***fat-1* transgenic zebrafish are protected from abnormal lipid deposition induced by high-vegetable oil feeding**

Shouxiang Sun¹, Filipe Castro^{3,4}, Óscar Monroig⁵, Xiaojuan Cao^{1,2}, Jian Gao^{1,2*}

¹College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education/Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, China.

²College of Fisheries, Hubei Provincial Engineering Laboratory for Pond Aquaculture, Huazhong Agricultural University, Wuhan 430070, China.

³CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

⁴FCUP - Faculty of Sciences, Department of Biology, University of Porto, Portugal

⁵Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellon 12595, Spain

*Corresponding author

TEL: 86(027)87282113. Email address: gaojian@mail.hzau.edu.cn (J. Gao)

Postal address: No.1 Shizishan Stress, Hongshan District, Wuhan 430070, Hubei Province, China

Abstract: High dietary concentration of vegetable oil, particularly those rich in n-6 polyunsaturated fatty acids (PUFAs), can induce negative physiological effects including excessive lipid deposition in teleost fish. Omega-3 desaturase (Fat-1) of *Caenorhabditis elegans* is able to convert n-6 PUFAs to n-3 PUFAs and thus induces a low n-6/n-3 PUFAs ratio alleviating lipid deposition. In this study, we investigated the effects of dietary n-6 PUFAs on lipid metabolism of *fat-1* transgenic zebrafish (Tg:*fat-1*), to explore the role of *fat-1* in fish lipid metabolism. We first generated Tg:*fat-1* zebrafish and assayed the effects of a low-fat diet (LFD) and a high-fat diet (HFD) prepared from soybean oil. Wild type zebrafish (WT) fed with HFD (HFD-WT) exhibited increased obesity and lipid deposition, especially in the abdominal cavity and liver. These defects were absent from HFD-Tg:*fat-1*. For each diet group, Tg:*fat-1* exhibited significantly decreased levels of almost all hepatic lipid classes compared with WT. Expression levels of lipid synthesis-related genes and lipid deposition-related genes were markedly lower in liver of HFD-Tg:*fat-1* compared with HFD-WT. In contrast, the steatolysis-related genes significantly up-regulated in HFD-Tg:*fat-1*. Then expression profiles of mitochondrial energy metabolism-related genes and ATP contents in livers from LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* were determined. Our findings suggest that *fat-1* protects fish from abnormal lipid deposition induced by high-vegetable oil feeding, through endogenously converting n-6 PUFAs to n-3 PUFAs.

Key words: *fat-1*, zebrafish, vegetable oil-feeding, lipid deposition, mitochondrial energy metabolism

Key Points

- *fat-1* transgenic zebrafish (Tg:*fat-1*) can endogenously convert n-6 PUFAs to n-3 PUFAs.
- Tg:*fat-1* avoid serious abnormal lipid deposition induced by high-vegetable oil feeding.
- *fat-1* transgenesis effectively improved lipid metabolism and mitochondrial energy metabolism in zebrafish.

Introduction

Aquaculture provides a significant share of long-chain polyunsaturated fatty acids (LC-PUFAs) for human consumption (Leaver et al. 2008). Therefore, aquaculture production needs to steadily grow to meet people's demands (Bostock et al. 2010). In recent years, with the development of aquafeed industry, the increasing demand for fish oil (FO) has imposed an immense pressure on fishery resources, beyond their maximum sustainable limit (Leaver et al. 2008). Vegetable oil (VO), with relatively considerable output, acceptable price, low organic contaminant status, and high content of unsaturated fatty acids, is considered a promising alternative to FO (Orsavova et al. 2015). Yet, high-fat diets (HFDs) with high inclusion of VO have been increasingly used for cost-effective farming in aquaculture in recent years. At the core of its use is the notion that dietary protein can be spared for growth whereas dietary lipids are utilized as energy source (Boujard et al. 2004; Li et al. 2012). A downside of this strategy is that HFDs often lead to ectopic lipid accumulation in farmed fish tissues, especially in liver and abdominal adipose tissue, and induce metabolic disturbances and aquatic diseases (Ruedajasso et al. 2004; Montero et al. 2015; Zuo et al. 2015).

Evidence has been accumulating that abnormal lipid deposition and altered lipid oxidation in the liver is often associated with an impaired mitochondrial energy metabolism (Holmström et al. 2012; Matsuda and Shimomura 2013; Putti et al. 2015). Mitochondria are the central organelle for energy metabolism (Wang et al. 2016). Lipid metabolism largely depends on mitochondria to metabolize the nutrients and generate cellular energy in the form of adenosine triphosphate (ATP) (Osellame et al. 2012). When excessive caloric intake increases the mitochondrial substrate load, or induces mitochondrial dysfunction and respiratory chain disorder, uncoupling protein 1 (*ucp-1*) uncouples mitochondrial respiration from ATP production by causing protons to leak across the inner mitochondrial membrane, enabling energy dissipation in the form of heat (Echtay et al. 2002). Mitochondrial energy metabolism disorder can be mitigated in n-3 PUFA-rich tissue (Demaision et al. 1994). The replacement of fish oil with linoleic acid (LA)-rich vegetable oil significantly decreases n-3 PUFA content in the fish. We previously identified the lipids accumulation occurred in the fish fed with high-LA diet (Li et al. 2015).

It has been reported that lipid deposition in liver was negatively correlated with dietary n-3/n-6 PUFAs ratio (Ruedajasso et al. 2004). These indicated that n-3 PUFAs were closely related to lipid deposition alleviating in fish.

Omega-3 desaturase gene (*fat-1*) encodes an n-3 fatty acid desaturase found in the *Caenorhabditis elegans* genome (Spychalla et al. 1997; Kang et al. 2004). A string of studies have previously shown that *fat-1* transgenic mice, pig, carp and zebrafish had an increasing content of n-3 PUFAs (Kim et al. 2012; Pang et al. 2014; Li et al. 2018; Zhang et al. 2018). In mammals, endogenously synthesized n-3 PUFAs in *fat-1* transgenic mice could ameliorate non-alcoholic fatty liver disease induced by HFDs (Song et al. 2008; Kim et al. 2012). However, whether endogenously synthesized n-3 fatty acids by *fat-1* transgenesis dramatically prevent the abnormal lipid deposition caused by high-VO diets in farmed fish is still unclear, and an evident gap has remained regarding the overall molecular mechanism.

In this study, we used a *fat-1* transgenic zebrafish (Tg:*fat-1*) to dissect the molecular landscape and mechanisms governing lipid deposition under two types of diets (an HFD and a low-fat diet (LFD)) prepared from soybean oil, rich in 18:2n-6. We compared fatty acid composition of muscle and liver, and hepatic lipidome between wild type zebrafish (WT) and Tg:*fat-1*, and determined lipid accumulation in the liver and abdominal cavity. The molecular mechanism for *fat-1* reducing lipid deposition in zebrafish was explored based on the expression profiles of lipid metabolism-related genes, mitochondrial energy metabolism-related genes and ATP contents in livers of wild type zebrafish fed with LFD (LFD-WT), LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. Our results provide useful information for the treatment of abnormal lipid deposition in fish induced by high-vegetable oil feeding.

