholic acid	86·95^{c}	118·24^{b}	164·92^{a}	11·79	0·000
Taurochenodeoxycholic acid	3·23^{b}	4·68^{b}	8·42^{a}	0·83	0·002
Taurodeoxycholic acid	1·04^{b}	1·52^{b}	2·74^{a}	0·28	0·008

M-TAU, medium-taurine group; H-TAU, high-taurine group.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

Hepatic relative mRNA expression

Regarding the expression of genes related to bile acid and cholesterol metabolism, the hepatic relative mRNA expression of *cyp7a1*, *hmgcr*, *abcg5*, *abcg8* and *fxr* significantly ($P < 0·05$) increased with increasing dietary TAU levels (Fig. 2). All these genes have the lowest expression in the control group and the highest expression in the group H-TAU.

The hepatic mRNA expression of lipoproteins, ApoA1 and ApoA4, also significantly ($P < 0·05$) increased with increasing dietary TAU levels (Fig. 3), while other lipoprotein-related genes, *apob100*, *mttp*, *apoe1*, *ldlr*, *scarb1* and *hdlbp*, showed no significant difference in hepatic gene expression among dietary groups.

Regarding the lipid metabolism-related genes, group H-TAU showed significantly ($P < 0·05$) higher hepatic gene expression of fatty acid synthase (*fas*) than the control group, as well as significantly ($P < 0·05$) higher gene expression of *pparγ* and bile salt-activated lipase (*bsal*) than groups control and M-TAU (Fig. 4).

Regarding the key proteins in MAPK signalling pathways, the gene expression of *erk2* and *jnk2* significantly ($P < 0·05$) increased with increasing dietary TAU levels, while *mek1* had significantly ($P < 0·05$) higher gene expression in group H-TAU than in groups control and M-TAU (Fig. 5).

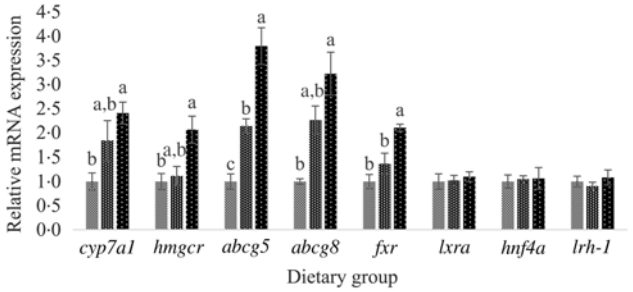

Fig. 2. Relative mRNA expression of genes related to bile acid and cholesterol metabolism in the liver of experimental fish. ^{a,b} Mean values for each gene with unlike letters were significantly different ($P < 0·05$). ▨, Control group; ▩, medium-taurine group; ■, high-taurine group. *cyp7a1*, Cholesterol 7*α*-hydroxylase; *hmgcr*, 3-hydroxy-3-methylglutaryl-CoA reductase; *abcg*, ATP-binding cassette subfamily G; *fxr*, farnesoid X receptor; *lxra*, liver X receptor alpha; *hnf4a*, hepatocyte nuclear factor 4, alpha; *lrh-1*, liver receptor homolog-1.

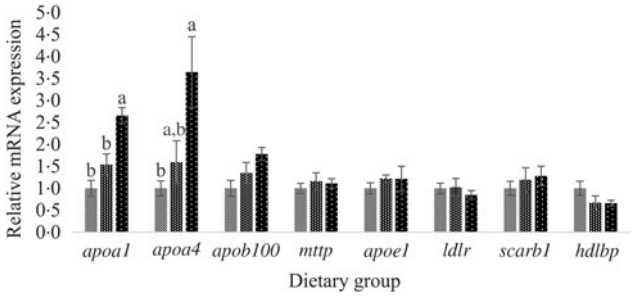

Fig. 3. Relative mRNA expression of lipoprotein-related genes in the liver of experimental fish. [a,b] Mean values for each gene with unlike letters were significantly different ($P < 0·05$). ▩, Control group; ▩, medium-taurine group; ■, high-taurine group. *mttp*, Microsomal TAG transfer protein; *ldlr*, LDL receptor; *scarb1*, scavenger receptor class B, member 1; *hdlbp*, HDL-binding protein.

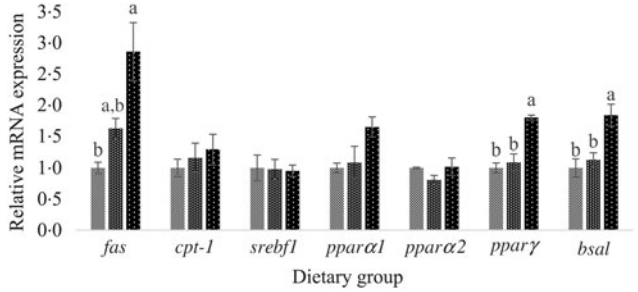

Fig. 4. Relative mRNA expression of lipid metabolism-related in the liver of experimental fish. [a,b] Mean values for each gene with unlike letters were significantly different ($P < 0·05$). ▩, Control group; ▩, medium-taurine group; ■, high-taurine group. *fas*, Fatty acid synthase; *cpt-1*, carnitine palmitoyltransferase-1; *srebf1*, sterol regulatory element-binding factor 1; *bsal*, bile salt-activated lipase-like.

