Aquaculture Reports 43 (2025) 102881

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effects of estradiol exposure on lipid metabolism in male and female sea cucumber *Apostichopus japonicus*

Shuangyan Zhang [a,b,c,d,e], Libin Zhang [a,c,d,e,*], David Gonçalves [f], Jialei Xu [g]

[a] CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b] Zibo Vocational Institute, Zibo 255300, China
[c] Laboratory for Marine Ecology and Environmental Science, Qingdao Marine Science and Technology Center, Qingdao 266237, China
[d] Key Laboratory of Breeding Biotechnology and Sustainable Aquaculture (CAS), Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[e] University of Chinese Academy of Sciences, Beijing 100049, China
[f] Institute of Science and Environment, University of Saint Joseph, Macao 999078, China
[g] Zhongke Tonghe (Shandong) Ocean Technology Co., Ltd, Dongying 257200, China

ARTICLE INFO

Keywords:
Sea cucumber
Sex difference
Physiology
Lipid metabolism
Estradiol

ABSTRACT

Lipids are important energy storage nutrients and signalling molecules for reproduction. Steroid hormones, including estradiol (E_2), have been shown to play a key regulatory role in lipid metabolism in vertebrates. However, not much is known on the action of sex steroids in lipid metabolism in invertebrates. This study investigated the impact of exogenous E_2 administration in lipid metabolism of male and female sea cucumber *Apostichopus japonicus*. Control females had higher crude lipid content in the body wall, but not in the intestines, as compared to control males. E_2 administration significantly reduced the total lipid content in the intestine and body wall of females, but not males. The analysis of intestinal metabolites and gene expression of *A. japonicus* showed that E_2 significantly affected the expression of genes and the levels of metabolites related with lipid metabolism pathway. The metabolism of sex hormones, triglycerides and fatty acids in females was more active as compared to males. The fatty acid degradation and glycerophospholipid metabolism pathways were disturbed by E_2 administration in males. E_2 significantly inhibited the activity of catalase (CAT) in the coelomic fluid, and it is speculated that E_2 may have disrupted lipid metabolism by inducing oxidative imbalance in males by targeting the glycerophospholipid metabolism pathway. The study confirms that sex steroids play a significant role in regulating physiological functions in echinoderms and, in particular, that E_2 might modulate lipid metabolism, as described for vertebrates. The results also suggest that exogenous administration of sex steroids could be used as a tool to regulate reproductive function to improve the rearing of *A. japonicus*.

1. Introduction

During reproduction, most of the energy required for embryo and larval development comes from parental nutrient reserves, and the storage of essential nutrients in the parent determines the efficiency of gonadal development (Rowe et al., 1991; Duray et al., 1994; Huang et al., 2010). Lipids are indispensable nutrients for energy storage and important signaling molecules in life processes, including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Carvalho et al., 2012). Fatty acids, which belong to the fatty acyl group of the lipid classification, are important energy substrates and are involved in several important physiological processes in animal reproduction. For energy storage, triglycerides play a major role. Sterols are hydroxyl-containing steroids, while cholesterol is a common sterol lipid. Cholesterol, an important component of lipoproteins, is a precursor of steroid hormones, which not only have important regulatory roles in reproductive processes but also promote lipid metabolic processes (Yepiz-Plascencia et al., 2000). When the organism is subjected to environmental stress, lipid homeostasis in the body can be disrupted or even cause disruption of the overall organism's physiological state (Lee et al., 2018). Therefore, lipid metabolism is often used as an indicator of immune stress induced by environmental factors.

Steroid hormones have a key regulatory role in lipid metabolism and

* Corresponding author at: CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China.
E-mail address: zhanglibin@qdio.ac.cn (L. Zhang).

https://doi.org/10.1016/j.aqrep.2025.102881
Received 8 December 2023; Received in revised form 30 April 2025; Accepted 12 May 2025
Available online 16 May 2025

are involved in fat deposition, lipolysis, fat transport, and lipoprotein synthesis (Sheridan and Kao, 1998). Estrogen, as a major sex hormone, can be involved in regulating lipid metabolic processes (Palmisano et al., 2017). It has been reported that estradiol (E_2) has an important regulatory role in feeding and energy homeostasis in mammals, and is also involved in the regulation of triglyceride and lipoprotein levels in lipids (Brown and Clegg, 2010). E_2 is also able to regulate physiological processes in aquatic animals, and has an important role in regulating spermatheca development in juvenile rainbow trout and promoting lipoprotein synthesis (Riazi and Fremont, 1988; Bouma et al., 2003). However, E_2 may disrupt the structure of the spermatheca in male zebrafish, leading to a reduction in spermatocytes and causing males to exhibit feminization accompanied by significant fat deposition (Sun et al., 2020). However, the effect of E_2 in zebrafish was concentration-dependent, with an inflammatory response occurring only at high concentrations of stress, whereas at lower concentrations E_2 was detected to promote the lipid deposition process (Sun et al., 2019). For Nile tilapia (*Oreochromis niloticus*), E_2 was found to accelerate the lipid transport process (Zhang et al., 2020). Overall, E_2 has an important regulatory role in both aquatic animal lipogenesis, catabolism, and deposition, and the relative level of the hormone in the body determines the positive or negative effects on lipid metabolism in both male and female individuals.

It has recently been demonstrated in the sea cucumber *Holothuria scabra* that sex steroids, including E_2, were present in neural and gonadal tissues, suggesting that these hormones may also play a significant role in physiological regulation in echinoderms (Thongbuakaew et al., 2021). Although previous studies emphasized the important role of lipid metabolism and sex differences in *Apostichopus japonicus*, and clarified the effects of exogenous E_2 uptake on feeding and gonad development of *A. japonicus*.(Zhang et al., 2021; Zhang et al., 2023a; Zhang et al., 2023b), the physiological effects of E_2 on lipid metabolism remains largely to be investigated. Here, the effects of exogenous administration of E_2 on lipid metabolism of male and female *A. japonicus* was assessed. The crude lipid content in the intestine and body wall, metabolome and transcriptome profiling in the intestines and immunoenzymes activity and malondialdehyde content in the body cavity fluid were evaluated to gain an integrative view on the changes in lipid metabolism induced by E_2 in *A. japonicus*. It was revealed for the first time through which pathway differences E_2 affects lipid metabolism homeostasis in male and female individuals, and explained how sex hormone interference interacts with the intestinal immune-oxidative stress cascade response.

2. Materials and methods

2.1. Sample collection

The sea cucumbers used in the experiment were purchased from Zhongke Tonghe Marine Science and Technology Co Ltd (Dongying, Shandong, China). The wet weight of each individual was recorded after 1 week of temporary rearing before the start of the experiment. One control group and one experimental group were set up. Each group was provided with three parallel culture tanks, and each parallel culture tank was provided with three repeated tanks (n = 3 tanks/group, 10 sea cucumbers/tank). Based on the E_2 content data of sea cucumber coelomic fluid and gonads during the reproductive period (unpublished data from the laboratory), the experimental group was set to expose sea cucumber to 1 μg/L E_2 for 30 days. Among them, E_2 was first dissolved in dimethyl sulfoxide (DMSO) and then added to the breeding water. The same dose of DMSO was added to the control group. During the experiment, ½ of the water in each tank was exchanged, and the aquaculture water of the experimental group was configured in advance and then replaced. The dissolved oxygen was maintained at 8.5 mg/L by continuous oxygenation, and the salinity and pH values were maintained at 30 ‰ and 8.0, respectively. Animals were fed with compound feed (sea mud: feed =3:1, w/w) three times a day, and the feeding conditions were observed to ensure normal feeding during the experiment. At the end of the experiment, the wet weight of the sea cucumbers was recorded again, animals were anesthetized with ice water and dissected, the body walls, coelomic fluid and intestinal tissues were sampled, weighed, and preserved, and the experimental samples were selected from the gonadal growth stage for subsequent analysis. The collected tissue samples were immediately frozen rapidly in liquid nitrogen after sampling and stored at −80°C for subsequent analysis. Twelve spiny sea cucumbers (6 females and 6 males) of about 153.27 ± 14.65 g were selected from each group. Males and females were labelled as M1 and F1 for the control group and as M2 and F2 for the experimental group, respectively.

