**SUPPLEMENTARY INFORMATION:**

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

Houguo Xu1,2, Qinggong Zhang1, Shin-Kwon Kim3, Zhangbin Liao1, Yuliang Wei1,2, Bo Sun1, Linlin Jia1, Shuyan, Chi4, Mengqing Liang1,2\*

*1Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao 266071, China*

*2Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Qingdao 266000, China*

*­­­­3Aquaculture Research Division, National Institute of Fisheries Science, 216 Gijanghaean Road, Gijang-eup 619705, Republic of Korea*

*4College of fisheries, Guangdong Ocean University, 1 Haida Road, Zhanjiang 524088, China*

\*Corresponding author: M. Liang, Tel./fax: +86-532-85822914, email: liangmq@ysfri.ac.cn

**Supplementary methodological material:**

**Quantitative analysis of hepatic lipidomics:**

To extract lipid metabolites from each sample, 20 mg liver tissue was homogenized in 400 μL distilled water, and then 200 μL homogenate was transferred to a new EP tube added with 200 μL distilled water and 960 μL extract solution (VMTBE: Vmethanol = 5: 1). Internal standards, 9 μL d7-PE(15:0/18:1) (10 μg mL-1), 9μL d7-LPC(18:1) (10 μg mL-1), 9 μL d7-TG(15:0/18:1/15:0) (100 μg mL-1) were included in the 960 μL extract solution. After vortex for 30 s and sonication for 10 min in ice-water bath, the samples were centrifuged (833 *g*) for 15 min at 4 °C. 500 μL homogenate was transferred to a new EP tube added with 500 μL MTBE, and then vortex, sonication, and centrifugation were repeated similarly as previously described. Then, 500 μL homogenate was subjected to the last step one more time. The supernatants were combined and dried in a vacuum dryer at 37 °C. Then, the dried samples were re-dissolved in 200 μL methanol dichloromethane solution (1:1), and subjected to vortex for 30 s, sonication for 10 min in ice-water bath, and centrifugation (833 *g*) for 15 min at 4 °C. 75 μL final supernatant was used for LC/MS analysis.

LC-MS/MS analyses were performed using an UHPLC system (1290, Agilent Technologies) with a Phenomen Kinetex C18 column (2.1 × 100 mm, 1.7 μm) coupled to TripleTOF 6600 mass spectrometry (AB Sciex).

The mobile phase A was 40% water + 60% acetonitrile (with 10 mmol L-1 ammonium formate), and phase B was 10% acetonitrile + 90% isopropanol (with 50 mL 10 mmol L-1 ammonium formate in every 1000 mL mixed solvent). The elution gradient was as follows: 0 ~ 12.0 min, 40% ~ 100% B; 12.0 ~ 13.5 min, 100% B; 13.5 ~ 13.7 min, 100% ~ 40% B; 13.7 ~ 18.0 min, 40% B. The column temperature and auto-sampler temperature was 45 °C and 4 °C, respectively. The injection volume for positive ion (POS) and negative ion (NEG) modes and was 0.5 μL and 2 μL, respectively. MS/MS spectra was acquired with triple Time of Flight (TOF) mass spectrometer on an information-dependent basis (IDA) during the LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluates the full scan-survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. In each cycle, the most intensive 12 precursor ions with intensity above 100 were chosen for MS/MS at collision energy (CE) of 30 eV (12 MS/MS events with accumulation time of 50 msec each). ESI source conditions were set as following: Gas 1: 40 psi; Gas 2: 80 psi; Curtain Gas: 25 psi; Source Temperature: 650 °C; Ion Spray Voltage Floating (ISVF): 5000 V and - 4000 V in positive and negative modes, respectively; Declustering potential: 60 V.

MS raw data (.d) files were converted to the mzXML format using ProteoWizard, and processed by R package XCMS (minfrac=0.5, cutoff=0.6). Peak detection was first applied to the MS1 data, and the CentWave algorithm in XCMS was used for peak detection. With the MS/MS spectrum, lipid identification was achieved through a spectral match using an in-house MS/MS spectral library. The absolute quantitation of lipids can be calculated using the peak area, SIL-IS and RF information.