Materials and methods

Construction of the *fat-1* transgenic zebrafish

The *fat-1* sequences were amplified by PCR using a pair of primers (*fat-1F* and *fat-1R*) from *C. elegans* cDNA (Supplemental Table S1). The bipartite CMV promoter and SV40 poly (A) was the backbone of *fat-1* expression vector. SV40 poly (A) regulated *fat-*

l and an enhanced green fluorescent protein gene (*egfp*) expression cassette. The *egfp* expression cassette was used to evaluate the efficiency of *fat-1* expression level. Briefly, pTol2-CMV:*fat-1*-SV40 poly (A) + CMV: *egfp*-SV40 poly(A) on the basis of pTol2-SV40 was generated (Supplemental Fig. S1A). The CMV promoter from pIRES2-*egfp* was amplified by primers of CMV-F and CMV-R containing *Vsp* I and *Nhe* I sites (Supplemental Table S1) and inserted into the plasmid. CMV: *egfp*-SV40 poly(A) was obtained by digesting pCMV-*egfp* vector, and then inserted into pTol2-CMV-SV40. Finally, the *fat-1* sequences were inserted into pTol2-CMV-SV40 poly(A)+CMV: *egfp*-SV40 poly(A). The *fat-1* construct in this study was the same as the one developed by Pang et al. (2014) and different from the one developed by Kang et al. (2004) in which a chicken beta-actin promoter was used. Approximately 1 nl of DNA and RNA mixtures containing 25 ng/μl expression constructs and 25ng/μl Tol2 transposase mRNA was injected into zebrafish fertilized embryos at single-cell stage. The embryos and fry of *fat-1* transgenic zebrafish were observed by light and fluorescence microscope. Under the fluorescence microscope, the *fat-1* transgenic F0 zebrafish with green fluorescent were screened (Supplemental Fig. S1B). The experimental fish used here were chosen from F2 generation of Tg:*fat-1*.

The *fat-1* expression levels in six tissues (liver, muscle, intestine, gonad, eye and brain) of two-month-old Tg:*fat-1* and WT (AB strain, obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China) were checked by semi-quantitative real-time PCR (Xu et al. 2019) to confirm the success of the transgenic zebrafish's generation. Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was used as the reference gene.

Vegetable oil-feeding experiment

A total of 45 two-month-old WT and 45 two-month-old Tg:*fat-1* with uniform-size were fed with an LFD (contained with 3.0% soybean oil) in triplicate (15 fish/tank) for 8 weeks. Meanwhile, 45 two-month-old WT and 45 two-month-old Tg:*fat-1* with uniform size were fed with an HFD (contained with 13.0% soybean oil) in triplicate (15 fish/tank) for 8 weeks. Fish (WT and Tg:*fat-1*) were fed three times per day to apparent satiation. The formulations of the LFD and HFD diets are listed in Supplemental Table S2.

After the feeding experiment, body weights of LFD-WT, LFD-Tg:*fat-1*, HFD-WT

and HFD-Tg:*fat-1* were measured, and then anatomical observation of visceral lipid deposition were performed. The dorsal muscles were sampled for fatty acid analysis. The weighed intact livers were used for fatty acid analysis, histological observation, lipidomic analysis, gene expression and ATP analysis. Since most of the *fat-1* transgenic fish are females, female zebrafish were used for all the analyses in this study, which could avoid the influence by sex.

Fatty acid analysis

The total lipids of whole bodies of WT and Tg:*fat-1*, livers and muscles of the four experimental groups (LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*) were extracted with chloroform/methanol (1:2, vol/vol). Fatty acid methyl esters (FAMES) were prepared by separately incubating the total lipid with 2 ml of methylation reagent containing 5 % H₂SO₄ (vol/vol) and 95 % methanol with 0.005 % butylated hydroxytoluene. The fatty acid compositions were determined using gas chromatography (Shimadzu emit Co., Ltd, Tokyo, Japan) according to the method of Li et al. (2019). The samples (1.0 µl) were automatically injected into the injection port and identified fatty acids were presented as area percentage of total fatty acids.

Total lipid measurement

Total lipid contents of whole bodies (percent weight) of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* were measured using the Dyer and Bligh procedure as previously described (Dyer and Bligh 1959).

Histology and Nile red staining

Liver samples from LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* were fixed in 4% formalin and embedded in paraffin. Sections (4 µm thick) were cut and stained with hematoxylin and eosin (H&E) staining. For Oil Red O staining, liver samples were embedded in optimal cutting temperature medium and cryosectioned following a standard protocol with modifications by Wang et al. (2006). Frozen sections were subjected to standard Oil Red O staining (Koopman et al. 2001), then prepared for light microscopy. Nile red staining was performed here according to a previous report (Peng et al. 2017). Nile red (N3013, Sigma, St. Louis, USA) was dissolved in acetone at 1 mg/ml as the stock solution. The LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* were immersed in

system water containing 0.1 µg/ml Nile red overnight at 26°C in the dark. Before photo taking, the zebrafish were transferred to clean water to wash the Nile red dye adhered. The images were obtained using an stereo microscope (Olympus SZX16 FL, Tokyo, Japan) at an excitation wavelength of 488 nm.

Lipidomic analysis

For lipidomic analysis, lipids were extracted from fresh liver (~20 mg) of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* with chloroform/methanol (1/2, v/v) according to the published protocols (Li et al. 2019). The liquid chromatography/tandem mass spectrometry (LC-MS, Shimadzu emit co., Ltd, Tokyo, Japan) was applied as analysis platform. A Kromasil 100-2.5-C18 column (2.1 mm×100 mm; 2.5 µm, part number: MH2CLD10, Kromasil, Bohus, Sweden) was used as a reversed phase to separate the lipids. Mobile phase A was ultrapure water with 5 mmol/L ammonium acetate. The mobile phase B was methanol with 5 mmol/L ammonium acetate. The HPLC system was coupled to a TRIPLE QUADTM 3500 mass spectrometer (AB Sciex, Redwood City, CA, USA) via electrospray ionization (ESI) source and was ran in negative ion mode under multiple reaction monitoring (MRM), and the LC/MS spectra were obtained by using enhanced production scan mode at a scan speed of 1000 Da/s. The data obtained were extracted and preprocessed with lipid search software (AB Sciex, Redwood City, CA, USA Multi Qantion 3.0.2) (Li et al. 2019). They were then normalized and edited into two-dimensional data matrix by Excel 2010 software (Microsoft, Dallas, Texas, USA) (Zhao et al. 2020), including the lipid class, peak intensity and fatty acid chains.