2.2. Quantification of total lipid content

The body wall and intestines were weighed and placed in a test tube with 8 mL of hydrochloric acid to determine total lipid content. The tubes were placed in a water bath at 70–80°C until the samples were completely digested, the tubes were removed and 10 mL of ethanol added. After cooling, the solution was transferred to a measuring cylinder with a stopper, 25 mL of anhydrous ether was added and shaken, and left to stand for a few minutes. The supernatant was removed and placed in a constant weight bottle, 5 mL of anhydrous ether was added and the extraction was repeated. The anhydrous ether was recovered from the solution and the supernatant was dried in a water bath and then dried at 100 ± 5°C to constant weight. The total lipid content was calculated according to the following formula:

$$X = \frac{m_2 - m_1}{m} \times 100$$

Where m_1 is the original weight of the flask, m_2 is the weight of the flask after final drying, and m is the weight of the sample under test.

2.3. Metabolites detection and analysis

2.3.1. Metabolite extraction

Samples of intestinal tissue were collected separately from male and female sea cucumbers, with 6 individuals of each sex per group. From each sample, 50 mg were used for metabolite detection. The samples were placed into a centrifuge tube containing 1 mL of extraction solvent (acetonitrile: methanol: water = 2:2:1) containing the standard. The samples were first vortexed for 30 s and homogenized at 45 Hz for 4 min, and sonicated in an ice water bath for 5 min, and this process was repeated three times. The samples were incubated at −20 °C for 1 h and centrifuged at 12,000 rpm for 15 min at 4 °C. At the end of centrifugation, the supernatant was transferred to an LC-MS vial and stored at −80°C.

2.3.2. LC-MS/MS analysis

A UHPLC system (1290, Agilent Technologies, USA) equipped with a UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm) and Q Exactive (Orbitrap MS, Thermo) was used. In mobile phase A, the positive pole was 0.1 % formic acid aqueous solution, the negative pole was 5 mmol/L ammonium acetate aqueous solution, and mobile phase B was acetonitrile. The flow rate and injection volume were 0.5 mL/min and 2 μL, respectively. The elution gradient was set to 1 % B at 0 min, 1 % B at 1 min, 99 % B at 8 min, 99 % B at 10 min, 1 % B at 10.1 min, and 1 % B at 12 min. The QE mass spectrometer was operated in the information-dependent acquisition mode (Information Dependent Acquisition, IDA) and was used to record MS/MS spectra. The acquisition software (Xcalibur 4.0.27, Thermo) continuously evaluated and collected full-scan measurements of MS data to record the acquisition of MS/MS spectra. In the Electrospray Ionisation (ESI) source, the capillary temperature was set to 320°C, the flow rates of the sheath gas and the auxiliary gas were 45 Arb and 15 Arb, respectively, and the full MS resolution and the MS/MS resolution were 70,000 and 17,500, respectively, with a

collision energy of 20/40/60 eV. In the model, the spray voltage was 3.8 kV (positive ion mode (POS)), −3.1 kV (negative ion mode (NEG)).

2.3.3. Data preprocessing

MS raw data were converted to mzML format using ProteoWizard and then processed using XCMS (version 3.2) in R for retention time alignment, peak detection, and peak matching. Data were filtered when the number of samples containing metabolites was less than 50 % of all samples in the group. Each sample was normalized against an internal standard. By default, missing values (Not Available, NA) were replaced with half of the smallest value that could be found in the data set. A data matrix was generated from the preprocessing results, including retention times, mass-to-charge ratio values, and peak intensities. After data processing using MS/MS database, peak annotation was performed using OSI-SMMS (version 1.0).

2.3.4. Differential metabolite and KEGG pathway analysis

Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) was used for multivariate statistical analysis to identify differential metabolites between groups. The OPLS-DA model was further validated cross-validation and 200 permutation test. The Variable Importance in Projection (VIP) score of the OPLS model was applied to rank the most distinguishable metabolites between the two groups, where the threshold of VIP was set to 1. T-tests were used to screen for differential metabolites, and metabolites with a $P < 0.05$ and a VIP ≥ 1 were considered to be differential metabolites between the two groups. Metabolites were mapped to KEGG for pathway enrichment analysis (Kanehisa et al., 2007), which identifies metabolic pathways or signaling pathways that are significantly enriched in different metabolites compared to the background. The calculating formula is as follows:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

Where N is the quantity of metabolites annotated by KEGG, n is the quantity of differential metabolites in N, M is the quantity of all metabolites annotated as specific pathways, and m is the quantity of differential metabolites in M. The calculated p-value was corrected by FDR, and FDR< 0.05 was taken as a threshold. The pathways that meet this condition were defined as those that are significantly enriched in the differential metabolites.

2.4. Differential gene expression detection and analysis

2.4.1. RNA extraction and cDNA library construction

The total RNA of the intestinal samples was extracted according to the instructions of the Trizol kit (Ingenuity Life Technologies Ltd.), and RNA quality was assessed using a bioanalyzer (2100, Agilent Technologies Ltd.) and RNase agarose gel electrophoresis. mRNA was enriched using Oligo dT magnetic beads, fragmented using fragmentation buffer and reverse transcribed into cDNA using random primers. Second-strand cDNA was synthesized using DNA polymerase I, RNase H, dNTP, and buffer, and cDNA fragments were purified using the QIAquick PCR Recovery Kit Purification.

2.4.2. Transcriptome sequencing, quality control, and sequence alignment

Raw reads were obtained by sequencing using the Illumina HiSeq2500 instrument from Kidio Biotechnology Ltd. And clean reads were filtered using fastp (version 0.18.0) (Chen *et al.* 2018). The HISAT2.2.4 package was used to compare RNA-seq sample reads against *A. japonicus* reference genome (ASM275485v1) (Kim *et al.* 2015). Reads for each sample were assembled using StringTie v1.3.1 based on the reference genome (Pertea *et al.* 2015). Transcriptome raw data (Bio project: PRJNA951353) were uploaded to NCBI.

2.4.3. Gene expression and pathway enrichment analysis

FPKM (Fragments Per Kilobase of transcript per Million mapped reads) was used to quantify the expression levels of genes. FPKM values were calculated using StringTie software, and different groups were compared by DESeq2 (Love et al., 2014). Genes with False Discovery Rate (FDR) < 0.05 and absolute fold change ($\log_2$ fc) ≥ 2 were considered differentially expressed. Metabolic pathways and signal transduction pathways were used to identify significantly enriched metabolic pathways and signal transduction pathways relative to the genomic background through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

2.5. Immuno-enzyme viability assay

2.5.1. Intestinal tissue homogenate preparation

Intestinal samples were taken for digestive enzyme viability assay. For this, 0.1 g of the sample was weighed and rinsed with 1 × PBS buffer (I010–1–1, Nanjing Jiancheng Bioengineering Institute) for 2–3 times, transferred into 1.5 mL centrifuge tubes to be sheared, and 900 μL of PBS buffer was added. Three small steel beads with a diameter of 3 mm were added to each centrifuge tube, which was transferred into a pre-cooled grinding adapter, and the tissue was fully ground using a multi-sample tissue grinder at a low temperature of 65 Hz for 10 min. After grinding, the beads were removed, and the sample centrifuged at 3500 r/min for 15 min at 4 ℃. The supernatant was transferred into a 2 mL centrifuge tube and stored in at 4 ℃, with the assay being completed within 24 hours.