**Quantitative analysis of hepatic bile acid omics:**

For the metabolites extraction, 50 mg sample was added into 1000 μL extract solvent (methanol/acetonitrile/water=2:2:1, containing 1% formic acid and 50 nmol L-1 internal standards). After vortexed for 30 s, the samples were homogenized with steel balls at 45 Hz for 4 min, and sonicated for 5 min in ice-water bath. The homogenization and sonication circle was repeated for 3 times, followed by incubation for 1 h at -20 °C and centrifugation (3333 *g*) for 15 min at 4 °C. The resulting supernatants were transferred to LC-MS vials and stored at -80 °C prior to UHPLC-QE Orbitrap/MS analysis.

Stock solutions were individually prepared by dissolving or diluting each standard substance to generate a final concentration of 10 mmol L-1. Aliquots of each stock solution were transferred to a 10 mL flask to form a mixed working standard solution. A series of calibration standard solutions were then prepared by stepwise dilution of this mixed standard solution.

 The UHPLC separation was carried out using an Agilent 1290 Infinity series UHPLC System (Agilent Technologies), equipped with a Waters ACQUITY UPLC BEH C18 column (150 × 2.1 mm, 1.7 μm, Waters). The mobile phase A was 0.1% acetic acid solution, and phase B was acetonitrile. The column temperature and auto-sampler temperature was 45 oC and 4 oC, respectively. The injection volume was 3 μL.

Q Exactive Focus mass spectrometer (Thermo Fisher Scientific) and Parallel Reaction Monitoring (PRM) were applied for MS assay. Typical ion source parameters were: spray voltage = +3500 / -3100 V; sheath gas (N2) flow rate = 40; aux gas (N2) flow rate = 15, sweep gas (N2) flow rate = 0; aux gas (N2) temperature = 350 °C; capillary temperature = 320 °C.

The PRM parameters for each targeted analyte were optimized by injecting the standard solutions of the individual analytes into the API source of the mass spectrometer. Since most of the bile acid analytes did not show product ion acceptable for quantification, the precursor ion in high resolution was selected for quantification. Calibration solutions were subjected to UPLC-PRM-MS/MS analysis using the methods described above to draw the calibration curve.

For all the analytes, the lowest limit of detection and quantitation ranged from 0.24 to 7.81 nmol L-1 and from 0.49 to 15.62 nmol L-1, respectively; Correlation coefficients (R2) of regression fitting were > 0.9931; Recoveries were 77.1% ~ 114.7%; and relative standard deviation < 10.9%.

**Supplementary TABLE 1** Fatty acid compositions in liver of tiger puffer fed experimental diets (% total fatty acids, mean ± standard error, n=3).

|  |  |  |  |
| --- | --- | --- | --- |
| Fatty acid | Control | M-TAU | H-TAU |
| C14:0 | 1.54±0.2 | 1.68±0.08 | 1.56±0.15 |
| C16:0 | 16.08±0.66 | 16.67±0.98 | 15.1±1.26 |
| C18:0 | 6.73±0.46 | 7.94±0.39 | 8.05±0.63 |
| C20:0 | 2.15±0.13 | 2.09±0.15 | 2.16±0.13 |
| ∑SFA | 26.49±0.36 | 28.37±1.55 | 26.86±0.87 |
| C16:1n-7 | 3.84±0.4 | 3.77±0.41 | 3.83±0.53 |
| C18:1n-9 | 18.01±1.01 | 18.22±0.88 | 19.53±1.17 |
| C18:1n-7 | 1.2±0.17 | 1.47±0.45 | 1.75±0.13 |
| ∑MUFA | 23.06±0.79 | 23.45±0.72 | 25.12±0.92 |
| C18:2n-6 | 18.63±1.26 | 18.86±0.53 | 18.05±0.2 |
| C20:4n-6 | 0.59±0.05 | 0.51±0.06 | 0.55±0.06 |
| ∑n-6 PUFA | 19.22±1.22 | 19.37±0.48 | 18.59±0.23 |
| C18:3n-3 | 1.83±0.12 | 1.66±0.11 | 1.85±0.31 |
| C20:5n-3 | 2.1±0.08 | 2.2±0.09 | 2.15±0.09 |
| C22:6n-3 | 5.17±0.43 | 5.63±0.35 | 5.14±0.21 |
| ∑n-3 PUFA | 9.11±0.59 | 9.49±0.47 | 9.15±0.21 |
| ∑n-3/∑n-6 | 0.48±0.05 | 0.49±0.04 | 0.49±0.01 |

SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; n-6 PUFA: n-6 poly-unsaturated fatty acids; n-3 PUFA: n-3 poly-unsaturated fatty acid.