Real-time quantitative PCR (qPCR)

Total RNA was isolated from livers of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* using RNA isoPlus (Takara Bio Inc, Shiga, Japan) following the manufacturer's instructions. Quantity and quality of isolated RNA were ascertained by electrophoresis and spectrophotometry and then reverse-transcribed with PrimeScript RT reagent Kit (Takara Bio Inc, Shiga, Japan). Complementary DNA was diluted 1:5 (vol/vol) prior to use as template for qPCR analyses. HieffTM qPCR SYBR Green Master Mix was purchased from YEASEN Biotech Co., Ltd. (Shanghai, China). The qPCR reaction solution consisted of 5 µl HieffTM qPCR SYBR Green Master Mix (2×), 0.2 µl PCR forward primer (20 µM),

0.2 µl PCR reverse primer (10 µM), 1.0 µl cDNA solution, and 3.6 µl ddH₂O. The reaction conditions were as followed: 95 °C for 30 s followed by 40 cycles consisting of 95 °C for 5 s and 57 °C for 30 s. The florescent flux was then recorded, and the reaction continued at 72 °C for 6 s and 95 °C for 5 s. The 2^{-ΔΔCT} method was used to analyze the expression levels of the target genes, namely those for sterol regulatory element binding transcription factor 1 (*srebf1*), fatty acid synthetase (*fas*), acetyl-CoA carboxylase (*acc*), stearoyl CoA desaturase (*scd*), peroxisome proliferator-activated receptor gamma (*ppary*), diacylglycerol *O*-acyltransferase 1 (*dgat-1*), *dgat-2*, patatin like phospholipase domain containing 2 (*atgl*), lipase, hormone-sensitive a (*lipea*), acyl-CoA oxidase (*aco*), peroxisome proliferator-activated receptor-γ coactivator-1a (*pgc-1a*), nuclear respiratory factor (*nrf-1*), carnitine palmitoyl transferase 1 (*cpt-1*), *ucp-1*, mitochondrial complex I special genes NADH dehydrogenase, subunit 6 (*mt-nd6*), succinate dehydrogenase complex flavoprotein subunit a (*mt-sdha*), *mt-sdhb*, mitochondrion cytochrome b (*mt-cyb*), special gene surfet locus protein 1 (*surf1*), ATP synthase 5 (*atp5*), with *β-actin* and *gapdh* as reference genes (Goulao et al. 2012; Li et al. 2010). Each qPCR analysis was repeated in triplicates. The primer sequences for qPCR are listed in Supplemental Table S1.

ATP levels measurement

Fresh liver aliquots (~20 mg) from LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* were used for determination of ATP on a bioluminescence ATP assay kit (Beyotime, Shanghai, China). The liver tissues were disrupted in 200 µl of lysis buffer and centrifuged at 12,000 g to collect the cell supernatant. An aliquot (100 µl) of an ATP detection working solution was added to each well of a black 96-well culture plate, and the plate was then incubated for 3 min at room temperature. Then, 20 µl samples of the lysate were added to the wells, and the luminescence was measured immediately.

Statistical analysis

Statistical analyses were conducted by *T* tests and one-way ANOVA in IBM SPSS statistics 22 software (SPSS Inc., Chicago, USA). The data were expressed as the means ± SD, and a probability of *p* < 0.05 was considered to be significant.

Results

Tg:*fat-1* generation

Supplemental Fig. S1C shows that *fat-1* was strongly expressed in various tissues of Tg:*fat-1*, confirming the success of *fat-1* transgenesis. The percentage of LA (18:2n-6) in Tg:*fat-1* decreased about 3.6 % compared with WT. With the *fat-1* transgenesis, the percentage of α -linolenic acid (ALA, 18:3n-3) presented a significant increase from 3.5 % to 5.1 %. Furthermore, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) content also displayed a remarkable increase in Tg:*fat-1*. Compared with WT, the level of total n-6 PUFAs of Tg:*fat-1* decreased 4% and total n-3 PUFAs raised about 7% (Supplemental Fig. S1D). As shown in Supplemental Table S3, no significant differences in saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) contents were found between WT and Tg:*fat-1*. These results strongly suggested that Tg:*fat-1* could endogenously convert n-6 PUFAs to n-3 PUFAs.

***fat-1* prevented from lipid deposition induced by HFD feeding**

The effect of *fat-1* on lipid deposition was tested in fish fed with LFD and HFD, varying in their lipid content (Supplemental Table S2). There was no difference in the amount eaten between WT and Tg:*fat-1* fish. For each diet group, the total n-3 PUFAs contents in liver and muscle of Tg:*fat-1* were significantly higher than those of WT, while the opposite occurred in levels of total n-6 PUFAs (Fig. 1A). The ratios of n-6/n-3 PUFAs in liver and muscle of LFD-Tg:*fat-1* and HFD-Tg:*fat-1* were significantly lower than those of LFD-WT and HFD-WT, respectively (Fig. 1B). Supplemental Table S4 and Supplemental Table S5 show detailed fatty acid compositions in dorsal muscle and liver, respectively, of LFD-WT, HFD-WT, LFD-Tg:*fat-1* and HFD-Tg:*fat-1*. The above results indicated that LFD-Tg:*fat-1* and HFD-Tg:*fat-1* could effectively convert n-6 PUFAs to n-3 PUFAs.

LFD-Tg:*fat-1* showed a slightly lower body weight ($p = 0.609$) and total lipid content ($p = 0.181$), compared with LFD-WT. However, HFD-Tg:*fat-1* presented significantly lower body weight (9.3% reduction) and total lipid content (33.2% reduction) than HFD-WT (Fig. 1C and D). Nile red staining for adipose tissue was performed (Supplemental Fig. S2). Visceral adipose tissue was most abundant in HFD-WT in comparison with HFD-Tg:*fat-1*. An obvious abdominal lipid deposition was visible to the naked eye in HFD-WT

and almost no abdominal obesity had found in HFD-Tg:*fat-1* and LFD-Tg:*fat-1* (Fig. 1E). Lipid accumulation was remarkably decreased in livers of HFD-Tg:*fat-1*, compared with HFD-WT (Fig. 2). In addition, the liver weight of HFD-Tg:*fat-1* was significantly lower than that of HFD-WT (Supplemental Fig. S3).

Taken together, these findings suggested that the lipid deposition in HFD-WT were noticeably attenuated by *fat-1* transgenesis. In other words, *fat-1* could prevent zebrafish from abnormal lipid deposition induced by HFD feeding, through endogenously converting n-6 PUFAs to n-3 PUFAs.

Hepatic lipidomics

To fully understanding the changes of lipid compositions in Tg:*fat-1*, lipidomic analysis of liver was carried out (Fig. 3). Eight lipid classes (phosphatidyl inositol (PI), lyso-phosphatidyl ethanolamine (LPE), phosphatidyl ethanolamine (PE), lyso-phosphatidyl choline (LPC), phosphatidyl choline (PC), triacylglycerol (TAG), free fatty acid (FFA), and cholesteryl ester (CE)), and 336 individual lipid species were identified from liver tissues of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. A heatmap showed the variations for each lipid species content among the four groups (Fig. 3A).