2.5.2. Detection of intestinal immune-enzyme activity and malondialdehyde content

The reagents used for the determination of superoxide dismutase, catalase activity, and malondialdehyde content in this experiment were purchased from Nanjing Jiancheng Bioengineering Institute, and the relevant experimental operations were carried out according to the instructions of the kit, and the absorbance was used to calculate the relevant data. The assays used are shown in Table 1.

3. Results

3.1. Total lipid content in tissues

Exposure to E_2 decreased the total lipid content both in the intestine and body wall of females (Fig. 1). No significant differences were found in males. In addition, the analysis of the factor sex revealed that there was a significant sex difference in total lipid content only in the body wall and only in the control group, with no significant differences between the other groups.

3.2. Changes in intestinal lipid metabolic pathways

3.2.1. Analysis of differential intestinal metabolites

Significant changes in gut metabolites were observed in both male and female sea cucumbers under E_2 exposure (Fig. 2). In females, 18 metabolites were up-regulated and 15 metabolites were down-regulated in F2 compared to F1, including 16 metabolites up-regulated and 13 metabolites down-regulated in POS mode and 2 metabolites up-regulated and 2 metabolites down-regulated in NEG mode. In males,

Table 1
Detection methods of immune enzyme activity and malondialdehyde content.

Test name	model number	Detection Methods
Total protein (TP)	A045–2	Bradford Assay
Superoxide dismutase (SOD)	A001–1	Hydroxylamine method
Catalase (CAT)	A007–1	Ammonium molybdate method
Malondialdehyde (MAD)	A003–2	Thiobarbituric acid method

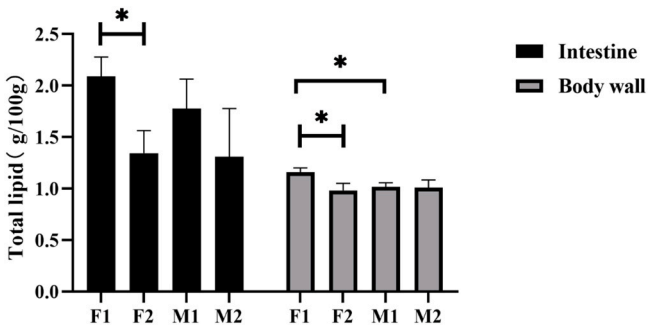

Fig. 1. Total lipid content in the intestine and body wall of male and female sea cucumbers. F – female; M – male; 1 –control; 2 - E_2 exposed.

28 metabolites were up-regulated and 31 metabolites were down-regulated in M2 compared to M1, of which 20 metabolites were up-regulated and 29 metabolites were down-regulated in the POS mode, and 8 metabolites were up-regulated and 2 metabolites were down-regulated in the NEG mode. In addition, sex differences in associated intestinal metabolites were found in both E_2.exposed and control animals. In controls, 22 metabolites were up-regulated and 44 metabolites were down-regulated in F1 compared to M1, with 20 metabolites up-regulated and 38 metabolites down-regulated in the POS mode and 2 metabolites up-regulated and 6 metabolites down-regulated in the NEG mode. In E_2.exposed, 13 metabolites were up-regulated and 24 metabolites were down-regulated in F2 compared to M2, with 10 metabolites up-regulated and 17 metabolites down-regulated in the POS mode and 2 metabolites up-regulated and 7 metabolites down-regulated in the NEG mode.

KEGG analysis of differential metabolites revealed (Fig. 3) that differential metabolites in the intestines of sea cucumbers were mainly enriched in metabolic processes including xenobiotic degradation and metabolism, carbohydrate metabolism, amino acid metabolism, cofactor and vitamin metabolism, nucleotide metabolism, lipid metabolism, terpene and polyketide metabolism, energy metabolism, other amino acid metabolism and other biosynthetic pathways of secondary metabolites. Further screening of the secondary classification of lipid metabolism pathways by enrichment pathways revealed that differential metabolites were mainly enriched in arachidonic acid metabolism, ether lipid metabolism, glycerophospholipid metabolism, linoleic acid metabolism, linolenic acid metabolism, and fatty acid biosynthesis processes.

3.2.2. *Analysis of differentially expressed genes in the intestine*

Differential gene expression analysis revealed (Fig. 4A) that 2473 genes were up-regulated and 852 genes were down-regulated in controls compared to E_2-treated females, and 1062 genes were up-regulated and 668 genes were down-regulated in males for the same comparison. In addition, sex differences in associated intestine-expressed genes were found in both groups. In controls, F1 had 3 genes upregulated and 6 genes downregulated compared to M1, while in E_2-treated F2 had 144 genes up-regulated and 277 genes downregulated compared to M2. KEGG analysis of differentially expressed genes revealed (Fig. 4B) that differential metabolites in the gut of *A. japonicus* were mainly enriched in metabolic processes, including lipid metabolism, sugar biosynthesis metabolism, carbohydrate metabolism, other amino acid metabolism, xenobiotic degradation metabolism, amino acid metabolism, cofactor and vitamin metabolism, energy metabolism, nucleotide metabolism, terpenoid and polyketide metabolism, and other secondary metabolite biosynthesis.

The lipid metabolism pathway was enriched with the most differentially expressed genes in the metabolic classification, and further screening of the secondary classification of lipid metabolism pathways by the enriched pathways revealed that the differentially expressed genes were mainly related to arachidonic acid metabolism, biosynthesis of unsaturated fatty acids, fatty acid metabolism, fatty acid degradation, fatty acid elongation, glycerolipid metabolism, glycerophospholipid metabolism, linoleic acid metabolism, primary bile acid biosynthesis, sphingolipid metabolism, steroid biosynthesis, steroid hormone biosynthesis, and linolenic acid metabolism. Further analysis revealed (Table 2) that differentially expressed genes related to lipid metabolism included *Ugt1a1* (MSTRG.37503), *Pnlip* (AJAP02920, AJAP19672, AJAP22259), *PnlipRP1* (AJAP07124, AJAP17786), *ACADL* (AJAP25403), *betB* (AJAP06596), *FASN* (AJAP22470, AJAP27225), *gpd1* (AJAP02336), and *ECI1* (AJAP04214), and the heatmaps of the differentially expressed genes are shown in Fig. 5.