**Supplementary TABLE 2** Lipids with significantly different contents among experimental groups, assayed with lipidomics method.

|  |  |  |  |
| --- | --- | --- | --- |
| Lipid | Content (ng mg-1 wet liver weight) | Fold Change | *P*-value |
| *M-TAU vs Control* |
|  | Mean of Control | Mean of M-TAU |  |  |
| PC(18:2/0:0) | 1.21×10-5 | 7.35×10-6 | 0.61 | 0.014 |
| PC(20:2/0:0) | 1.09×10-6 | 6.66×10-7 | 0.61 | 0.042 |
| PE(18:2/0:0) | 5.28×10-4 | 1.56×10-4 | 0.30 | 0.017 |
| PC(22:5/0:0) | 2.55×10-6 | 1.21×10-6 | 0.47 | 0.049 |
| PC(18:1/20:5) | 6.75×10-3 | 4.34×10-3 | 0.64 | 0.042 |
| TG(18:0/22:6/22:6) | 2.36×10-3 | 3.07×10-3 | 1.30 | 0.007 |
| TG(16:0/17:1/20:5) | 1.92×10-5 | 2.21×10-5 | 1.15 | 0.043 |
| TG(18:1/18:1/18:3) | 2.45×10-4 | 3.09×10-4 | 1.26 | 0.023 |
| TG(18:0/18:1/20:1) | 2.57×10-4 | 3.20×10-4 | 1.25 | 0.017 |
| TG(18:1/18:1/22:5) | 8.10×10-4 | 1.00×10-3 | 1.24 | 0.005 |
|  |  |  |  |  |
| *H-TAU vs Control* |
|  | Mean of Control | Mean of H-TAU |  |  |
| PE(18:2/0:0) | 5.28×10-4 | 1.56×10-4 | 0.30 | 0.017 |
| PC(18:1/20:5) | 6.75×10-3 | 4.34×10-3 | 0.64 | 0.042 |
| TG(17:0/18:2/20:5) | 1.22×10-3 | 9.48×10-4 | 0.78 | 0.048 |
| TG(19:0/20:1/20:1) | 2.68×10-6 | 4.50×10-6 | 1.68 | 0.039 |
| TG(20:0/20:0/22:0) | 1.14×10-5 | 1.51×10-5 | 1.33 | 0.010 |
| Cer(t15:0/22:0) | 2.47×10-5 | 5.16×10-5 | 2.09 | 0.002 |
| *H-TAU vs M-TAU* |
|  | Mean of M-TAU | Mean of H-TAU |  |  |
| PC(22:6/12:0) | 3.24×10-4 | 7.27×10-6 | 0.02 | 0.000 |
| PC(16:1/18:3) | 5.68×10-4 | 7.27×10-6 | 0.01 | 0.003 |
| PE(18:4/16:0) | 3.00×10-4 | 8.38×10-5 | 0.28 | 0.048 |
| PE(16:0/20:1) | 1.77×10-4 | 5.23×10-5 | 0.29 | 0.047 |
| PE(20:0/18:2) | 3.52×10-4 | 9.78×10-5 | 0.28 | 0.048 |
| PE(22:2/18:0) | 2.88×10-3 | 7.31×10-4 | 0.25 | 0.046 |
| PE(25:0/18:1) | 1.12×10-3 | 7.27×10-6 | 0.01 | 0.001 |
| TG(12:0/14:0/16:1) | 4.50×10-5 | 2.60×10-5 | 0.58 | 0.018 |
| TG(16:0/16:1/16:1) | 2.23×10-5 | 9.90×10-6 | 0.44 | 0.026 |
| TG(16:1/16:1/18:2) | 1.76×10-6 | 3.00×10-6 | 1.70 | 0.045 |
| TG(20:1/20:1/20:5) | 1.23×10-5 | 1.68×10-5 | 1.37 | 0.037 |
| Cer(t18:1/24:4) | 1.89×10-5 | 4.20×10-5 | 2.23 | 0.018 |

PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triglyceride; Cer, ceramide.