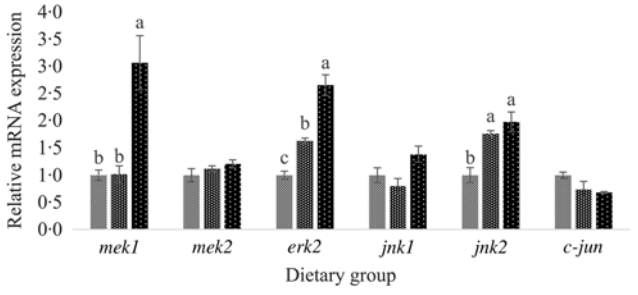

Fig. 5. Relative mRNA expression of key proteins in mitogen-activated protein kinase (MAPK) signalling pathways in the liver of experimental fish. [a,b] Mean values for each gene with unlike letters were significantly different ($P < 0·05$). ▩, Control group; ▩, medium-taurine group; ■, high-taurine group. *mek1*, Mitogen-activated protein kinase kinase 1 (*map2k1*); *mek2*, mitogen-activated protein kinase kinase 2 (*map2k2*); *erk2*, mitogen-activated protein kinase 1 (*mapk1*); *jnk1*, mitogen-activated protein kinase 8 (*mapk8*); *jnk2*, mitogen-activated protein kinase 9 (*mapk9*); *c-jun*, c-Jun protein.

Hepatic concentration of lecithin cholesterol acyl transferase

The hepatic concentration of LCAT assayed by the ELISA method was significantly ($P < 0·05$) higher in group H-TAU compared with groups control and M-TAU, and no significant difference was observed between the latter two groups (Fig. 6).

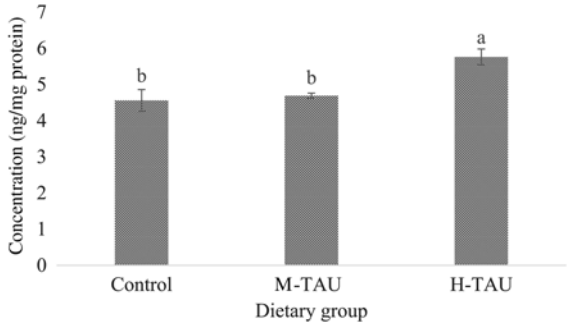

Fig. 6. Concentration of lecithin cholesterol acyl transferase in the liver of experimental fish, assayed with the ELISA method. [a,b] Mean values with unlike letters were significantly different ($P < 0·05$). M-TAU, medium-taurine group; H-TAU, high-taurine group.

Discussion

As expected, in the present study, dietary TAU supplementation stimulated the hepatic bile acid biosynthesis in tiger puffer, which was evidenced by both the increased hepatic bile acid contents and the up-regulated hepatic gene expression of CYP7A1, the rate-limiting enzyme for bile acid synthesis[36]. The up-regulated gene expression of ATP-binding cassette subfamily G5 and ATP-binding cassette subfamily G 8, which play essential roles in the selective sterol excretion by the liver into bile[37,38], by TAU supplementation provided additional evidences. These results were in good accordance with other fish studies with species such as Japanese flounder[7], red sea bream[8] and turbot[9]. These studies have demonstrated that TAU can stimulate the biosynthesis and excretion of bile acids, and this stimulation could be due to the roles of TAU in conjugating with bile acids. In teleosts, virtually all described bile acid conjugations occur with TAU[39]. For example, in the marine teleost Japanese flounder, TAU has been found to be the major compound to conjugate with cholesterol derivatives in the liver to produce bile salts[7]. Similarly, for tiger puffer in the present study, TAU, which was well accumulated in the liver, rather than glycine was the only compound to conjugate with bile acids at the current detection level. Only three conjugated bile acids, taurocholic acid, taurochenodeoxycholic acid and taurodeoxycholic acid, was detected in the present study. This result was similar to most species of aquaculture interest, which only secrete C24 bile acids, cholic acid and chenodeoxycholic acid, although noteworthy exceptions exist in sturgeons, paddlefish, cyprinids, gilthead sea bream and red sea bream[39].

In contrast with the hepatic bile acid content, the TBA concentration in the serum decreased with increasing dietary TAU levels. The enterohepatic circulation of bile acids has been well known[39,40]. It has been demonstrated that most bile acids are re-absorbed in distal ileum, and the re-absorbed bile acids are then routed to the liver where they will be recycled to the gallbladder. Studies with rats have showed that TAU inhibited the re-absorption of bile acids from the distal ileum[41–43]. Same mechanisms could be used to explain the reduction of serum bile acids by TAU in the present study.