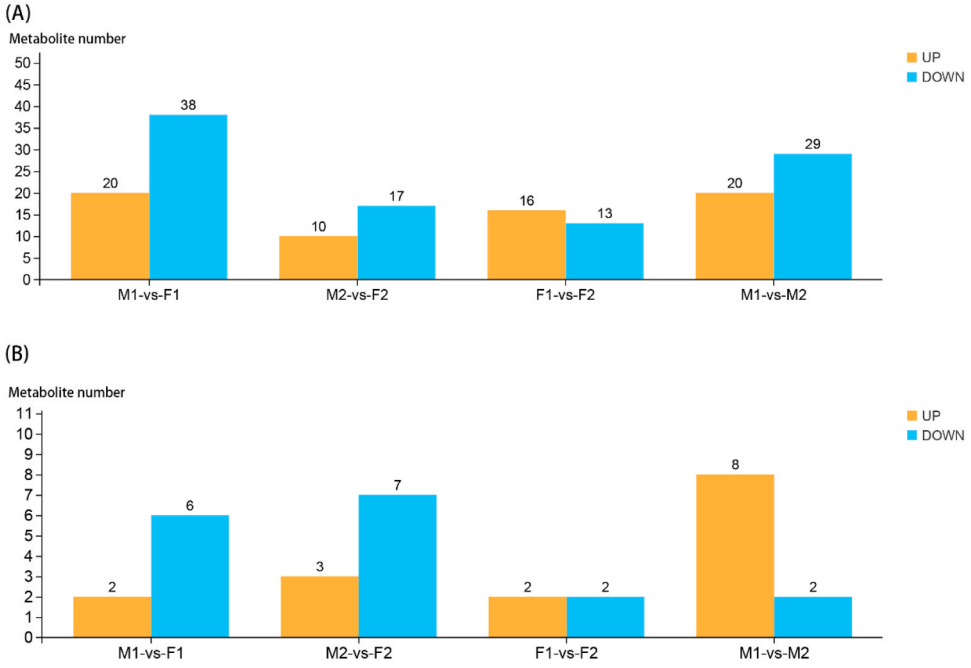

Fig. 2. Histogram of differential metabolites in the intestine of sea cucumbers in each group: (A) POS mode; (B) NEG mode. F – female; M – male; 1 –control; 2 - E_2 exposed.

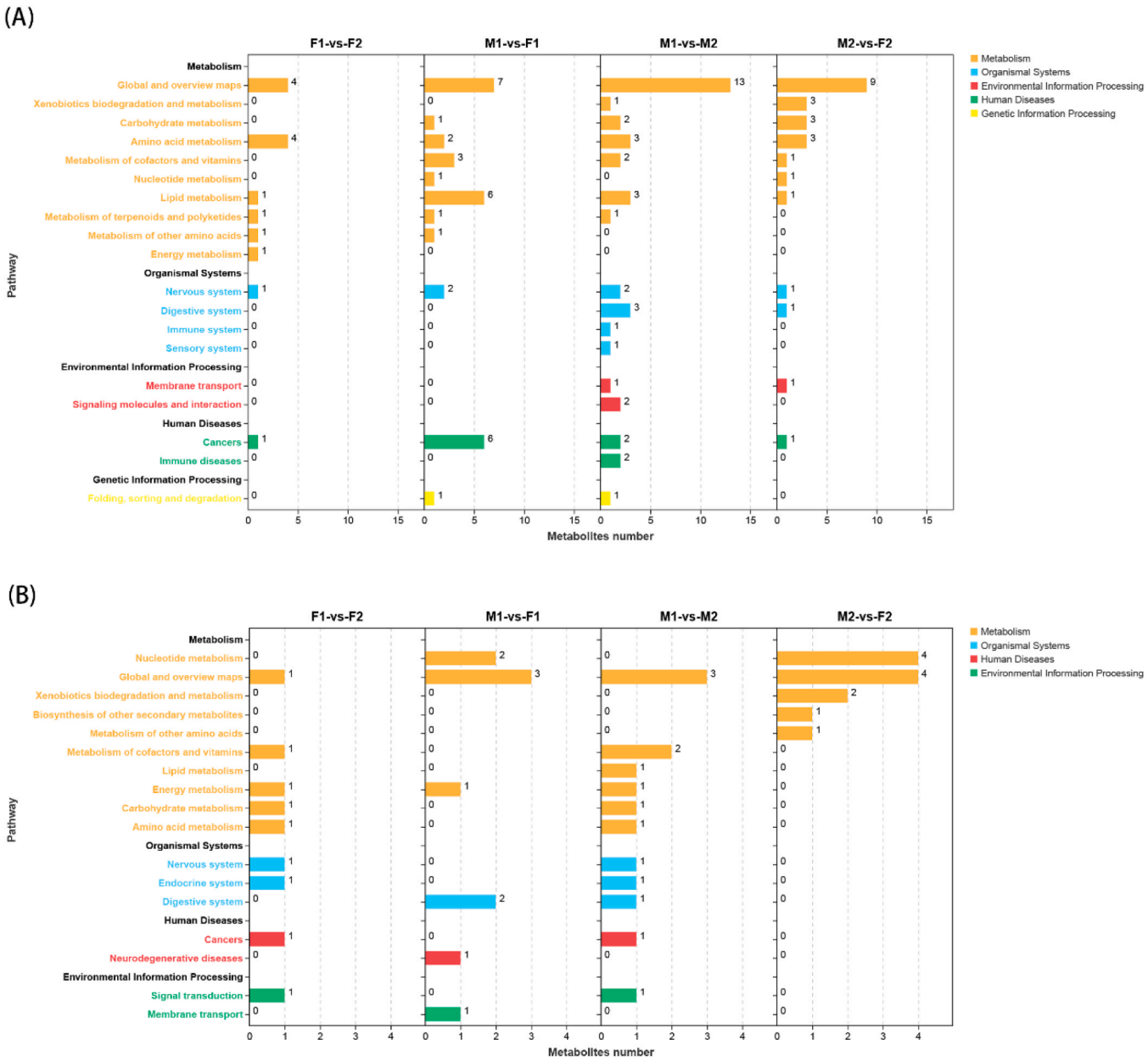

Fig. 3. KEGG enrichment pathway of differential metabolites: (A) POS mode; (B) NEG mode. F – female; M – male; 1 –control; 2 - E_2 exposed.

3.2.3. *Metabolite and differential gene expression analysis*

Combining the differential intestinal metabolites and expressed genes, E_2 had a significant effect on the intestinal lipid metabolism of sea cucumber. Among them, in females, *ugt1a1* is more active in the steroid hormone synthesis pathway, and this gene is involved in regulating the metabolism of estradiol. After a combined analysis of gene expression and metabolites (Fig. 6), it was found that the activity of *gpd1* in Glycerophosphoric acid synthesis was enhanced, the content of lipid metabolites such as lysophosphatidylcholine (LysoPC), octanoic acid, and choline was significantly altered in males exposed to E_2. The up-regulation of choline and LysoPC might be mainly affected by the regulation of glycerophospholipid metabolism. The intestinal lipid metabolism pathways of sea cucumber affected by E_2 mainly included triglyceride metabolism, glycerophospholipid metabolism, fatty acid biosynthesis, fatty acid degradation, steroid hormone synthesis, and sphingolipid metabolism. The sex hormone, triglyceride, and fatty acid metabolism were upregulated in female sea cucumber, and fatty acid degradation and glycerophospholipid metabolism were downregulated in male sea cucumber.

3.3. *Immune enzyme activity and MDA content assay*

The SOD and CAT enzyme activity, and MDA content in the coelomic fluid of the sea cucumbers were examined (Fig. 7), and only the SOD enzyme activity was not significantly different between the groups. In males, but not in females, CAT enzyme activity was reduced by E_2, resulting in a significant sex difference in the activity of this enzyme in the body cavity fluid between males and females exposed to E_2. The results for MDA showed that there was a significant sex difference in levels between male and female sea cucumbers, which were not affected by E_2.

