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Protective effects of aerobic swimming training on high-fat diet induced nonalcoholic fatty liver disease: Regulation of lipid metabolism via PANDER-AKT pathway

Hao Wu [a, 1], Meihua Jin [b, 1], Donghe Han [c], Mingsheng Zhou [d], Xifan Mei [e], Youfei Guan [f, g], Chang Liu [a, *]

[a] Department of Endocrinology, First Affiliated Hospital of Liaoning Medical University, Jinzhou, Liaoning, China
[b] Department of Immunology, Liaoning Medical University, Jinzhou, Liaoning, China
[c] Department of Neurobiology, Liaoning Medical University, Jinzhou, Liaoning, China
[d] Department of Physiology, Liaoning Medical University, Jinzhou, Liaoning, China
[e] Department of Orthopedics, First Affiliated Hospital of Liaoning Medical University, Jinzhou, Liaoning, China
[f] Department of Physiology and Pathophysiology, Peking University Diabetes Center, Peking University Health Science Center, Beijing, China
[g] Shenzhen University Diabetes Center, Shenzhen University Health Science Center, Shenzhen, China

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ABSTRACT

This study aimed to investigate the mechanism by which aerobic swimming training prevents high-fat-diet-induced nonalcoholic fatty liver disease (NAFLD). Forty-two male C57BL/6 mice were randomized into normal-diet sedentary (ND; n = 8), ND exercised (n = 8), high-fat diet sedentary (HFD; n = 13), and HFD exercised groups (n = 13). After 2 weeks of training adaptation, the mice were subjected to an aerobic swimming protocol (60 min/day) 5 days/week for 10 weeks. The HFD group exhibited significantly higher mRNA levels of fatty acid transport-, lipogenesis-, and β-oxidation-associated gene expressions than the ND group. PANDER and FOXO1 expressions increased, whereas AKT expression decreased in the HFD group. The aerobic swimming program with the HFD reversed the effects of the HFD on the expressions of thrombospondin-1 receptor, liver fatty acid-binding protein, long-chain fatty-acid elongase-6, Fas cell surface death receptor, and stearoyl-coenzyme A desaturase-1, as well as PANDER, FOXO1, and AKT. In the HFD exercised group, PPARα and AOX expressions were much higher. Our findings suggest that aerobic swimming training can prevent NAFLD via the regulation of fatty acid transport-, lipogenesis-, and β-oxidation-associated genes. In addition, the benefits from aerobic swimming training were achieved partly through the PANDER-AKT-FOXO1 pathway.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by accumulation of triglycerides within hepatocytes in the form of macrovesicle and microvesicle lipids with subsequent inflammation (i.e., nonalcoholic steatohepatitis), which can lead to fibrosis, cirrhosis, and liver failure [1]. NAFLD is mostly accompanied by obesity, type 2 diabetes, and dyslipidemia [2] because of metabolism imbalance in fatty acids in adipose and liver tissues, which can result in hepatic insulin resistance and enhanced liver lipogenesis [3]. However, effective therapeutic strategies for preventing disease progression in NAFLD patients are still lacking.

Lifestyle interventions have become an important strategy for the treatment of NAFLD [4]. Recently, a review that described the relationship between exercise and its implications for treatment of fatty liver disorders concluded that the effect of exercise on the reduction of liver fat compared favorably with most of the available pharmacological therapies [5]. It reported that exercise training prevents the development of hepatic steatosis in rats fed a high-fat diet [6] and significantly reduces hepatic steatosis in obese patients with NAFLD [7]. Although increasing evidence has shown that

* Corresponding author. Department of Endocrinology, First Affiliated Hospital of Liaoning Medical University, Jinzhou 121000, Liaoning Province, China. Fax: +86 04164673162.
E-mail address: cliuxmei@outlook.com (C. Liu).
[1] Both authors contributed equally to this work.

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physical exercise can reduce liver lipid and metabolic control [8,9], studies on the underlying mechanisms by which aerobic exercise training prevents the development of NAFLD remain largely unexamined.

Recently, our collaborators and other researchers have revealed the roles of the PANDER (pancreatic derived factor, FAM3B) in diabetic and obese animals. It demonstrated that inactivation of hepatic PANDER markedly reduced steatosis, insulin resistance, and hyperglycemia in db/db mice [10], and overexpression of PANDER could promote liver steatosis by activating some lipid metabolic-related genes, such as FAS (Fas cell surface death receptor) and CD36 (thrombospondin-1 receptor) through a decrease in AKT (protein kinase B, PKB) [11]. Based on these results, we hypothesized that enhanced physical activity could suppress liver fat accumulation by downregulating lipid synthesis, and transporting and stimulating fatty acid oxidation through the PANDER-AKT pathway.