Different from the bile acid results which showed good consistency between fish and mammals, the effects of TAU supplementation on cholesterol content in tiger puffer seemed different

from those observed in mammals. The hypocholesterolaemic effect of TAU has been consistently observed in many independent experiments performed on rat and mouse with exogenous hypercholesterolaemia caused by high-cholesterol–sodium cholate loading diet[1]. However, in the present study, the TC content in both liver and serum of tiger puffer was increased by TAU. Meanwhile, this was the first time in fish observing the stimulating effect of TAU on hepatic gene expression of 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Since normal or even low-cholesterol diets were used in the present study, the difference in abundance of dietary cholesterol could be a factor resulting in different results regarding effects of dietary TAU on cholesterol contents. In a study with turbot using exogenous cholesterol supplementation, when 1·0 % exogenous cholesterol was added, TAU supplementation decreased the serum TC concentration[9]. However, another fish study with juvenile totoaba-fed normal diets showed that plasma TC content increased linearly with dietary TAU (R^2 0·75, $P < 0·001$)[13], which was similar to the present study. Nevertheless, the effects of TAU on cholesterol content in fish studies seemed much varied. A study with yellow catfish-fed low-cholesterol diets showed that serum TC content decreased with increasing dietary TAU levels[12], whereas the study with white seabream-fed low-cholesterol diets showed that the plasma TC concentration was not affected by dietary TAU supplementation[14]. Protein sources, especially soya products, have been reported to interact with cholesterol in the intestine[44–48], whereas most TAU studies with fish used high levels of plant protein sources in the diets. The difference in protein source profile may contribute to the varied results among different fish studies. Another probable explanation of the inconsistent results among fish studies was that the effects of TAU on cholesterol metabolism in fish could be species-specific. Similar to tiger puffer, totoaba also have low lipid contents in the muscle and viscera, relatively high-lipid level in the liver and relatively low lipid requirement[49–52]. Whether these characteristics are associated with the stimulation of cholesterol synthesis by TAU warrants further studies.

Besides the TC contents, the HDL-cholesterol contents in both liver and serum were also elevated by the TAU supplementation. Elevation of HDL-cholesterol by TAU has been widely observed in mammal studies[53–57], and in most of these studies, the elevation of HDL-cholesterol was concomitant with the decrease of serum TC. HDL particles have been demonstrated to be able to transport cholesterol to liver for bile acid synthesis and thus reduce its accumulation in blood. In the present study, in accordance with the increase of HDL-cholesterol, the hepatic transcription of ApoA1 and the hepatic LCAT concentration were increased by dietary TAU. ApoA1 is the major apo of plasma HDL[58]. The increase of HDL-cholesterol by TAU may be initiated from the stimulation of ApoA1 expression. Required for remodelling HDL particles into their spherical forms[59], LCAT is a central enzyme synthesised mainly in the liver and functioning on the surface of HDL[60,61], where it converts cholesterol and phosphatidylcholines to cholesteryl esters and lysophosphatidylcholines, for the backward transport of cholesterol ester to the liver. The up-regulation of hepatic LCAT by dietary TAU evidenced the up-regulation of serum cholesterol removal. The still increase of serum TC by dietary TAU suggested that the removing effects of TAU on serum cholesterol may be masked by the stimulation of cholesterol biosynthesis *per se* by TAU.

Regarding LDL-cholesterol, in the present study, neither the LDL-cholesterol content in serum and liver nor the hepatic transcription of LDL receptor, which removes VLDL and LDL from circulation via binding to ApoB100 and ApoE moieties on them, was affected by dietary TAU supplementation. The hepatic mRNA expression of ApoB100 and ApoE1, as well as microsomal triglyceride TAG transfer protein, which is required for the secretion of plasma lipoproteins that contain ApoB[62], was also not affected by TAU. These results were obviously different from what observed in mammal studies. In mammal studies, it has been reported that TAU lowers cholesterol concentration by reducing ApoB and VLDL secretion from the liver and improves cholesterol clearance from the circulation by up-regulating LDL receptor-binding capacity led to increase of LDL uptake[3,4,20,56,63]. The decrease of plasma LDL-cholesterol has also been observed in a study with turbot, but in that study a high-cholesterol level was used in the diet[9]. The non-response of LDL to dietary TAU in the present study could be related to the significant stimulation of cholesterol biosynthesis. The necessity of transport of excess cholesterol out of liver via LDL may counteract the opposite transportation effects of TAU. Another possibility was that TAU does not affect LDL metabolism at all in fish species that store lipids in the liver.

Regarding the effects of dietary TAU supplementation on lipid accumulation, neither the lipid content in the whole body, liver and muscle, nor the TAG content in the serum and liver was affected by dietary TAU supplementation. Different from the mammal studies, most of which extensively demonstrated the hypolipidaemic effects of TAU[2,64–66], the relevant fish studies showed various results regarding the effects of TAU on lipid accumulation.