For each diet group, Tg:*fat-1* possessed significantly decreased levels of almost all lipid classes (except the FFA in LFD group), compared with WT (Fig. 3B). Interestingly, the extent to which lipid classes were decreased in Tg:*fat-1* varied between diets. HFD-Tg:*fat-1* presented a 53% decrease in hepatic TAG content, compared with HFD-WT, while LFD-Tg:*fat-1* had a 19% decrease compared with LFD-WT.

Lipid metabolism-related genes expression in liver tissues from WT and Tg:*fat-1* fed on LFD and HFD

To explore the molecular mechanism through which *fat-1* prevents zebrafish from abnormal lipid deposition induced by HFD feeding, we firstly analyzed the mRNA expression levels of 10 lipid metabolism-related genes in liver tissues from LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* (Fig. 4). Four lipid synthesis-related genes (*srebfl-1*, *fas*, *scd* and *ppary*) were significantly down-regulated in HFD-Tg:*fat-1*, compared with HFD-WT. Expression levels of two lipid deposition-related genes (*dgat-1* and *dgat-2*) in HFD-Tg:*fat-1* were significantly lower than those in HFD-WT. Three steatolysis-

related genes (*atgl*, *lipea* and *aco*) were significantly up-regulated in HFD-Tg:*fat-1*, compared with HFD-WT. Expression profiles of the lipid deposition-related genes and steatolysis-related genes in LFD group were similar as those of HFD group, but the expression patterns of lipid synthesis-related genes were different. These findings can explain why HFD-Tg:*fat-1* had obviously less lipid deposition compared with HFD-WT.

Expression levels of mitochondrial energy metabolism-related genes and ATP contents in liver of WT and Tg:*fat-1* fed on LFD and HFD diets

To investigate the role of *fat-1* in preventing mitochondrial energy metabolism disorder, we further investigated the expression levels of mitochondrial energy metabolism-related genes in livers of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. The mRNA expression levels of *pgc-1a*, *nrf-1* and *cpt-1* in HFD-Tg:*fat-1* were significantly higher than those of HFD-WT (Fig. 5A). Moreover, for each diet group, the expression of thermogenic gene *ucp-1* was significantly up-regulated in Tg:*fat-1* compared with WT (Fig. 5B). The expression levels of mitochondrial respiratory chain genes including *mt-nd6* (complex I), *sdha* (complex II), *sdhb* (complex II), *mt-cyb* (complex III), *surfl* (complex IV) and *atp5* (ATP synthase) were also investigated (Fig. 5C). However, there was no significantly difference in expression level of *mt-nd6* between HFD-WT and HFD-Tg:*fat-1*. *sdha*, *sdhb*, *mt-cyb*, *surfl* and *atp5* were significantly up-regulated in HFD-Tg:*fat-1*, compared with HFD-WT, while the same situation happened in the LFD group. Moreover, hepatic ATP contents were determined in LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1* (Fig. 5D). ATP contents in LFD-Tg:*fat-1* and HFD-Tg:*fat-1* were significantly higher than those in LFD-WT and HFD-WT, respectively. These findings indicated that *fat-1* can increase mitochondrial energy metabolism to reduce lipid deposition in zebrafish.

Discussion

In this study, *fat-1* transgenic zebrafish fed with a high-fat diet effectively converted n-6 PUFAs to n-3 PUFAs, in accordance with previous studies of *fat-1* transgenic mice, pig and carp (Kim et al. 2012; Li et al. 2018; Zhang et al. 2018). *fat-1* transgenic mice presented increased content of serum n-3 PUFAs and significantly improved body weight (Ji et al. 2009). Bandarra et al. (2011) reported that hepatic lipid deposition of gilthead

seabream (*Sparus aurata*) was significantly increased with decreased dietary n-3/n-6 PUFA ratios. Thus, the increase of n-3/n-6 PUFA ratio in HFD-Tg:*fat-1* was accompanied by a steep decrease of lipid deposition, mainly manifested in abdominal cavity, liver and dorsal muscle tissues. In this context, *fat-1* could protect fish from abnormal lipid deposition induced by high-vegetable oil feeding, through endogenously converting n-6 PUFAs to n-3 PUFAs.

To our knowledge, this study was the first report to investigate the effects of *fat-1* on lipid metabolism of fish fed with diets of high n-6 PUFAs and its mechanism. The liver plays a central role in the maintenance of systemic lipid homeostasis. Our results show that compared with HFD-WT, HFD-Tg:*fat-1* presented an obvious decrease of lipid deposition, accompanied by significantly down-regulated lipid-synthesis related genes (*srebfl*, *fas*, *acc*, *scd* and *ppary*), markedly down-regulated lipid-deposition related genes (*dgat-1* and *dgat-2*), and significantly up-regulated steatolysis genes (*atgl*, *lipea* and *aco*) in liver tissues. Thus, the decreased lipid deposition in HFD-Tg:*fat-1* was attributable to both decreased lipogenesis and increased lipolysis. Similarly, it has reported that expressions of many genes related to lipid metabolism were influenced in *fat-1* transgenic cattle by through transcriptome analysis (Liu et al. 2015). These genes were steatolysis related genes (i.e. *aco*, lipoprotein lipase (*lpl*) and *cpt-1b*) and lipid-synthesis related genes (i.e. *fas* and *scd*). The expressions of the most genes (*aco*, *lpl*, *cpt-1b* and *fas*) in the *fat-1* transgenic cattle showed similar trends as those in our transgenic zebrafish. But the *scd* expression profile was very different between these two transgenic animals, which may be due to the differences of species and diets used. In addition, *fat-1* transgenic mice showed attenuated expressions of genes involved lipogenesis (López-Vicario et al. 2013). It has been reported that *srebfl*, an important transcription activator of lipid synthesis (Horton et al. 2002), can be inhibited by n-3 PUFAs in mouse (Xu et al. 1999). Therefore, it is possible that in HFD-Tg:*fat-1*, which display a higher content of n-3 PUFAs, *srebfl* expression is inhibited thus decreasing the expression levels of other lipid-synthesis related genes, such as *fas*, *acc*, *scd*, resulting in lipid synthesis reduction (Kim et al. 1999; Bennett et al. 1995). The result of lipidomic analysis also showed the lipids, especially TAG which is a major energy storage form (Beenackers et al. 1985), were significantly decreased in HFD-Tg:*fat-1* compared

with HFD-WT.

Compared with LFD-WT, LFD-Tg:*fat-1* had significantly lower expression levels of the lipid deposition-related genes and higher expression levels of steatolysis-related genes in liver tissues, which was similar as HFD-WT vs HFD-Tg:*fat-1*. However, up-regulations of lipid synthesis-related genes (*fas*, *acc*, *scd*) and higher FFA levels were found in liver tissues of LFD-Tg:*fat-1* compared with LFD-WT. It has reported that the mRNA expression level of *srebf1* and protein level of nSREBF1 will be up-regulated in cells treated with low contents of lipids (Horton et al. 2002). Our results showed an equivalent expression level of *srebf1* in LFD-Tg:*fat-1* as LFD-WT. This might be explained by excessive lipid consumption resulted in a lipid-synthesis compensation in liver of LFD-Tg:*fat-1*.