Therefore, the primary purpose of the present study was to examine if and how aerobic swimming training attenuates the development of NAFLD via the regulation of lipid metabolism-associated genes. The secondary purpose was to investigate the regulating pathways of aerobic swimming training on NAFLD.

2. Materials and methods

2.1. Animals

Forty-two 8-week-old male wild-type C57BL/6 mice were housed in a temperature-controlled room (20–23 °C; 35–55% humidity) with a 12-h light/dark cycle and free access to food and water. The body weight was measured weekly (Mondays, 12 PM). Body fat mass was measured by magnetic resonance imaging (MRI) with an EchoMRI-500™ (Echo Medical Systems, Shanghai, China). All animals were sacrificed under anesthetic conditions (anesthetized with diethyl ether) at the end of the experimental period. Blood samples were rapidly obtained by cardiac puncture from the right atria. The liver, and epididymal fat deposits (adipose tissue surrounding the ureters, bladder, and epididymis) on both sides of the animal were removed and weighed. The tissues of interest were snap-frozen in liquid nitrogen immediately after resection and stored at −80 °C. All procedures were approved by the Institutional Animal Care and Use Committee of the Peking University Health Science Center.

2.2. Diets and exercise protocol

Both the normal diet and high-at diet were obtained from Shanghai Laboratory Animal Center (SLAC), Chinese Academy of Sciences (Shanghai, China). Animals were randomly assigned into four groups fed with normal diet (containing 10% lipid, 76% carbohydrate, and 14% protein, 3.75 kcal/g of chow) or High-fat diet (containing 60% lipid, predominantly from animal fat, 26% carbohydrate, and 14% protein, 5.40 kcal/g of chow): normal diet sedentary group (ND; n = 8), normal diet exercised group (ND-Ex; n = 8), high-fat diet sedentary group (HFD; n = 13), and high-fat diet exercised group (HFD-Ex; n = 13). The exercise protocol consisted of 12 weeks of training. The first 2 weeks were an adaptation period, with a progressive increase of the time exercising (6 min/day until reaching 60 min/day, 5 times/week) without an increase of weight in the tail (Fig. 1). This exercise training protocol corresponds to approximately 40–60% of the maximum oxygen uptake of a sedentary, untrained subject who has at least one risk factor for developing cardiovascular disease, and it emphasized exercising at a low to moderate intensity for a long duration [12]. The temperature of the water was maintained between 30 °C and 32 °C.

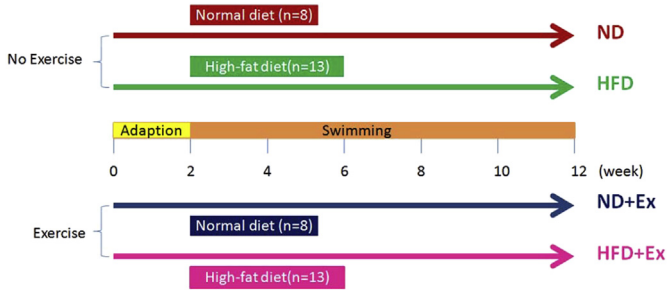

Fig. 1. Experimental design. The training program consisted of two parts, adaption and main program. The first 2 weeks were referred to adaptation with a progressive increase of the time exercising (6 min/day until reaching 60 min/day, 5 times/week). After 2 weeks adaption, mice were subjected to main exercise training (60 min/day, 5 times/week).

2.3. Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed at the beginning (week 0), at weeks 4 and 8, and at the end (week 12) of the swimming training to evaluate the effects of diet and exercise on the glucose tolerance. Animals were fasted for 6 h (8 AM–2 PM) before receiving an oral administration of glucose (3 g/kg body weight). The blood was collected through a small incision in the tail tip, and a FreeStyle brand glucometer (Abbott Laboratories, Shanghai, China) was used to obtain the glucose plasma concentrations before administering the glucose and 30, 60, 90, and 120 min afterwards. The area under the curve was calculated (GraphPad Prism version 5) to assess the glucose intolerance.