In the study with totoaba, plasma TAG increased quadratically (R^2 0·53, $P < 0·001$) with dietary TAU[13], but in the studies with white seabream and yellow catfish, dietary TAU supplementation decreased the plasma TAG level[14], while the blood TAG of Japanese flounder was not affected by dietary TAU[10]. In white grouper, TAU supplementation decreased the livers' total lipids but appeared to have little effect on lipid stores in the muscle[11]. With respect to body lipid content, while there is a general trend of decreasing body lipid content in response to limiting levels of dietary TAU[9,67–70], the opposite trend or lack of effect has also been observed in different species such as Atlantic salmon and sable fish[67,71,72]. Evidence also suggests differences in the lipid-regulating effects of TAU depending on fish size[69]. These results strongly suggested that the effects of TAU on lipid accumulation in fish could vary with many factors such as fish species, fish size, tissue type and dietary formulation.

In the present study, in spite of being not effective in regulating lipid contents, TAU supplementation increased the hepatic transcription of lipogenic genes *fas* and *pparγ*, indicating the potential of TAU in accelerating lipogenesis. The lipidomics results also showed that TAU up-regulated the concentration of many TAG. In addition, the transcription of bile salt-activated lipase-like, which is a major lipase in fish, and that of ApoA4,

which acts primarily in intestinal lipid absorption, were up-regulated by the TAU supplementation, indicating the potential of TAU in facilitating lipid digestion. The biological activities of TAU in facilitating lipid emulsion and absorption via bile salts have been well documented[73–75]. The increase of lipid content in tiger puffer by TAU supplementation may appear after a longer feeding period. Moreover, the present results indicated again the discrepancy in lipid-regulating effect of TAU between high-fat mammal models and fish-fed normal diets. Besides lipogenesis, this discrepancy is also observed in fatty acid β-oxidation and energy expenditure. In mammals, increasing fatty acid β-oxidation and energy expenditure in adipose tissues is one of the mechanisms underlying the TAG ameliorating and anti-obesity effects of TAU[64,76,77]. However, in the present study, the transcription of *cpt-1* and *pparα2*, which play important roles in fatty acid β-oxidation, was not affected by dietary TAU.

Additionally, the lipidomics results showed that on the contrary to TAG, however, the hepatic abundance of several phosphatidylcholines and phosphatidylethanolamines, including some lysophospholipids, was down-regulated by dietary TAU supplementation. The decrease in phospholipids by TAU could be related to the increase of HDL-cholesterol. It was possible that more hepatic phospholipids were transported to blood to facilitate the function of HDL. However, it was difficult to explain the regulation of each individual phospholipid by TAU based on our current knowledge. These phospholipids regulated by TAU may be preferences of cholesterol metabolism on the surface of HDL.

Moreover, from the lipidomics results, we can see that the concentration of ceramide, Cer(t18:1/24:4) and Cer(t15:0/22:0), was higher in the high-TAU group compared with other groups. It has been well known that TAU has considerable accumulation in animal brain[7,78] and can serve as neurotransmitter and neuromodulator[73,79]. The present result indicated that Cer(t18:1/24:4) and Cer(t15:0/22:0) might have important roles in neuromodulation of tiger puffer.

The mechanisms involved in the regulation of bile acids by TAU have been investigated in a number of previous studies[1,80]. In the promoters of mammal CYP7A1 genes, binding sites for transcription factors such as liver X receptor α and hepatocyte nuclear factor 4, α, and liver receptor homolog-1 have been observed and these transcription factors have been reported to be important regulators of CYP7A1 transcription[81–84]. However, in the present study, transcription of all these transcription factors was not affected by dietary TAU in spite of the increase of CYP7A1 transcription. The promoter sequence of tiger puffer CYP7A1 has not been available. Differences in binding sites for the transcription factors mentioned above probably exist between tiger puffer and mammals.

However, the present results showed that the transcription of FXR, which is a bile acid receptor and plays a critical role in the regulation of bile acid synthesis and homoeostasis, was significantly increased by dietary TAU. FXR acts as a negative regulator of CYP7A1. Stimulation of FXR transcription by dietary TAU reflected feedback regulation of CYP7A1 by increased hepatic bile acid pool. In C57BL/6 mice, however, Lam *et al.*[42] reported that TAU may interrupt the activation of FXR via some unknown pathways. Mammal studies showed that bile acids such as lithocholic acid, chenodeoxycholic acid and deoxycholic acid are potential ligands that activate FXR[81–85]. However, it remains unclear which bile acid is effective ligand of FXR in tiger puffer.

Regarding the hepatic receptors of lipoproteins, besides LDL receptor, scavenger receptor class B, member 1 and HDL-binding protein, which are potential receptors of HDL, were also analysed. The function of scavenger receptor class B, member 1 and HDL-binding protein has not been well understood. The significant effects of TAU on HDL-cholesterol content but not on transcription of *scarb1* and *hdlbp* in the present study indicated that there might be no high-affinity interaction between HDL and these receptors.