Holmström et al. (2012) found that obese mice had a decrease in hepatic mitochondrial respiratory capacity, along with *pgc-1a* expression reduction and mitochondrial fission increasing. To assess the effects of *fat-1* transgenesis on hepatic mitochondrial metabolism in zebrafish, the transcript levels of *nrf-1*, through which *pgc-1a* controls mitochondrial biogenesis and lipid oxidation, and *cpt-1*, an enzyme of catalyzing the transfer of the acyl group of a long-chain fatty acyl-CoA from coenzyme A to l-carnitine, were assayed here (Nguyen et al. 2007; Scarpulla 2011). These results showed that endogenous n-3 PUFAs synthesis effectively increased mitochondrial fatty acid β -oxidation process and *fat-1* alleviated the mitochondrial metabolism disorder. *fat-1* reducing lipid deposition effect may be due to an increase in mitochondrial steatolysis ability. Next, the expression profiles of mitochondrial respiratory chain genes and *ucp-1*, a protein responsible for thermogenic respiration, indicated that the steatolysis was increased by *fat-1* (Fedorenko et al. 2012), resulting in higher caloric production. Meanwhile, there was a significant increase in hepatic ATP concentration, indicating that at the premise of maintaining normal ATP requirement in liver, *fat-1* could help to consume the excess energy in the form of heat.

Appropriate lipid content in economic exploited teleost fish species is beneficial for a healthy growth and meat quality increase. Yet, excessive lipid deposition induced by high-vegetable oil feeding is often associated with aquatic animal diseases such as tissue

inflammation. Not only does it affect the economic benefits of fisheries, but also does harm to human health (Durborow 1999). Here, we show that *fat-1* transgenic fish can effectively regulated lipid metabolism and alleviated abnormal lipid deposition. A detailed schematic presentation about the mechanism of *fat-1* alleviating lipid deposition is shown in Fig. 6. HFD-WT showed an obvious obesity and abnormal lipid deposition in the liver after fed with a high n-6 PUFAs diet. Meanwhile, high n-3 PUFAs content and ratio of n-3/n-6 PUFAs by converted the n-6 PUFAs of high-fat diet into n-3 PUFAs were found in HFD-Tg:*fat-1*. A high proportion of n-3 PUFAs in HFD-Tg:*fat-1* increased the mitochondrial energy metabolism and improved lipid metabolism, resulting in a reduction of TAG synthesis in liver. Finally, serious lipid deposition induced by high fat diet feeding was avoided in HFD-Tg:*fat-1*. This study provides important information for the role of *fat-1* gene in the resolution of excessive lipid deposition in fish induced by high fat-diet feeding. And it can also provide additional evidence for the feasibility of transgenic biotechnology in optimizing animal quality.

Authors contributions

J.G. and S.S. designed research; S.S. and J.G. performed research; S.S. analyzed data; S.S. and X.C. wrote the paper; J.G. acquisition of funding; J.G., X.C., F.C., Ó.M., and S.S. involved in the discussion; X.C., F.C., Ó.M., and J.G., revised the paper. All authors read and approved the manuscript.

Compliance with Ethical Standards

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Conflict of interest

The authors declare no conflict of interests.

Ethical approval

This study was conducted in strict accordance with the recommendations in the guide for the Care and Use of Laboratory Animals of Huazhong Agricultural University. All efforts were made to minimize suffering of the fish.

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Figure 1. *fat-1* transgenesis in zebrafish attenuated the n-6 PUFAs levels, the gains in body weight, total lipid contents, and visceral lipid deposition induced by fat-diet feeding.

(A) Total n-6 PUFAs and n-3 PUFAs levels in liver and muscle tissues of wild type zebrafish (WT) and *fat-1* transgenic zebrafish (*Tg:fat-1*) fed with low-fat diet (LFD) or high-fat diet (HFD) for 8 weeks. Results obtained here indicated that % of total fatty acids. (B) The ratios of n-6/n-3 PUFAs in liver and muscle tissues of WT and *Tg:fat-1* fed with LFD and HFD for 8 weeks. (C) The body weights of WT fed with LFD (LFD-WT), LFD-*Tg:fat-1*, HFD-WT and HFD-*Tg:fat-1*. (D) The total lipid contents of whole bodies of LFD-WT, LFD-*Tg:fat-1*, HFD-WT and HFD-*Tg:fat-1*. (E) Excessive visceral adipose tissues (white dotted lines) of LFD-WT, LFD-*Tg:fat-1*, HFD-WT and HFD-*Tg:fat-1* viewed with light microscope. Scale bar=500μm. Data are given as means ± SD of 3 independent experiments. The statistical analyses were conducted by one-way ANOVA with Tukey's post hoc test. The different letters above the bars indicated significant difference ($p < 0.05$).

fat-1: omega-3 desaturase gene; PUFAs, polyunsaturated fatty acids.

Figure 2. Histological structures of livers from wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg;*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks.

Arrows indicated fat vacuoles. Scale bar=100μm. *fat-1*: omega-3 desaturase gene; H&E, hematoxylin and eosin.

Figure 4. Hepatic expression patterns of lipid metabolism-related genes in wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg:*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks.

Data are given as means $\pm$ SD of 3 independent experiments. The statistical analyses were conducted by one-way ANOVA with Tukey's post hoc test. The different letters above the bars indicated significant difference ($p < 0.05$).

fat-1: omega-3 desaturase gene; *srebf1*, sterol regulatory element binding transcription factor 1; *fas*, fatty acid synthetase; *acc*, acetyl-CoA carboxylase; *scd*, stearoyl CoA desaturase; *ppary*, peroxisome proliferator-activated receptor gamma; *dgat-1*, diacylglycerol *O*-acyltransferase 1; *atgl*, patatin like phospholipase domain containing 2; *lipea*, lipase, hormone-sensitive a; *aco*, acyl-CoA oxidase.