2.4. Measuring the hepatic and serum triglyceride and cholesterol content

The hepatic lipids were extracted by homogenization of weighed pieces of liver tissue in chloroform:methanol (2:1 vol/vol) solvent (Beijing Chemical Technology). Aliquots from the clear supernatant were used for estimation of cholesterol and triglycerides after the solvent was dried off under a stream of nitrogen. the total triglycerides (TG) and total cholesterol (TC) were extracted from the mouse liver and quantitated by the use of TG and TC assay kits (Nanjing Jiancheng Biotechnology Co.,Ltd. Nanjing, China). The serum lipid profile was determined by fast-performance liquid chromatography (FPLC) (Hitachi 7170A, Tokyo, Japan), as previously described [13].

2.5. RNA extraction and quantitative PCR

Total RNA was isolated from the mouse livers using TRIzol reagent. For quantitative real-time PCR (qPCR), 5 μg of each RNA sample was reverse transcribed, and the cDNA samples were then used as templates for qPCR. The PCR reactions were carried out at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s with a final extension at 72 °C for 5 min β-actin was used as an internal control. The primer sequences are shown in Table 1.

2.6. Statistical analysis

The data are presented as the mean ± standard error. The differences among the groups were tested with one-way ANOVA and the post hoc test of Tukey (GraphPad Prism, GraphPadSoftware, San Diego, CA, USA). A P-value < 0.05 was considered statistically significant.

Table 1
Primers used for the gene expression analysis.

Genes[a]	Sequences[b] (5′–3′)
CD36	F: AAACTGCATGGCAGCTTTGG
	R: CAGAAGGGTGCACAGGAGAG
FATP5	F: GATGCTTTAGAGCGGCAAGC
	R: AACTTGGCCAACCCCAGAAA
LFABP	F: GCAGAGCCAGGAGAACTTTGAG
	R: TTTGATTTTCTTCCCTTCATGCA
DGAT2	F: AGGCCCTATTTGGCTACGTT
	R: GATGCCTCCAGACATCAGGT
ELOVL6	F: TGCCATGTTCATCACCTTGT
	R: TGCTGCATCCAGTTGAAGAC
FAS	F: GCCATGCCCAGAGGGTGGTT
	R: AGGGTCGACCTGGTCCTCA
SCD1	F: GCGATACACTCTGGTGCTCA
	R: CCCAGGGAAACCAGGATATT
PPARα	F: CTCCTCTAGGCCCCTCCTTT
	R: AGCTCCCTTCTGCCTGGATA
AOX	F: TGTCATTCCTACCAACTGTC
	R: CCATCTTCTCAACTAACACTC
KT	F:CAACAGACAGTGTTCATCG
	R:TCACAGGCACAATCTCAG
PANDER	F: TCCCTGCTGTTCATGGTGACT
	R: GGCTTCTATGGCATCCTTTGC
AKT	F: TCTTTGCTGGCATTGTTTGGC
	R: GCTGTCATCTTGGTCAGGAGGAGT
FOXO1	F: TCAGGCTAGGAGTTAGTGAGCA
	R: GGGGTGAAGGGCATCTTT
β-actin	F: TGGAATCCTGTGGCATCCATGAAAC
	R: TAAAACGCAGCTCAGTAACAGTCCG

[a] CD36, thrombospondin 1 receptor; FATP5, fatty acid transporter protein 5; LFABP, liver fatty acid-binding protein; DGAT2, diacylglycerol O-acyltransferase 2; ELOVL6, long-chain fatty acid elongase 6; FAS, Fas cell surface death receptor; SCD1, stearoyl-coenzyme A desaturase 1; PPARα, peroxisome proliferator activated receptor α; AOX, acyl-coenzyme A oxidase; KT, 3-ketoacyl-coenzyme A thiolase; PANDER, pancreatic-derived factor; AKT, Protein kinase B; and FOXO1, Forkheadbox 1.
[b] F, forward; R, reverse.

3. Results

3.1. Biometry

At the end of the experiment, the body masses attained in the ND, ND-Ex, HFD, and HFD-Ex groups were 29.04 ± 0.54 g, 25.11 ± 1.05 g, 36.32 ± 0.59 g, and 28.15 ± 0.87 g, respectively (Fig. 1A). In comparison to the HFD group, the swimming training was efficient in preventing an increase in body weight in the HFD-Ex group throughout the experimental period (Fig. 2A).