Regarding the cellular signalling transduction related to TAU function, very little information has been available. It has been reported that TAU stimulated alkaline phosphatase activity and collagen synthesis in cultured osteoblasts via activation of the extracellular signal-regulated kinase (ERK) pathway[86,87]. A study with HepG2 cell showed that TAU could enhance *cyp7a1* expression by inducing *hnf4α* and inhibiting *mek1/2* and *p-c-jun* expression[22]. In the present study, transcription of *mek1*, *erk2* and *jnk2* was up-regulated by dietary TAU. As the first time in fish, this result preliminarily indicated that the MAPK signalling pathway might be involved in the exertion of TAU function. Since the MAPK signalling pathway is involved in various physiological processes, future studies are needed to elucidate its precise role in regulating the effects of TAU on the metabolisms of bile acids, cholesterol and lipids.

In conclusion, dietary TAU supplementation stimulated the hepatic biosynthesis of both bile acids and cholesterol in tiger puffer and increased the HDL-cholesterol content in both liver and serum. TAU tended to increase the contents of some individual TAG and ceramides but decrease the contents of individual phosphatidylcholines and phosphatidylethanolamines. Taurocholic acid and taurochenodeoxycholic acid were the major bile acids in the liver of tiger puffer. It was possible that FXR, but not liver X receptor α, hepatocyte nuclear factor 4, α, and liver receptor homolog-1, was involved in the regulation of CYP7A1 by TAU and that the MAPK signalling pathway was involved in the exertion of TAU functions. As the state of knowledge about TAU physiology in fish remains fragmented and limited, additional research is evidently necessary to elucidate the mechanisms by which TAU exerts its functions.

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H. X. and M. L. designed this research. Q. Z. and Z. L. conducted the feeding trial. Q. Z. and Y. W. analysed the fish proximate composition and amino acid contents. H. X., Q. Z., B. S. and L. J. analysed other parameters and performed statistical analysis. H. X., S.-K. K. and S. C. wrote the manuscript. All

authors read and approved the final manuscript. H. X. and M. L. had primary responsibility for final content.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000161)