**Supplementary TABLE 3 The bile acids which can be detected with the method used in the present study.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | Name | Abbr. | CAS | Molecular formula | Type 1 Primary(P)/Secondary(S) | Type 2 Free(F)/Conjugated(C) |
| 1 | Dehydrolithocholic acid | DHLCA | 1553-56-6 | C24H38O3 | — | F |
| 2 | Allolithocholic acid | alloLCA | 2276-93-9 | C24H40O3 | S | F |
| 3 | Isolithocholic acid | isoLCA | 1534-35-6 | C24H40O3 | S | F |
| 4 | Lithocholic acid | LCA | 434-13-9 | C24H40O3 | S | F |
| 5 | 23-Nordeoxycholic acid | 23norDCA | 53608-86-9 | C23H38O4 | — | F |
| 6 | 7-Ketolithocholic acid | 7-ketoLCA | 4651-67-6 | C24H38O4 | S | F |
| 7 | 12-Ketolithocholic acid | 12-ketoLCA | 5130-29-0 | C24H38O4 | S | F |
| 8 | Apocholic acid | apoCA | 641-81-6 | C24H38O4 | — | F |
| 9 | Ursodeoxycholic acid | UDCA | 128-13-2 | C24H40O4 | P (murine), S (human) | F |
| 10 | Hyodeoxycholic acid | HDCA | 83-49-8 | C24H40O4 | S | F |
| 11 | Chenodeoxycholic acid | CDCA | 474-25-9 | C24H40O4 | P | F |
| 12 | Deoxycholic acid | DCA | 83-44-3 | C24H40O4 | S | F |
| 13 | Isodeoxycholic acid | isoDCA | 566-17-6 | C24H40O4 | — | F |
| 14 | Dehydrocholic acid | DHCA | 81-23-2 | C24H34O5 | — | F |
| 15 | 7,12-Diketolithocholic acid | 7,12-diketoLCA | 517-33-9 | C24H36O5 | S | F |
| 16 | 6,7-Diketolithocholic acid | 6,7-diketoLCA | - | C24H36O5 | S | F |
| 17 | 7-Ketodeoxycholic acid | 7-DHCA | 911-40-0 | C24H38O5 | S | F |
| 18 | 12-Dehydrocholic acid | 12-DHCA | 204023 | C24H38O5 | — | F |
| 19 | 3-Dehydrocholic acid | 3-DHCA | 2304-89-4 | C24H38O5 | — | F |
| 20 | Ursocholic acid | UCA | 2955-27-3 | C24H40O5 | — | F |
| 21 | α-Muricholic acid | α-MCA | 2393-58-0 | C24H40O5 | P | F |
| 22 | β-Muricholic acid | β-MCA | 2393-59-1 | C24H40O5 | P | F |
| 23 | λ-Muricholic acid | λ-MCA | 547-75-1 | C24H40O5 | P | F |
| 24 | Allocholic acid | ACA | 2464-18-8 | C24H40O5 | S | F |
| 25 | Cholic acid | CA | 81-25-4 | C24H40O5 | P | F |
| 26 | Glycolithocholic acid | GLCA | 24404-83-9 | C26H43NO4 | S | C |
| 27 | Glycoursodeoxycholic acid | GUDCA | 64480-66-6 | C26H43NO5 | S | C |
| 28 | Glycohyodeoxycholic acid | GHDCA | 38411-84-6 | C26H43NO5 | S | C |
| 29 | Glycochenodeoxycholic acid | GCDCA | 16564-43-5 | C26H43NO5 | P | C |
| 30 | Glycodeoxycholic acid | GDCA | 16409-34-0 | C26H43NO5 | S | C |
| 31 | Glycodehydrocholic acid | GDHCA | 3415-45-0 | C26H37NO6 | — | C |
| 32 | Glyco-λ-muricholic acid | GλMCA | - | C26H43NO6 | P | C |
| 33 | Glycocholic acid | GCA | 475-31-0 | C26H43NO6 | P | C |
| 34 | Taurolithocholic acid | TLCA | 6042-32-6 | C26H45NO5S | S | C |
| 35 | Tauroursodeoxycholic acid | TUDCA | 14605-22-2 | C26H45NO6S | S | C |
| 36 | Taurohyodeoxycholic acid | THDCA | 110026-03-4 | C26H45NO6S | S | C |
| 37 | Taurochenodeoxycholic acid | TCDCA | 516-35-8 | C26H45NO6S | P | C |
| 38 | Taurodeoxycholic acid | TDCA | 1180-95-6 | C26H45NO6S | S | C |
| 39 | Tauro α-Muricholic acid | T-α-MCA | 25696-60-0 | C26H45NO7S | P | C |
| 40 | Tauro β-Muricholic acid | T-β-MCA | - | C26H45NO7S | P | C |
| 41 | Taurocholic acid | TCA | 81-24-3 | C26H45NO7S | P | C |
| Internal standard | Taurochenodeoxycholic Acid-[d4]  |  | 6009-98-9 | C26H41D4NO6S |  |  |