The HFD group showed a heavier liver mass, (plus 40%, $P < 0.001$), body fat mass (plus 160%, $P < 0.001$), and epididymal fat mass (plus 140%, $P < 0.001$) than the ND group. The exercise reduced the liver mass by 12% ($P < 0.001$), body fat mass by 35% ($P < 0.001$), and epididymal fat mass by 43% ($P < 0.05$) in the HFD-Ex group, respectively. The differences in organ masses were no significant between ND and ND-Ex (Fig. 2B, C).

In comparison with the ND group, the HFD group had an increase of 40% in fasting glucose at 12th week ($P < 0.001$), while, the swimming training reduced the fasting glucose by 30% ($p < 0.05$) in the HFD-Ex groupin comparison with the ND group. At the end of the experimental period, the OGTT in the HFD group had a higher value than the ND group (plus36%, $p < 0.01$) and HFD-Ex group (plus 25%, $p < 0.05$). No difference was observed between the ND-Ex and ND groups (Fig. 2D).

3.2. Lipid metabolism

In comparison to the ND group, the livers of the HFD group showed a 69% higher TG level ($p < 0.001$) and a 110% higher TC level. However, the levels of TG and TG in HFD-Ex group were less than in the HFD group (27% for TG, $p < 0.05$; 39% for TC, $p < 0.05$). There were no differences in the liver TG and TC levels between the ND and ND-Ex group. In comparison to the ND group, the serum TG and TC levels were 45% and 69% ($p < 0.001$) higher in the HFD group, respectively. However, the swimming training reduced these levels by 30% (for TG, $p < 0.05$) and 18% (for TC, $p < 0.05$) in the HFD-Ex group. No difference was observed between the ND-Ex and ND groups (Fig. 3A). Oil red O staining of the liver sections showed the accumulated neutral lipid in the HFD group, which was improved (less lipid accumulation) in the HFD-Ex group (Fig. 3B).

3.3. Lipid metabolism-associated gene expression in the liver

3.3.1. Fatty acid transport-associated gene expression

In comparison to the ND group, the HFD resulted in an approximately 3.4-fold ($p < 0.001$) increase in the expression of the CD36. The exercise reduced its expression by 52% ($p < 0.05$) in the HFD-Ex group in comparison with its counterpart HFD group. In the HFD group, the liver fatty acid-binding protein (LFABP) expression increased in comparison to the ND group (≈1.6-fold; $p < 0.01$) and was reduced 30% ($p < 0.05$) in the HFD-Ex group. There were no significant differences between the ND-Ex and ND groups (Fig. 4A).

3.3.2. Lipogenesis-associated gene expression

The expression of diacylglycerol O-acyltransferase 2 (DGAT2), long-chain fatty acid elongase (ELOVL6), FAS, and stearoyl-coenzyme A desaturase 1 (SCD1) were significantly increased in the HFD group by ≈ 1.5-fold ($p < 0.001$), ≈1.6-fold ($p < 0.01$), 1.5-fold ($p < 0.05$), and ≈1.9-fold ($p < 0.05$), respectively. In comparison to the HFD group, the swimming training reduced the ELOVL6, FAS, and SCD1 expression by 31%, 43%, and 42% ($p < 0.01$), respectively. No difference was observed between the ND-Ex and ND groups (Fig. 4B).

3.3.3. Oxidation-associated gene expression

Peroxisome proliferator activated receptor α (PPARα), acyl-coenzyme A (CoA) oxidase (AOX), and 3-ketoacyl-CoA thiolase (KT) expression were increased in the HFD group by ≈ 2.1-fold ($p < 0.001$), ≈1.7-fold ($p < 0.01$), and ≈1.6-fold ($p < 0.05$), respectively. Swimming training upregulated the PPARα expression by ≈1.4-fold ($p < 0.05$), and ≈3.1-fold ($p < 0.05$) compare to the HFD and ND groups. The AOX expression significantly increased in the HFD-Ex group in comparison to the ND (≈2.7-fold, $p < 0.01$) and HFD (≈1.6-fold, $p < 0.01$) groups. However, it was not efficient in regulating the KT expression (Fig. 4C).

3.4. Expression of PANDER, AKT, and FOXO1

In comparison to the ND group, the expression of PANDER and FOXO1 were significantly increased in the HFD group by ≈ 2.3-fold ($p < 0.001$) and 1.6-fold ($p < 0.001$), respectively, while the expression of AKT was decreased by nearly 33% ($p < 0.05$). Swimming training reduced the expression of PANDER and FOXO1 by around 34% and 33% ($p < 0.01$), while the AKT expression was increased by ≈ 1.7 fold ($p < 0.05$) in comparison to HFD group (Fig. 4D). No difference was observed between the ND-Ex and ND groups.