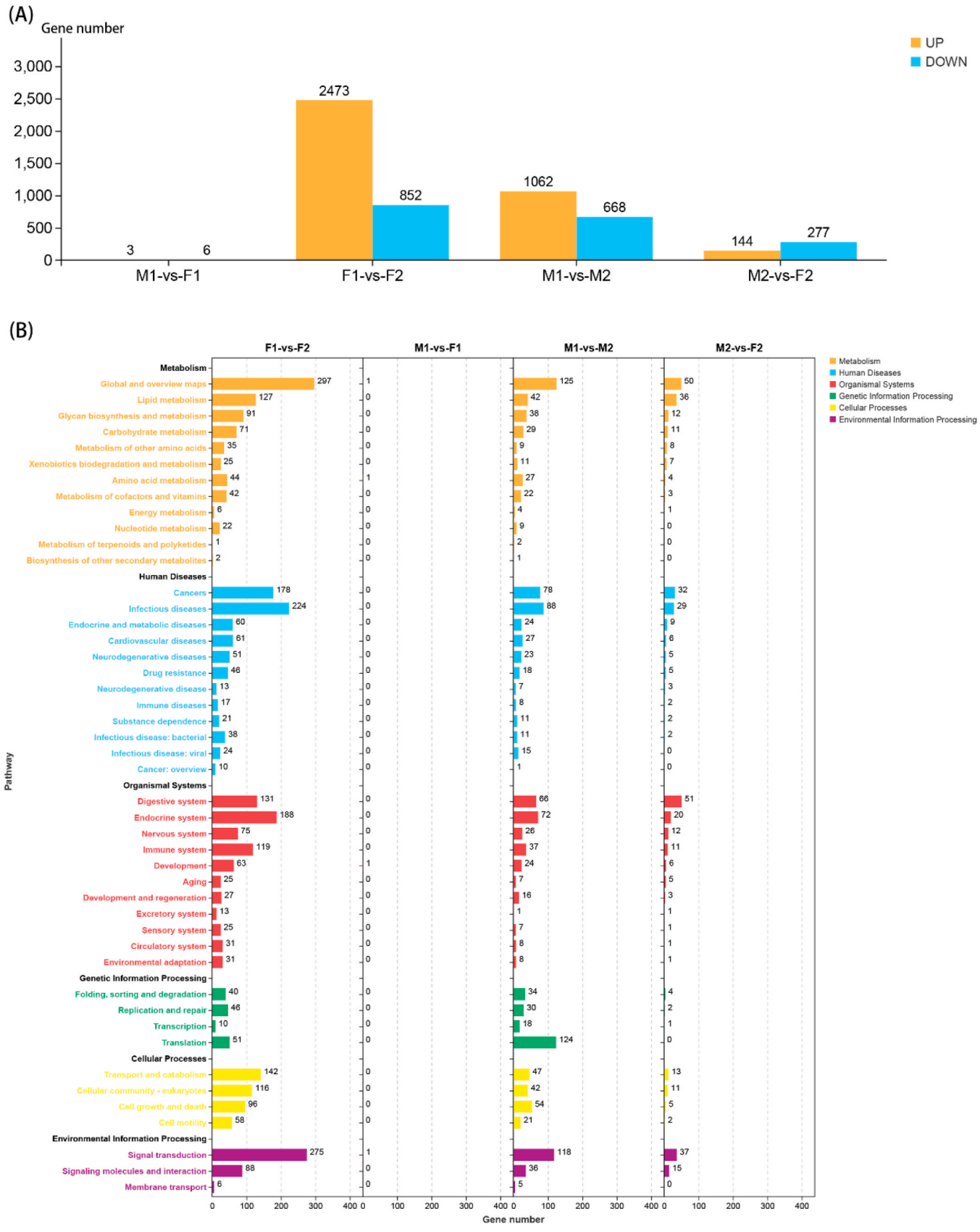

Fig. 4. Differentially expressed genes (A) and their KEGG enrichment pathways (B) among groups. F – female; M – male; 1 –control; 2 - E_2 exposed.

Table 2
Differentially expressed genes related to lipid metabolism pathway.

Gene id	Symbol	Function
MSTRG.37503	*Ugt1a1*	Steroid hormone synthesis
AJAP02920	*Pnlip*	Glycerol ester metabolism
AJAP19672		
AJAP22259		
AJAP07124	*PnlipRP1*	
AJAP17786		
AJAP25403	*ACADL*	Fatty acid degradation
AJAP06596	*betB*	Glyceride metabolism, fatty acid degradation
AJAP22470	*FASN*	Fatty acid biosynthesis
AJAP27225		
AJAP02336	*gpd1*	Glycerophospholipid metabolism
AJAP04214	*ECI1*	Fatty acid degradation

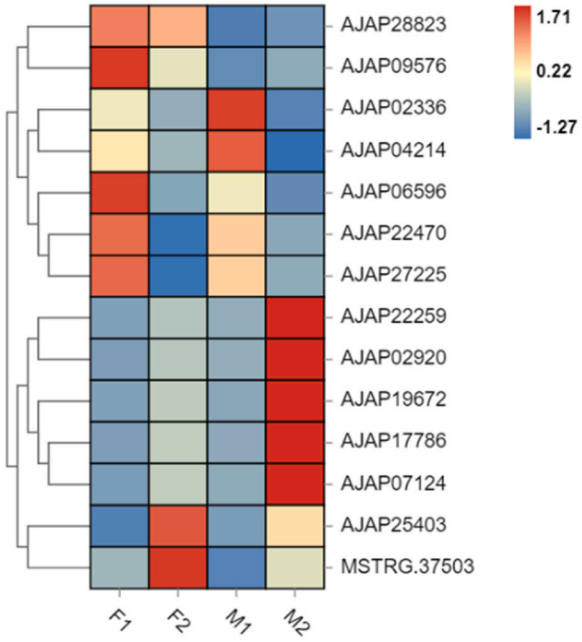

Fig. 5. Heatmap of differentially expressed genes related to lipid metabolism in the intestines of sea cucumbers. F – female; M – male; 1 –control; 2 - E_2 exposed.

4. Discussion

4.1. Estradiol is involved in lipolysis of A. japonicus

In mammals, lipid metabolism is an important part of estrogen-regulated energy metabolism, and estrogen activates the lipolysis pathway by binding to the estrogen receptor. Estrogen promotes the accumulation of liver fat in fish, and when excessive fat is accumulated in the liver, lipid peroxidation is triggered to induce fatty liver in fish, and lipid peroxidation also causes inflammation and oxidative stress, which may lead to increased mortality in severe cases (Adeyemi et al., 2014). However, it has also been found that estrogen promotes fish growth and improves metabolic efficiency. The effect of E_2 on lipid levels and lipid metabolism in aquatic animals is concentration-dependent. Thus, E_2 has an important regulatory role in aquatic animal lipogenesis, catabolism, and deposition, and the relative levels of this hormone in the body determine the effects on individual lipid metabolism (Hiroaki et al., 1993).