Figure 5. Expression levels of mitochondrial energy metabolism-related genes and ATP concentrations in liver tissues of wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg:*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks. (A) Expression levels of *pgc-1a*, *nr1f-1* and *cpt-1* in liver tissues of WT fed with LFD (LFD-WT), LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. (B) Expression levels of *ucp-1* in liver tissues of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. (C) Expression levels of mitochondrial respiratory chain genes (complex I : *mt-nd6*; complex II : *sdha*, *sdhb*; complex III : *mt-cyb*; complex IV : *surf1*; ATP synthase : *atp5*) in liver tissues of LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. (D) ATP concentrations in liver tissues from LFD-WT, LFD-Tg:*fat-1*, HFD-WT and HFD-Tg:*fat-1*. Data are given as means $\pm$ SD of 3 independent experiments. The statistical analyses were conducted by one-way ANOVA with Tukey's post hoc test. The different letters above the bars indicated significant difference ($p < 0.05$).

fat-1: omega-3 desaturase gene; *pgc-1a*, peroxisome proliferator-activated receptor- γ coactivator-1a; *nr1f-1*, nuclear respiratory factor; *cpt-1*, carnitine palmitoyl transferase 1; *ucp-1*, uncoupling protein 1; *mt-nd6*, mitochondrial complex I special genes NADH dehydrogenase, subunit 6; *sdha*, succinate dehydrogenase complex flavoprotein subunit a; *mt-cyb*, mitochondrion cytochrome b; *surf1*, special gene surfeit locus protein 1; *atp5*: ATP synthase 5.

Figure 6. A schematic presentation of *fat-1* could protect fish from abnormal lipid deposition and dyslipidaemia induced by high-vegetable oil feeding, through endogenously converting n-6 PUFAs to n-3 PUFAs which could affect lipid metabolism and increase mitochondrial energy metabolism to result in lipid deposition reduction.

fat-1: omega-3 desaturase gene; WT, Wild type zebrafish; Tg:*fat-1*, *fat-1* transgenic zebrafish; HFD, high-fat diet; TAG, triacylglycerol; PUFA, polyunsaturated fatty acids; *srebf1*, sterol regulatory element binding protein-1; *fas*, fatty acid synthetase; *acc*, acetyl-CoA carboxylase; *scd*, stearoyl CoA desaturase; *ppary*, peroxisome proliferator-activated receptor gamma; *dgat-1*, diacylglycerol *O*-acyltransferase 1; *atgl*, patatin like phospholipase domain containing 2; *lipea*, lipase, hormone-sensitive a; *aco*, acyl-CoA oxidase; *pgc-1a*, peroxisome proliferator-activated receptor- γ coactivator-1a; *nrf-1*, nuclear respiratory factor; *cpt-1*, carnitine palmitoyl transferase 1; *ucp-1*, uncoupling protein 1; *mt-nd6*, mitochondrial complex I special genes NADH dehydrogenase, subunit 6; *sdha*, succinate dehydrogenase complex flavoprotein subunit a; *mt-cyb*, mitochondrion cytochrome b; *surf1*, special gene surfet locus protein 1; *atp5*, ATP synthase 5; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

Applied Microbiology and Biotechnology – SUPPLEMENTARY INFORMATION

***fat-1* transgenic zebrafish are protected from abnormal lipid deposition induced by high-vegetable oil feeding**

Shouxiang Sun¹, Filipe Castro^{3,4}, Óscar Monroig⁵, Xiaojuan Cao^{1,2}, Jian Gao^{1,2*}

¹College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education/Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, China.

²College of Fisheries, Hubei Provincial Engineering Laboratory for Pond Aquaculture, Huazhong Agricultural University, Wuhan 430070, China.

³CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

⁴FCUP - Faculty of Sciences, Department of Biology, University of Porto, Portugal

⁵Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Ribera de Cabanes, Castellon 12595, Spain

*Corresponding author

TEL: 86(027)87282113. Email address: gaojian@mail.hzau.edu.cn (J. Gao)

Postal address: No.1 Shizishan Stress, Hongshan District, Wuhan 430070, Hubei Province, China

Supplemental Figure S1-S3

Supplemental Table S1-S5

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760 **Supplemental Figure S1. Construction of the *fat-1* transgenic zebrafish (*Tg:fat-1*).**

761 (A) The schema for constructing *Tg:fat-1*: the bipartite construct contained CMV promoter
 762 and SV40poly (A) which regulated *fat-1* expression cassette, and a cis-linked CMV
 763 promoter and SV40poly (A) which regulated *egfp* (enhanced green fluorescent protein)
 764 expression cassette. (B) *Tg:fat-1* embryos (24 hours after fertilization) and fry (96 hours
 765 after fertilization) viewed with light and fluorescence microscope. Scale bar=500μm. (C)
 766 Expression patterns of *fat-1* in different tissues (namely liver, muscle, intestine, gonad,
 767 eyes, brain) of wild type zebrafish (WT) and *Tg:fat-1*. The reference gene was *gapdh*
 768 (glyceraldehyde-3-phosphate dehydrogenase). (D) Fatty acid compositions in whole fish
 769 bodies of 2-month-old *Tg:fat-1* and WT. Results obtained here indicated that % of total
 770 fatty acids. Data are given as means ± SD of 3 independent experiments. The statistical
 771 analyses were conducted by *T* tests. The asterisks labeled above the error bars indicated
 772 significant differences (**p* < 0.05, ***p* < 0.01, ****p* < 0.001).

773 *fat-1*: omega-3 desaturase gene; PUFAs: polyunsaturated fatty acids.

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Supplemental Figure S2. Nile red fluorescence of wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg;*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks.

Arrowheads indicated visceral adipose tissue. Lipid was of yellow fluorescence. n=3. Scale bar=500μm. *fat-1*: omega-3 desaturase gene.

Supplemental Figure S3. The liver weights of wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg;*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks.

Data are given as means $\pm$ SD of 3 independent experiments. The statistical analysis was conducted by one-way ANOVA with Tukey's post hoc test. The different letters above the bars indicated significant difference ($p < 0.05$). *fat-1*: omega-3 desaturase gene.