References

1. Chen W, Guo JX & Chang P (2012) The effect of taurine on cholesterol metabolism. *Mol Nutr Food Res* **56**, 681–690.
2. Militante JD & Lombardini JB (2004) Dietary taurine supplementation, hypolipidemic and antiatherogenic effects. *Nutr Res* **24**, 787–801.
3. Kishida T, Miyazato S, Ogawa H, *et al.* (2003) Taurine prevents hypercholesterolemia in ovariectomized rats fed corn oil but not in those fed coconut oil. *J Nutr* **133**, 2616–2621.
4. Ebihara K, Miyazato S, Ogawa H & Kishida T (2006) Taurine increases cholesterol 7α-hydroxylase activity and fecal bile acids excretion but does not reduce the plasma cholesterol concentration in ovariectomized rats fed with coconut oil. *Nutr Res* **26**, 167–172.
5. Salze GP & Davis DA (2015) Taurine: a critical nutrient for future fish feeds. *Aquaculture* **437**, 215–229.
6. National Research Council (2011) *Nutrient Requirements of Fish*. Washington, DC: National Academies Press.
7. Kim S-K, Matsunari H, Takeuchi T, *et al.* (2007) Effect of different dietary taurine levels on the conjugated bile acid composition and growth performance of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*. *Aquaculture* **273**, 595–601.
8. Takagi S, Murata H, Goto T, *et al.* (2011) Role of taurine deficiency in inducing green liver symptom and effect of dietary taurine supplementation in improving growth in juvenile red sea bream *Pagrus major* fed non-fishmeal diets based on soy protein concentrate. *Fish Sci* **77**, 235–244.
9. Yun B, Ai Q, Mai K, *et al.* (2012) Synergistic effects of dietary cholesterol and taurine on growth performance and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets. *Aquaculture* **324–325**, 85–91.
10. Han Y, Koshio S, Jiang Z, *et al.* (2014) Interactive effects of dietary taurine and glutamine on growth performance, blood parameters and oxidative status of Japanese flounder *Paralichthys olivaceus*. *Aquaculture* **434**, 348–354.
11. Koven W, Peduel A, Gada M, *et al.* (2016) Taurine improves the performance of white grouper juveniles (*Epinephelus aeneus*) fed a reduced fish meal diet. *Aquaculture* **460**, 8–14.
12. Li M, Lai H, Li Q, *et al.* (2016) Effects of dietary taurine on growth, immunity and hyperammonemia in juvenile yellow catfish *Pelteobagrus fulvidraco* fed all-plant protein diets. *Aquaculture* **450**, 349–355.
13. Satriyo TB, Galaviz MA, Salze G, *et al.* (2017) Assessment of dietary taurine essentiality on the physiological state of juvenile, *Totoaba macdonaldi*. *Aquac Res* **48**, 5677–5689.
14. Magalhães R, Martins N, Martins S, *et al.* (2019) Is dietary taurine required for white seabream (*Diplodus sargus*) juveniles? *Aquaculture* **502**, 296–302.
15. Nishimura N, Umeda C, Oda H, *et al.* (2003) The effect of taurine on the cholesterol metabolism in rats fed diets supplemented with cholestyramine or high amounts of bile acid. *J Nutr Sci Vitaminol* **49**, 21–26.
16. Lam NV, Chen W, Suruga K, *et al.* (2006) Enhancing effect of taurine on CYP7A1 mRNA expression in Hep G2 cells. *Amino Acids* **30**, 43–48.
17. Yang SF, Tzang BS, Yang KT, *et al.* (2010) Taurine alleviates dyslipidemia and liver damage induced by a high-fat/cholesterol-dietary habit. *Food Chem* **120**, 156–162.
18. Murakami S, Fujita M, Nakamura M, *et al.* (2016) Taurine ameliorates cholesterol metabolism by stimulating bile acid production in high-cholesterol-fed rats. *Clin Exp Pharmacol Physiol* **43**, 372–378.
19. Parks DJ, Blanchard SG, Bledsoe RK, *et al.* (1999) Bile acids: natural ligands for an orphan nuclear receptor. *Science* **284**, 1365–1368.
20. Murakami S, Kondo Y, Toda Y, *et al.* (2002) Effect of taurine on cholesterol metabolism in hamsters: up-regulation of LDL receptor by taurine. *Life Sci* **70**, 2355–2366.
21. Wang Y, Mei X, Yuan J, *et al.* (2016) Taurine zinc solid dispersions enhance bile-incubated L02 cell viability and improve liver function by inhibiting ERK2 and JNK phosphorylation during cholestasis. *Toxicology* **366–367**, 10–19.
22. Guo J, Gao Y, Cao X, *et al.* (2017) Cholesterol-lowing effect of taurine in HepG2 cell. *Lipids Health Dis* **16**, 56.
23. Fernandes JMO, Mackenzie MG, Elgar G, *et al.* (2005) A genomic approach to reveal novel genes associated with myotube formation in the model teleost, *Takifugu rubripes*. *Physiol Genomics* **22**, 327–338.
24. Kai W, Kikuchi K, Fujita M, *et al.* (2005) A genetic linkage map for the tiger pufferfish *Takifugu rubripes*. *Genetics* **171**, 227–238.
25. Imai S, Sasaki T, Shimizu A, *et al.* (2007) The genome size evolution of medaka (*Oryzias latipes*) and fugu (*Takifugu rubripes*). *Genes Genet Syst* **82**, 135–144.
26. Wongwarangkana C, Fujimori KE, Akiba M, *et al.* (2015) Deep sequencing, profiling and detailed annotation of microRNAs in *Takifugu rubripes*. *BMC Genomics* **16**, 457.
27. Kaneko G, Yamada T, Han Y, *et al.* (2013) Differences in lipid distribution and expression of peroxisome proliferator-activated receptor gamma and lipoprotein lipase genes in torafugu and red seabream. *Gen Comp Endocrinol* **184**, 51–60.
28. Xu H, Mu Y, Zhang Y, *et al.* (2016) Graded levels of fish protein hydrolysate in high plant diets for turbot (*Scophthalmus maximus*): effects on growth performance and lipid accumulation. *Aquaculture* **454**, 140–147.
29. Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
30. Xu H, Cao L, Wei Y, *et al.* (2018) Lipid contents in farmed fish are influenced by dietary DHA/EPA ratio: a study with the marine flatfish, tongue sole (*Cynoglossus semilaevis*). *Aquaculture* **485**, 183–190.
31. Tu J, Yin Y, Xu M, *et al.* (2018) Absolute quantitative lipidomics reveals lipidome-wide alterations in aging brain. *Metabolomics* **14**, 5.
32. Han J, Liu Y, Wang R, *et al.* (2015) Metabolic profiling of bile acids in human and mouse blood by LC-MS/MS in combination with phospholipid-depletion solid-phase extraction. *Anal Chem* **87**, 1127–1136.
33. Xu H, Dong X, Ai Q, Mai K, *et al.* (2014) Regulation of tissue LC-PUFA contents, Δ6 fatty acyl desaturase (FADS2) gene expression and the methylation of the putative FADS2 gene promoter by different dietary fatty acid profiles in Japanese seabass (*Lateolabrax japonicus*). *PLOS ONE* **9**, e87726.
34. Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.