It was found that fat vacuoles and lipid deposition appeared in hepatocytes of swordtails under 50 μg/L E_2 exposure, and the degree of liver tissue damage changed with the duration of E_2 exposure, with more severe damage with longer exposure time (Wen et al., 2013). In zebrafish studies, significant fat deposition usually occurs in males after E_2 administration, but due to the concentration-dependent effect of E_2 on lipid deposition, this effect occurs only at relatively low concentrations, whereas higher concentrations directly trigger inflammatory and antioxidant responses (Sun et al., 2019, 2020). E_2 was also able to accelerate the fatty acid transport process in Nile tilapia (*O. niloticus*), inducing the secretion of LDLR (low density lipoprotein receptor) and LDL (low density lipoprotein) to promote fatty acid production by stimulating the expression of triglycerides, transferrin, and VLDL (Zhang et al., 2020). In addition, immature rainbow trout (*O. mykiss*) treated with E_2 also showed a significant increase in total plasma lipids (Mercure et al., 2001). Exogenous E_2 is not only capable of affecting the lipid content of fish plasma, but also interferes with the body lipid content of fish, and a significant increase in the lipid reserves of female golden shiner (*Notemigonus crysoleucas*) has been reported in response to E_2 administration (De Vlaming et al., 1977).

Although the effects of estrogens in lipid metabolism have been relatively well-investigated in vertebrates, the possibility that these hormones also play a similar role in sea cucumbers had not been investigated, although it is now clear that echinoderms also synthesize sex steroids, including E_2 (Thongbuakaew et al., 2021). In this research, E_2 was found to significantly affect the total lipid content in the intestines and body walls of sea cucumbers, while the total lipid content in the body walls of control animals showed a significant sex difference. In females, total lipid content was more affected by E_2, with a significant decrease in both intestines and body walls, while no significant changes were found in males. Thus, E_2 did not cause lipid deposition in the intestines and body walls of sea cucumbers but rather promoted lipolysis, which is consistent with the findings in the Japanese Blue Crab *Portunus trituberculatus* (Liu, 2016). Characteristics limiting fatty acid synthesis and/or restricting esterification processes have also been found in phytoestrogens, and adipogenesis in adipocytes is limited when using the same concentration of E_2 (Szkudelska et al., 2000). Walking catfish (*Clarias batrachus*) catabolize stored fat under starvation to provide free fatty acids for energy production (Lal et al., 2013). Since it has been found in previous studies that exogenous E_2 can inhibit the daily feeding rate of sea cucumber, inhibition of feeding behavior may be the main reason for the decrease of lipid content in sea cucumber tissues.

4.2. Estradiol can cause abnormal lipid metabolism in sea cucumbers

Lipids are important components for individuals to maintain normal physiological activities, and lipid homeostasis is affected by environmental factors (Lee et al., 2018). In recent years, environmental estrogenic pollutants, including exogenous E_2, and BPA, have received much attention from the mariculture industry, and there have been numerous studies on their effects on the growth, development, and reproduction of aquatic animals. Estrogens not only cause changes in fat content but also impact the metabolism related with sex differentiation and development of aquatic animals. For example, E_2 can disrupt the lipid metabolism pattern of male zebrafish gonads into a feminized pattern by regulating the expression of lipid metabolism-related genes. The digestive gland was found to be a target tissue for E_2 in studies on mussels (*Mytilus galloprovincialis*) (Canesi et al., 2007). Since the sea cucumber does not have liver tissue, the intestine was chosen to be analyzed for E_2 effects on lipid catabolism in the sea cucumber.

In accordance to the results obtained for total lipid content, analysis of differentially expressed genes in the gut revealed that the expression of the *FASN* gene, which is associated with fatty acid synthesis, was significantly downregulated by E_2 in both males and females. FASN activity determines the ability to synthesize fatty acids, and reduced levels of *FASN* gene expression leads directly to reduced lipid deposition (Menendez and Lupu, 2007). With an adequate supply of oxygen in the body, fatty acids can generate large amounts of energy through oxidative catabolism, a process known as β-oxidation of fatty acids (Madureira et al., 2016). Previous studies have suggested that the *FASN* gene is an important indicator of whether lipid metabolism is disturbed

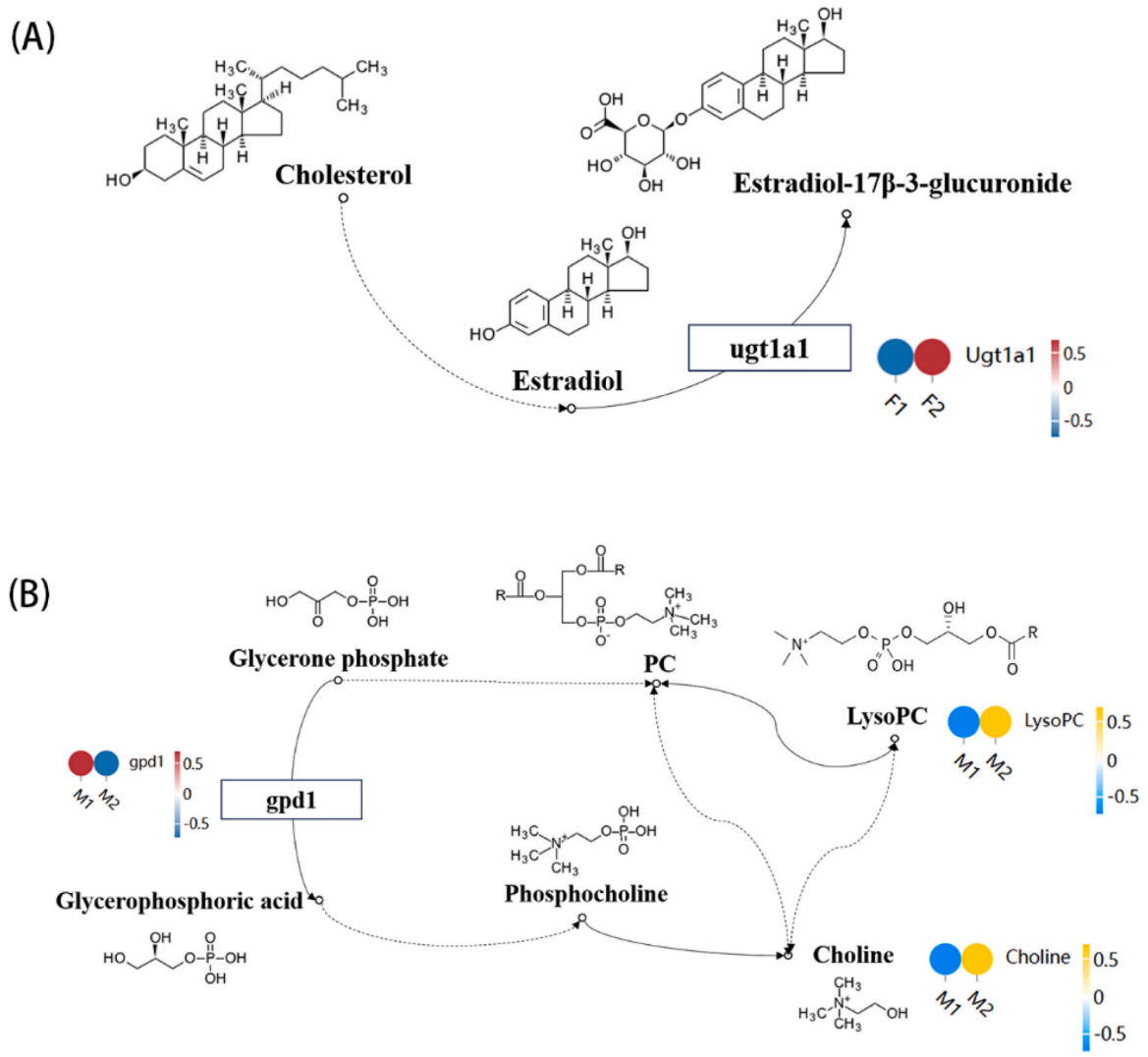

Fig. 6. The responses of E_2 on the intestinal lipid metabolism pathways of male and female sea cucumbers. (A) is a female sea cucumber; (B) is a male sea cucumber.