799 **Supplemental Table S1. Primers used in this study.**

Symbol	Forward	Reverse	Accession numbers
Construction of Tg: <i>fat-1</i>			
<i>fat-1</i>	ATGGTCGCTCATTCTCA	TAGAACGGCACGGGATAT	
CMV	CCGATTAATGCGTTATCCCCTG ATTCTGT	ACGCGCTAGCCGGATCTGAC GGTTCATAA	
Real-time quantitative PCR			
<i>fat-1</i>	GTGTACGAGGCTGATGAA	ATGAGATGGTAATGTGGG	NM_001028389.5
<i>β-actin</i>	CACCACCACAGCCGAAAGAG	ACCGCAAGATTCCATACCCA	AF057040.1
<i>gapdh</i>	TCCAGTACGACTCCACCCAT	TGACTCTCTTTGCACCACCC	NM_001115114.1
<i>srebfl</i>	TTCTTTCTTCGGATTGATG	GATGAGATGGTAATGTGGG	NM_001105129.1
<i>fas</i>	ATGCCCACCTTTGACTTAT	GACCGTGTTTGAACCTCTT	XM_009306807.3
<i>acc</i>	GCAGCTACACCACCTACA	CATAGTGAATACAGCCAGAC	NM_001271308.1
<i>scd</i>	AGCCACTTTACCTCTGCG	AGCTCTAGTTTGCGTCCT	NM_198815.2
<i>ppary</i>	ACTCTCCGCTGATATGGT	GTTCTCCTGTGTCCTGTAG	NM_131467.1
<i>dgat-1</i>	AGGAGCGAGTCTGTGAAG	ACTATTCTGTCTGTAGCG	NM_001255528.1
<i>dgat-2</i>	TAAGACTCATCAAGACGCATA	GCAGATTCTCCGCACAT	NM_001030196.1
<i>atgl</i>	ACACCTCCATCCAGTTCA	TCTTCATCTTCACCGTCAG	XM_005174256.4
<i>lipea</i>	ATCCTCATCAACAGCCTCT	CAATGCGGAAGCCACTATA	NM_001316725.1
<i>aco</i>	TGTGTCTTACCTGAACGAAT	TACTCTTGCTGTGCTGTAG	NM_001005933.2
<i>pgc-1a</i>	ATAGAGGAGAGGCGAGTG	GTGTAGCGGTAGGTGATG	AY998087.2
<i>nrf-1</i>	GCATCAGACGGAGCATAT	TGTCGGAGTCATCATAAGC	NM_001328540.1
<i>cpt-1</i>	ATGAGGAGCACCAAAGAA	GTGGGAAAAGCGTAAAGA	NM_001044854.1
<i>ucp-1</i>	CCAGTATAGTGAAGGAGACAT	GAGACAGTCATAGAGCCAAT	NM_199523.2
<i>mt-nd6</i>	CCAGCCGCCACAATTACAAC	GTTTGCTTATTCAGCGGCCC	GeneID: 140536
<i>sdha</i>	TCTCTATGGACGGTCTCTG	TGGCAATGACTGTGTTCTT	NM_200910.1
<i>sdhb</i>	TGTGTCTGCTGCTGTATG	TTGTGTTGCTGATAGTCTGA	NM_001098740.1
<i>mt-cyb</i>	ATTCGCATACGCCATTCTA	ATGTCTGCTACCAGTGTTT	GeneID: 140512
<i>surfl</i>	GACGACTTCAGTGCCAAT	CCTCTGTTCCACCAAGATAGT	NM_001080173.1
<i>atp5</i>	TCACAACCACCAAGAAGG	GGCTACATCATAATGCTCAG	NM_001024429.2

800 *fat-1*: omega-3 desaturase gene; Tg:*fat-1*, *fat-1* transgenic zebrafish; *gapdh*, glyceraldehyde-3-
801 phosphate dehydrogenase; *srebfl*, sterol regulatory element binding protein-1; *fas*, fatty acid synthetase;
802 *acc*, acetyl-CoA carboxylase; *scd*, stearoyl CoA desaturase; *ppary*, peroxisome proliferator-activated
803 receptor gamma; *dgat-1*, diacylglycerol O-acyltransferase 1; *atgl*, patatin like phospholipase domain
804 containing 2; *lipea*, lipase, hormone-sensitive a; *aco*, acyl-CoA oxidase; *pgc-1a*, peroxisome
805 proliferator-activated receptor-γ coactivator-1a; *nrf-1*, nuclear respiratory factor; *cpt-1*, carnitine
806 palmitoyl transferase 1; *ucp-1*, uncoupling protein 1; *mt-nd6*, mitochondrial complex I special genes
807 NADH dehydrogenase, subunit 6; *sdha*, succinate dehydrogenase complex flavoprotein subunit a; *mt-*
808 *cyb*, mitochondrion cytochrome b; *surfl*, special gene surfeit locus protein 1; *atp5*, ATP synthase 5.

813 **Supplemental Table S2. Formulations of the low-fat diet (LFD) and high-fat diet (HFD).**

Ingredients	Diets	
	LFD	HFD
Casein	28.0	28.0
Gelatin	7.0	7.0
Soybean oil	3.0	13.0
Corn starch	41.5	41.5
Vitamin premix ^a	1.5	1.5
Mineral premix ^b	4.5	4.5
CMC	3.0	3.0
Cellulose	11.0	1.0
Choline chloride	0.5	0.5
Total	100.0	100.0
Nutrient composition		
Moisture (%)	13.9	14.7
Crude protein (%)	33.2	34.3
Crude lipid (%)	3.1	13.4
Ash (%)	5.6	5.7

^aVitamin premix (mg or IU/kg): Vitamin A, 500,000 IU; Vitamin D₃, 50,000 IU; Vitamin E, 2500 mg; Vitamin K₃, 1000mg; Vitamin B₁, 5000 mg; Vitamin B₂, 5000 mg; Vitamin B₆, 5000 mg; Vitamin B₁₂, 5000 mg; Inositol, 25,000 mg; Pantothenic acid, 10,000 mg; Cholin, 100,000 mg; Niacin, 25,000 mg; Folic acid, 1000 mg; Biotin, 250 mg; Vitamin C, 10,000 mg.

^bMineral premix (g / kg): CaCO₃, 314.0 g; KH₂PO₄, 469.3 g; MgSO₄7H₂O, 147.4 g; NaCl, 49.8 g; Fe (II) gluconate, 10.9 g; MnSO₄H₂O, 3.12 g; ZnSO₄7H₂O, 4.67 g; CuSO₄5H₂O, 0.62 g; KCl, 0.16 g; CoCl₂6H₂O, 0.08 g; NH₄ molybdate, 0.06 g; NaSeO₃, 0.02 g. CMC, carboxy methyl cellulose.

Supplemental Table S3. Fatty acid compositions in whole bodies of two-month-old wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg;*fat-1*).

% of total fatty acids	WT	Tg: <i>fat-1</i>
14:0	4±0.2	3.8±0.3
16:0	15.0±0.4	14.4±0.2
18:0	6.6±0.6	6.5±0.1
20:0	1.7±0.1	1.5±0.0
Total SFAs	27.3±0.4	26.2±0.1
16:1n-7	8.9±0.7	7.8±0.6
18:1n-9	17.0±0.4	15.3±0.5
20:1n-9	1.6±0.1	1.3±0.2
22:1n-11	0.9±0.1	0.7±0.2
Total MUFAs	28.4±1.2	25.1±1.0
18:2n-6	18.1±0.5	14.5±0.1**
18:3n-6	1.6±0.3	1.2±0.3
20:2n-6	2.1±0.2	1.8±0.1
20:3n-6	2.3±0.2	0.9±0.1**
20:4n-6	2.5±0.0	2.4±0.1
22:5n-6	1.5±0.1	1.0±0.3
Total n-6 PUFAs	27.8±0.4	21.7±0.4**
18:3n-3	3.5±0.4	5.1±0.2*
18:4n-3	1.9±0.2	1.8±0.3
20:4n-3	1.9±0.5	2.4±0.1
20:5n-3	2.9±0.3	5.1±0.2*

22:5n-3	1.3±0.2	2.7±0.6*
22:6n-3	5.7±0.2	7.8±0.2**
Total n-3 PUFAs	17.2±0.9	24.7±0.7***
Total PUFAs	40.2±1.3	46.4±0.2*
n-6/n-3	1.6±0.0	0.9±0.0*

Data were mean ± SD (n=3). The asterisks labeled above the data indicated significant differences (*p* value: *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001). *fat-1*: omega-3 desaturase gene; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Supplemental Table S4. Fatty acid compositions in dorsal muscle of wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg:*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks.