35. Dunn WB, Broadhurst D, Begley P, *et al.* (2011) Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc* **6**, 1060–1083.
36. Chiang JYL (1998) Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *Front Biosci* **3**, 176–193.
37. Yu L, Hammer RE, Li-Hawkins J, *et al.* (2002) Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci USA* **99**, 16237–16242.
38. Wang J, Mitsche MA, Lütjohann D, *et al.* (2015) Relative roles of ABCG5/ABCG8 in liver and intestine. *J Lipid Res* **56**, 319–330.
39. Hofmann AF, Hagey LR & Krasowski MD (2010) Bile salts of vertebrates, structural variation and possible evolutionary significance. *J Lipid Res* **51**, 226–246.
40. Dawson PA (2018) Bile formation and the enterohepatic circulation. In *Hysiology of the Gastrointestinal Tract*, 6th ed., pp. 931–956 [HM Said, editor]. Cambridge MA: Academic Press.
41. Chen W, Nishimura N, Oda H, *et al.* (2003) Effect of taurine on cholesterol degradation and bile acid pool in rats fed a high-cholesterol diet. *Adv Exp Med Biol* **526**, 261–267.
42. Lam NV, Chen W, Suruga K, *et al.* (2006) Effects of taurine on mRNA levels of nuclear receptors and factors involved in cholesterol and bile acid homeostasis in mice. *Adv Exp Med Biol* **583**, 193–202.
43. Nishimura N, Yamamoto T & Ota T (2009) Taurine feeding inhibits bile acid absorption from the ileum in rats fed a high cholesterol and high fat diet. *Adv Exp Med Biol* **643**, 285–291.
44. Anderson JW & Bush HM (2011) Soy protein effects on serum lipoproteins: a quality assessment and meta-analysis of randomized, controlled studies. *J Am Coll Nutr* **30**, 79–91.
45. Nguyen HP, Khaoian P, Fukada H, *et al.* (2011) Effects of different soybean proteins on lipid digestion and growth of yellowtail *Seriola quinqueradiata*. *Fish Sci* **77**, 357–365.
46. El Khoury D & Anderson GH (2013) Recent advances in dietary proteins and lipid metabolism. *Curr Opin Lipidol* **24**, 207–213.
47. Kortner TM, Gu J, Krogdahl A, *et al.* (2013) Transcriptional regulation of cholesterol and bile acid metabolism after dietary soyabean meal treatment in Atlantic salmon (*Salmo salar* L.). *Br J Nutr* **109**, 593–604.
48. Murashita K, Akimoto A, Iwashita Y, *et al.* (2013) Effects of biotechnologically processed soybean meals in a nonfishmeal diet on growth performance, bile acid status, andmorphological condition of the distal intestine and liver of rainbow trout *Oncorbynchus mykiss*. *Fish Sci* **79**, 447–457.
49. Rueda-López S, Lazo JP, Reyes GC, *et al.* (2011) Effect of dietary protein and energy levels on growth, survival and body composition of juvenile *Totoaba macdonaldi*. *Aquaculture* **319**, 385–390.
50. Bañuelos-Vargas I, López LM, Pérez-Jiménez A, *et al.* (2014) Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on hepatic intermediary metabolism and antioxidant status of totoaba juveniles (*Totoaba macdonaldi*). *Comp Biochem Physiol* **170B**, 18–25.
51. Fuentes-Quesada JP, Viana MT, Rombenso AN, *et al.* (2018) Enteritis induction by soybean meal in, *Totoaba macdonaldi*, diets: effects on growth performance, digestive capacity, immune response and distal intestine integrity. *Aquaculture* **495**, 78–89.
52. Barreto-Curiel F, Focken U, D'Abramo LR, *et al.* (2019) Assessment of amino acid requirements for *Totoaba macdonaldi* at different levels of protein using stable isotopes and a non-digestible protein source as a filler. *Aquaculture* **503**, 550–561.
53. Mochizuki H, Oda H & Yokogoshi H (2001) Dietary taurine potentiates polychlorinated biphenyl-induced hypercholesterolemia in rats. *J Nutr Biochem* **12**, 109–115.
54. Nishimura N, Umeda C, Oda H, *et al.* (2002) The effect of taurine on plasma cholesterol concentration in genetic type 2 diabetic GK rats. *J Nutr Sci Vitaminol* **48**, 483–490.
55. Yokogoshi H, Mochizuki H, Nanami K, *et al.* (1999) Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. *J Nutr* **129**, 1705–1712.
56. Murakami S, Kondo Y & Nagate T (2000) Effects of long-term treatment with taurine in mice fed a high-fat diet: improvement in cholesterol metabolism and vascular lipid accumulation by taurine. *Adv Exp Med Biol* **483**, 177–186.
57. Chen W, Suruga K, Nishimura N, *et al.* (2005) Comparative regulation of major enzymes in bile acids biosynthesis pathways by cholesterol, cholic acid and taurine in mice and rats. *Life Sci* **77**, 746–757.
58. Chapman MJ (1986) Comparative analysis of mammalian plasma lipoproteins. *Methods Enzymol* **128**, 70–143.
59. Clay MA, Pyle DH, Rye KA, *et al.* (2000) Formation of spherical, reconstituted high density lipoproteins containing both apolipoproteins A-I and A-II is mediated by lecithin:cholesterol acyltransferase. *J Biol Chem* **275**, 9019–9025.
60. Kosek AB, Durbin D & Jonas A (1999) Binding affinity and reactivity of lecithin cholesterol acyltransferase with native lipoproteins. *Biochem Biophys Res Commun* **258**, 548–551.
61. Hirsch-Reinshagen V, Donkin J, Stukas S, *et al.* (2009) LCAT synthesized by primary astrocytes esterifies cholesterol on glia-derived lipoproteins. *J Lipid Res* **50**, 885–893.
62. Walsh MT, Iqbal J, Josekutty J, *et al.* (2015) Novel abetalipoproteinemia missense mutation highlights the importance of the N-terminal β-barrel in microsomal triglyceride transfer protein function. *Circ Cardiovasc Genet* **8**, 677–687.
63. Chang YY, Chou CH, Chiu CH, *et al.* (2011) Preventive effects of taurine on development of hepatic steatosis induced by a high-fat/cholesterol dietary habit. *J Agric Food Chem* **59**, 450–457.
64. Murakami S (2015) Role of taurine in the pathogenesis of obesity. *Mol Nutr Food Res* **59** 1353–1363.
65. Chen W, Matuda K, Nishimura N, *et al.* (2004) The effect of taurine on cholesterol degradation in mice fed a high-cholesterol diet. *Life Sci* **74**, 1889–1898.
66. Chen W, Guo J, Zhang Y, *et al.* (2016) The beneficial effects of taurine in preventing metabolic syndrome. *Food Funct* **7**, 1849–1863.
67. Espe M, Ruohonen K & El-Mowafi A (2012) Effect of taurine supplementation on the metabolism and body lipid-to-protein ratio in juvenile Atlantic salmon (*Salmo salar*). *Aquac Res* **43**, 349–360.
68. Lunger AN, McLean E, Gaylord TG, *et al.* (2007) Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). *Aquaculture* **271**, 401–410.
69. Qi G, Ai Q, Mai K, *et al.* (2012) Effects of dietary taurine supplementation to a casein-based diet on growth performance and taurine distribution in two sizes of juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture* **358–359**, 122–128.
70. Salze GP, Spangler E, Cobine PA, *et al.* (2016) Investigation of biomarkers of early taurine deficiency in Florida pompano *Trachinotus carolinus*. *Aquaculture* **451**, 254–265.
71. Espe M, Ruohonen K & El-Mowafi A (2012) Hydrolysed fish protein concentrate (FPC) reduces viscera mass in Atlantic salmon (*Salmo salar*) fed plant-protein-based diets. *Aquacult Nutr* **18**, 599–609.
72. Johnson RB, Kim SK, Watson AM, *et al.* (2015) Effects of dietary taurine supplementation on growth, feed efficiency, and nutrient composition of juvenile sablefish (*Anoplopoma fimbria*) fed plant based feeds. *Aquaculture* **445**, 79–85.
73. Huxtable RJ (1992) Physiological actions of taurine. *Physiol Rev* **72**, 101–163.