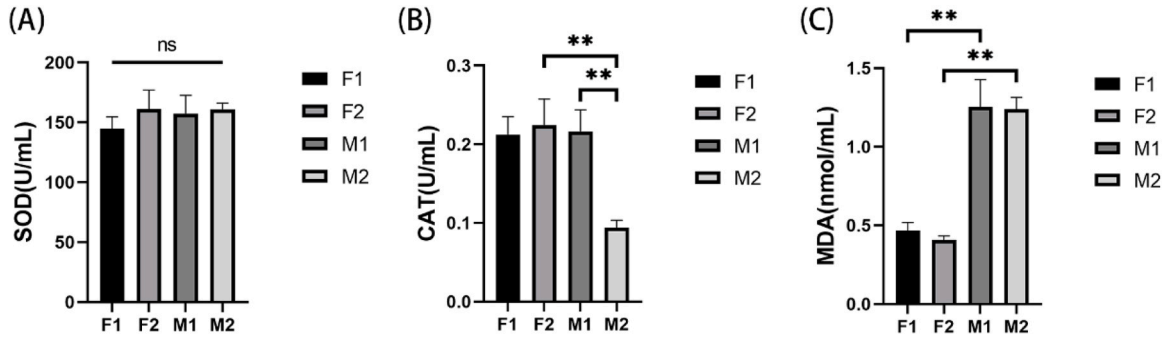

Fig. 7. SOD activity (A), CAT activity (B) and MDA content (C) in the coelomic fluid of sea cucumbers. F – female; M – male; 1 –control; 2 - E_2 exposed.

or not, and it was found in fish studies that BPA, a weak agonist for E_2 receptors, can affect lipid metabolism by inhibiting β-oxidation of fatty acids by interfering with the expression of *FASN* (Guan et al., 2016, 2019). In contrast, studies in zebrafish found that E_2 caused changes in metabolic pathways related to lipid metabolism and could significantly increase the expression of genes related to lipid synthesis, including the *FASN* gene (Sun et al., 2019, 2020). Differences between studies may relate to concentration-dependent effects of E_2 on lipid metabolism or to species-differences in E_2 action. In a study with carp (*Cyprinus. carpio*), high concentrations of estrogen analogs upregulate the expression of lipid synthesis-related genes while downregulating the expression of lipid catabolism genes, whereas lipid synthesis-related genes would be downregulated and lipid catabolism-related genes would be upregulated at low and medium concentrations (Yang et al., 2022). *ACADL* genes related to fatty acid degradation were significantly up-regulated in both male and female exposed to E_2.

Metabolomic and transcriptomic data in the gut revealed that sex hormone, triglyceride, and fatty acid metabolism were more active in

females than in males. The upregulation of *Ugt1a1, a* gene related to steroid hormone synthesis, and the significant increase in E_2 glucuronide levels in females were not observed in males, suggesting that female sea cucumbers have a better ability to balance exogenous E_2 than males. Fatty acid degradation and glycerophospholipid metabolism were found to be disturbed in males, and the expression of *gpd1* gene related to glycerophospholipid metabolism was significantly down-regulated in the intestine in animals exposed to E_2, with levels of choline, PC, and LysoPC significantly increased. This suggests that glycerophospholipid metabolism may be a potential target pathway of E_2 in male *A. japonicus*. In a study on *Macrobrachium rosenbergii,* fatty acid, and glycerophospholipid metabolism-related proteins were found to be highly correlated with peroxide levels (Liu et al., 2022). Free radicals in the body can react with phospholipids synthesized from glycerophospholipids to produce lipid peroxides, and when fat is over-accumulated it can produce harmful lipid peroxides, which, because cell membranes are enriched in phospholipids, can cause damage to the cells when combined with oxygen free radicals (Bidlack and Tappel, 1973; Dong et al., 2021). The content of MDA as well as SOD and CAT enzyme activities in the coelomic fluid were examined, and it was found that CAT enzyme activities in males were significantly reduced by E_2 administration, which indicated that male sea cucumbers exposed to E_2 reduced the antioxidant capacity in the coelomic fluid to a certain extent. Therefore, E_2 may have caused abnormal lipid metabolism by inducing oxidative imbalance in male sea cucumber.

5. Conclusion

The study shows that exogenous E_2 had a significant impact in lipid metabolism in sea cucumber. E_2 significantly affected the total lipid content in the intestine and body wall of female sea cucumber, and was able to promote lipid decomposition, resulting in sex differences in total lipid content of the body wall. It was found that E_2 significantly affected the expression of genes and levels of metabolites related with the lipid metabolic pathway, with the metabolism of sex hormones, triglycerides, and fatty acids being more active in females, while fatty acid degradation and glycerol-phospholipid metabolism pathway were disturbed in males. E_2 significantly inhibited the enzyme activity of catalase in the coelomic fluid and it was hypothesized that E_2 caused lipid metabolism disruption by inducing an oxidative imbalance in males, with the glycerophospholipid metabolism being identified as a potential target pathway. Overall, the results agree with recent evidence showing that sex steroids are synthesized by sea cucumber and play relevant physiological functions also in echinoderms, as described for vertebrates.

CRediT authorship contribution statement

Zhang Shuangyan: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Zhang Libin:** Writing – review & editing, Funding acquisition, Conceptualization. **Gonçalves David:** Writing – review & editing, Funding acquisition. **Xu Jialei:** Writing – review & editing, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42461160255), and the Macao Science and Technology Development Fund (FDCT0006/2024/AFJ), and Shandong Provincial Natural Science Foundation of China (ZR2024QD125). This work had the support of the Sino-Portugal Joint Laboratory in Marine and Environmental Sciences.