% of total fatty acids	LFD-WT	LFD-Tg: <i>fat-1</i>	HFD-WT	HFD-Tg: <i>fat-1</i>
14:0	1.3±0.1	1.5±0.1	1.2±0.2	1.1±0.2
16:0	18.1±0.5	18.3±0.3	17.6±2.5	16.7±0.6
18:0	6.9±0.9	6.1±0.7	6.1±0.6	5.9±1.1
20:0	1.1±0.1	0.9±0.0	1.2±0.1	0.8±0.0
Total SFAs	27.4±0.2	26.9±0.6	26.1±3.3	24.5±1.3
16:1n-7	3.3±1.1	4.5±0.7	3.9±0.2	4.4±0.6
18:1n-9	38.9±2.4	36.7±0.9	25.00±2.8	26.0±1.8
20:1n-9	0.4±0.1	0.6±0.0	1.2±1.2	0.4±0.1
22:1n-11	0.3±0.0	0.5±0.0	0.4±0.1	0.3±0.0
Total MUFAs	43.0±1.3	42.3±0.7	30.5±1.0	31.1±1.2
18:2n-6	15.2±0.4 ^b	14.1±2.5 ^a	26.3±1.1 ^c	23.1±1.0 ^c
18:3n-6	0.8±0.0 ^b	0.6±0.0 ^a	1.1±0.0 ^c	0.9±0.3 ^b
20:2n-6	0.7±0.0 ^d	0.6±0.0 ^c	0.3±0.0 ^b	0.2±0.1 ^a
20:3n-6	0.6±0.2 ^b	0.4±0.0 ^a	0.6±0.1 ^b	0.5±0.1 ^b
20:4n-6	2.9±0.1 ^b	1.3±0.1 ^a	3.5±0.3 ^b	2.9±0.6 ^a
Total n-6 PUFAs	20.8±0.4 ^b	17.6±2.4 ^a	32.1±2.5 ^d	27.6±0.7 ^c
18:3n-3	1.7±0.3 ^a	2.6±0.4 ^b	2.8±0.2 ^b	4.3±0.6 ^c
18:4n-3	0.3±0.1	0.5±0.2	0.5±0.1	0.8±0.1
20:4n-3	1.6±0.2	2.3±0.1	2.0±0.1	1±0.6

20:5n-3	1.6±0.9 ^a	2.5±1.0 ^b	2.0±0.8 ^a	3.2±0.5 ^c
22:5n-3	1.1±0.3	0.6±0.2	0.5±0.1	0.9±0.2
22:6n-3	2.4±0.1 ^a	3.7±0.5 ^c	3.3±0.0 ^b	5.33±0. ^c
Total n-3 PUFAs	8.8±1.0 ^a	12.3±1.8 ^{ab}	11.2±2.0 ^b	15.5±0.9 ^c
n-6/n-3	2.4±0.2 ^c	1.4±0.2 ^a	2.9±0.2 ^d	1.77±0.1 ^b
Total PUFAs	29.6±1.3	29.9±0.7	43.8±0.8	43.2±1.3

Data were mean ± SD (n=3). The different letters in each row indicated significant differences ($p < 0.05$). *fat-1*: omega-3 desaturase gene; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Supplemental Table S5. Fatty acid compositions in liver of wild type zebrafish (WT) and *fat-1* transgenic zebrafish (Tg:*fat-1*) fed with low-fat diet (LFD) and high-fat diet (HFD) for 8 weeks.

% of total fatty acids	LFD-WT	LFD-Tg: <i>fat-1</i>	HFD-WT	HFD-Tg: <i>fat-1</i>
14:0	1.8±0.2	2.0±0.1	1.6±0.2	1.9±0.1
16:0	19.0±2.8	19.2±0.7	16.4±2.1	14.3±0.6
18:0	9.1±0.5	8.5±1.6	7.1±1.1	8.5±1.
20:0	1.6±0.0	1.5±0.0	1.4±0.0	1.3±0.0
Total SFAs	31.6±2.1	31.4±1.7	24.5±1.9	24.0±1.3
16:1n-7	6.8±0.9	6.8±0.3	5.8±0.2	6.1±0.6
18:1n-9	41.3±2.5	40.3±2.4	31.4±1.8	32.3±1.8
20:1n-9	0.4±0.1	0.5±0.1	0.4±0.1	0.3±0.1
22:1n-11	0.2±0.0	0.3±0.1	0.2±0.0	0.2±0.0
Total MUFAs	48.7±3.5	48.3±2.1	37.7±1.9	39.0±1.2
18:2n-6	10.3±0.4 ^b	8.4±2.1 ^a	22.7±2.2 ^d	19.0±1.0 ^c
18:3n-6	0.4±0.1 ^a	0.8±0.1 ^b	1.5±0.1 ^c	1.9±0.3 ^c
20:2n-6	0.9±0.1 ^b	0.9±0.2 ^b	0.4±0.0 ^a	0.4±0.1 ^a
20:3n-6	0.5±0.6 ^a	0.4±0.05 ^b	0.6±0.3 ^b	0.6±0.1 ^a
20:4n-6	3.2±1.1 ^b	2.5±0.3 ^a	4.0±1.8 ^c	2.9±0.6 ^a
Total n-6 PUFAs	15.5±1.6 ^b	12.6±2.5 ^a	30.2±0.3 ^d	25.2±0.7 ^c
18:3n-3	0.9±0.1 ^a	2.1±0.1 ^b	1.6±0.7 ^a	3.0±0.6 ^b
18:4n-3	0.1±0.0 ^a	0.1±0.0 ^a	0.2±0.1 ^b	0.3±0.1 ^c
20:4n-3	1.0±0.2	1.7±0.2	1.4±1.2	2.7±0.5
20:5n-3	0.3±0.1 ^a	0.5±0.1 ^b	0.9±0.5 ^b	1.2±0.5 ^c
22:5n-3	0.5±0.4	0.5±0.2	0.5±0.2	0.6±0.2
22:6n-3	1.2±0.6 ^a	2.2±0.1 ^b	2.1±0.3 ^a	3.5±0.5 ^c
Total n-3 PUFAs	4.7±0.9 ^a	7.6±0.2 ^c	7.7±0.3 ^b	11.4±0.9 ^c
n-6/n-3	3.2±0.3 ^c	1.6±0.2 ^a	4.6±0.16 ^d	2.3±0.2 ^b
Total PUFAs	20.2±2.3	19.5±1.5	38.0±1.6	36.9±1.2

Data were mean ± SD (n=3). The different letters in each row indicated significant differences ($p < 0.05$). *fat-1*: omega-3 desaturase gene; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.