74. Bouckenooghe T, Remacle C & Reusens B (2006) Is taurine a functional nutrient? *Curr Opin Clin Nutr Metab Care* **9**, 728–733.
75. Richard N, Colen R & Aragão C (2017) Supplementing taurine to plant-based diets improves lipid digestive capacity and amino acid retention of Senegalese sole (*Solea senegalensis*) juveniles. *Aquaculture* **468**, 94–101.
76. Puigserver P, Wu Z, Park CW, *et al.* (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**, 829–839.
77. Fukuda N, Yoshitama A, Sugita S, *et al.* (2011) Dietary taurine reduces hepatic secretion of cholesteryl ester and enhances fatty acid oxidation in rats fed a high-cholesterol diet. *J Nutr Sci Vitaminol* **57**, 144–149.
78. Pasantes-Morales H, Chatagner F & Mandel P (1980) Synthesis of taurine in rat liver and brain *in vivo*. *Neurochem Res* **5**, 441–451.
79. Kuriyama K (1980) Taurine as a neuromodulator. *Fed Proc* **39**, 2680–2684.
80. Hoang MH, Jia Y, Jun HJ, *et al.* (2012) Taurine is a liver X receptor-α ligand and activates transcription of key genes in the reverse cholesterol transport without inducing hepatic lipogenesis. *Mol Nutr Food Res* **56**, 900–911.
81. Lehmann JM, Kliewer SA, Moore LB, *et al.* (1997) Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem* **272**, 3137–3140.
82. Stroup D, Crestani M & Chiang JYL (1997) Identification of a bile acid response element in the cholesterol 7 alpha-hydroxylase gene CYP7A. *Am J Physiol* **273**, G508–G517.
83. Peet DJ, Truly SD, Ma W, *et al.* (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* **93**, 693–704.
84. Chiang JYL, Kimmel R & Stroup D (2001) Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). *Gene* **262**, 257–265.
85. Miyazaki T, Honda A & Matsuzaki Y (2014) Regulation of taurine conjugation and biosynthesis by bile acids through farnesoid X receptor activation. *Hepatol Res* **44**, E1–E2.
86. Park S, Kim H & Kim S-J (2001) Stimulation of ERK2 by taurine with enhanced alkaline phosphatase activity and collagen synthesis in osteoblast-like UMR-106 cells. *Biochem Pharmacol* **62**, 1107–1111.
87. Yuan LQ, Lu Y, Luo XH, *et al.* (2007) Taurine promotes connective tissue growth factor (CTGF) expression in osteoblasts through the ERK signal pathway. *Amino Acids* **32**, 425–430.

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