Data availability

I have shared links to my data in the manuscript

References

Adeyemi, J.A., Adewale, O.O., Oguma, A.Y., 2014. Mortality, oxidative stress and hepatotoxicity in juvenile African catfish, *Clarias gariepinus* Burchell, exposed to lead and cypermethrin. Bull. Environ. Contam. Toxicol. 92, 529–533.
Bidlack, W.R., Tappel, A.L., 1973. Damage to microsomal membrane by lipid peroxidation. Lipids 8, 177–182.
Bouma, J., Cloud, J., Nagler, J., 2003. In vitro effects of estradiol-17β on myoid and fibroblastic cell proliferation in the immature rainbow trout testis. Fish. Physiol. Biochem. 28, 191–192.
Brown, L., Clegg, D., 2010. Central effects of estradiol in the regulation of food intake, body weight, and adiposity. J. Steroid Biochem. Mol. Biol. 122, 65–73.
Canesi, L., Borghi, C., Fabbri, R., Ciacci, C., Lorusso, L.C., Gallo, G., Vergani, L., 2007. Effects of 17β-estradiol on mussel digestive gland. Gen. Comp. Endocrinol. 153, 40–46.
Carvalho, M., Sampaio, J.L., Palm, W., Brankatschk, M., Eaton, S., Shevchenko, A., 2012. Effects of diet and development on the *Drosophila* lipidome. Mol. Syst. Biol. 8, 600.
De Vlaming, V., Shing, J., Paquette, G., Vuchs, R., 1977. In vivo and in vitro effects of oestradiol-17β on lipid metabolism in *Notemigonus crysoleucas*. J. Fish. Biol. 10, 273–285.
Dong, X., Qin, W., Fu, Y., Ji, P., Wang, J., Du, X., Miao, S., Sun, L., 2021. Effects of dietary betaine on cholesterol metabolism and hepatopancreas function in gibel carp (*Carassius gibelio*) fed with a high-fat diet. Aquac. Nutr. 27, 1789–1797.
Duray, M., Kohno, H., Pascual, F., 1994. The effect of lipid-enriched broodstock diets on spawning and on egg and larval quality of hatchery-bred rabbitfish (*Siganus guttatus*). Philipp. Sci. 31, 42–57.
Guan, Y., Gao, J., Zhang, Y., Chen, S., Yuan, C., Wang, Z., 2016. Effects of bisphenol A on lipid metabolism in rare minnow *Gobiocypris rarus*. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 179, 144–149.
Guan, Y., Zhang, T., He, J., Jia, J., Zhu, L., Wang, Z., 2019. Bisphenol A disturbed the lipid metabolism mediated by sterol regulatory element binding protein 1 in rare minnow *Gobiocypris rarus*. Aquat. Toxicol. 207, 179–186.
Hiroaki, C., Kenji, I., Kazuo, H., Kohei, Y., 1993. Effects of dietary estradiol-17β on feminization, growth and body composition in the Japanese eel (*Anguilla japonica*). Comp. Biochem. Physiol. Part A: Physiol. 106, 367–371.
Huang, X., Yin, Y., Shi, Z., Li, W., Zhou, H., Lv, W., 2010. Lipid content and fatty acid composition in wild-caught silver pomfret (*Pampus argenteus*) broodstocks: effects on gonad development. Aquaculture 310, 192–199.
Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T., 2007. KEGG for linking genomes to life and the environment. Nucleic Acids Res. 36, D480–D484.
Lal, B., Sarang, M.K., Kumar, P., 2013. Malathion exposure induces the endocrine disruption and growth retardation in the catfish, *Clarias batrachus* (Linn.). Gen. Comp. Endocrinol. 181, 139–145.
Lee, M.-C., Park, J.C., Lee, J.-S., 2018. Effects of environmental stressors on lipid metabolism in aquatic invertebrates. Aquat. Toxicol. 200, 83–92.
Liu, M., Sun, C., Zheng, X., Zhou, Q., Liu, B., Zhou, Y., Xu, P., 2022. Comparative proteomic analysis revealed the mechanism of tea tree oil targeting lipid metabolism and antioxidant system to protect hepatopancreatic health in macrobrachium rosenbergii. Front. Immunol. 13, 906435.
Liu, M., 2016. Effects of estradiol on ovarian development and gene expression of estrogen related receptor of swimming crab, *Portunus trituberculatus*. Shanghai Ocean University.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 1–21.
Madureira, T.V., Castro, L.F.C., Rocha, E., 2016. Acyl-coenzyme A oxidases 1 and 3 in brown trout (Salmo trutta f. fario): can peroxisomal fatty acid β-oxidation be regulated by estrogen signaling? Fish. Physiol. Biochem. 42, 389–401.
Menendez, J.A., Lupu, R., 2007. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat. Rev. Cancer 7, 763–777.
Mercure, F., Holloway, A.C., Tocher, D.R., Sheridan, M.A., Kraak Van der, G., Leatherland, J.F., 2001. Influence of plasma lipid changes in response to 17β-oestradiol stimulation on plasma growth hormone, somatostatin, and thyroid hormone levels in immature rainbow trout. J. Fish. Biol. 59, 605–615.
Palmisano, B.T., Zhu, L., Stafford, J.M., 2017. Role of estrogens in the regulation of liver lipid metabolism. Sex and gender factors affecting metabolic homeostasis. Diabetes Obes. 227–256.
Riazi, A., Fremont, L., 1988. Serum vitellogenin and yolk proteolipid complex composition in relation to ovarian growth in rainbow trout *Salmo gairdneri* (Rich.). Comp. Biochem. Physiol. Part B: Comp. Biochem. 89, 525–529.
Rowe, D., Thorpe, J., Shanks, A., 1991. Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci. 48, 405–413.
Sheridan, M.A., Kao, Y.-h, 1998. Regulation of metamorphosis-associated changes in the lipid metabolism of selected vertebrates. Am. Zool. 38, 350–368.
Sun, S.-X., Wu, J.-L., Lv, H.-B., Zhang, H.-Y., Zhang, J., Limbu, S.M., Qiao, F., Chen, L.-Q., Yang, Y., Zhang, M.-L., 2020. Environmental estrogen exposure converts lipid

metabolism in male fish to a female pattern mediated by AMPK and mTOR signaling pathways. J. Hazard. Mater. 394, 122537.
Sun, S.-X., Zhang, Y.-N., Lu, D.-L., Wang, W.-L., Limbu, S.M., Chen, L.-Q., Zhang, M.-L., Du, Z.-Y., 2019. Concentration-dependent effects of 17β-estradiol and bisphenol A on lipid deposition, inflammation and antioxidant response in male zebrafish (*Danio rerio*). Chemosphere 237, 124422.
Szkudelska, K., Nogowski, L., Szkudelski, T., 2000. Genistein affects lipogenesis and lipolysis in isolated rat adipocytes. J. Steroid Biochem. Mol. Biol. 75, 265–271.
Thongbuakaew, T., Suwansa-ard, S., Chaiyamoon, A., Cummins, S.F., Sobhon, P., 2021. Sex steroids and steroidogenesis-related genes in the sea cucumber, *Holothuria scabra* and their potential role in gonad maturation. Sci. Rep. 11, 2194.
Wen, R., Zhong, Y., Fanf, Z., Zhang, Z., 2013. Effects of 17β-estradiol on the development of the testis and liver in male swordtail fish (*Xiphophorus helleri*). Chin. J. Zool. 48, 174–181.
Yang, L., Zhang, W., Zhi, S., Liu, M., Zhao, M., Qin, C., Yan, X., Feng, J., Nie, G., 2022. Effects of genistein on glucose and lipid metabolism of common carp (*Cyprinus carpio*. L) in vivo and in vitro. Aquac. Rep. 22, 100930.
Yepiz-Plascencia, G., Vargas-Albores, F., Higuera-Ciapara, I., 2000. Penaeid shrimp hemolymph lipoproteins. Aquaculture 191, 177–189.
Zhang, S., Ru, X., Zhang, L., Gonçalves, D., Yang, H., Xu, J., 2023b. Effects of a high-fat diet on intestinal and gonadal metabolism in female and male sea cucumber *Apostichopus japonicus*. Biology 12, 212.
Zhang, S., Ru, X., Zhang, L., Gonçalves, D., Yang, H., 2023a. Examination of sex-related differences in intestinal and gonadal lipid metabolism in the sea cucumber *Apostichopus japonicus*. Aquaculture 562, 738787.
Zhang, S., Zhang, L., Ru, X., Ding, K., Feng, Q., 2021. Transcriptome analysis of gender-biased CYP genes in gonads of the sea cucumber *Apostichopus japonicus*. Comp. Biochem. Physiol. Part D: Genom. Proteom. 38, 100790.
Zhang, X., Zhong, H., Han, Z., Tang, Z., Xiao, J., Guo, Z., Wang, F., Luo, Y., Zhou, Y., 2020. Effects of waterborne exposure to 17β-estradiol on hepatic lipid metabolism genes in tilapia (*Oreochromis niloticus*). Aquac. Rep. 17, 100382.