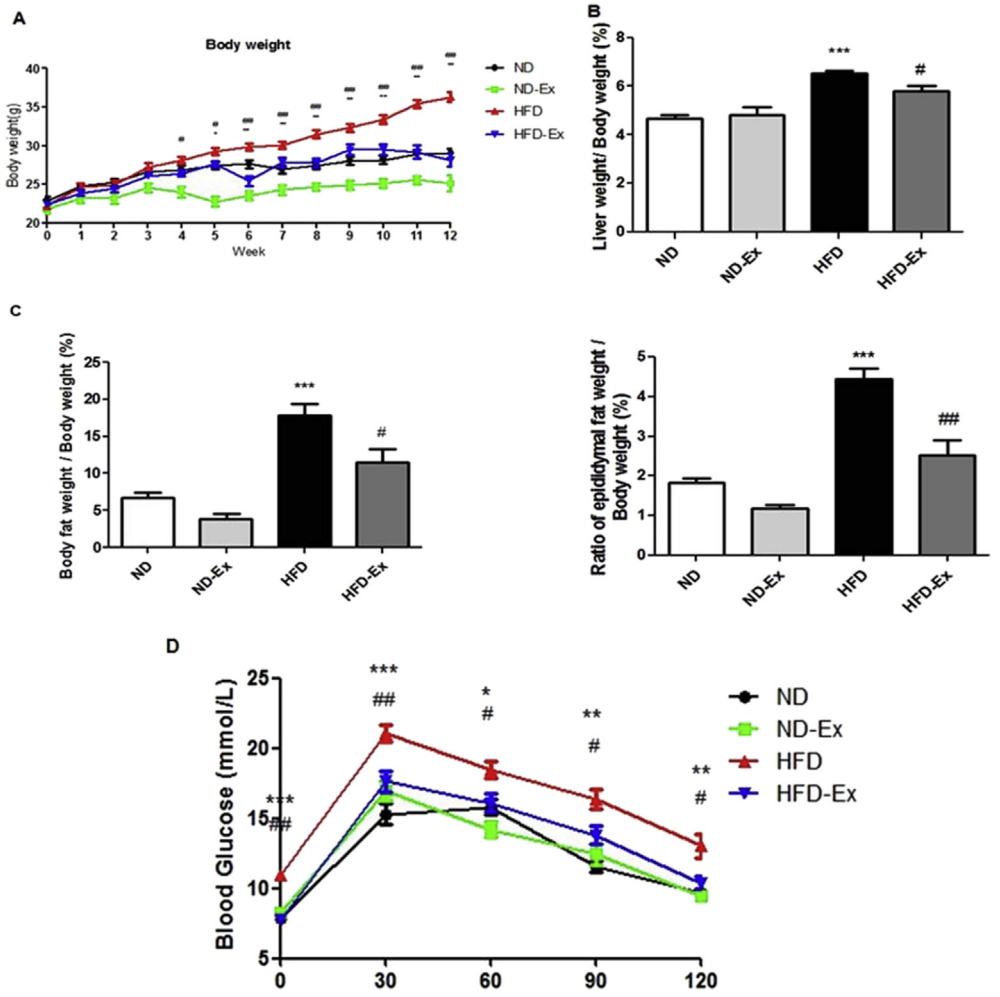

Fig. 2. (A) Body weight. There were significant differences ($p \leq 0.05$ for the same week) when comparisons with the normal diet (*) and high-fat diet exercised (#) groups were made. (B) The ratio of liver weight (%). (C) The ratios of fat weights (%). (D) Blood glucose (week 12). Results are expressed as means ± S.E. ($n = 8$ or13 for each group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. ND group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. HFD group. ND, normal diet; ND-Ex, normal diet exercised; HFD, high-fat diet; HFD-Ex, high-fat diet exercised.

4. Discussion

4.1. Swimming training ameliorates high-fat diet-induced NAFLD by regulating the lipid metabolism-related genes

Because excess fatty acid uptake is the first step in the development of NAFLD, we investigated the expressions of CD36 and LFABP, which can promote the fatty acid uptake in the liver [14–16]. In accordance with the previous studies that reported their enhanced expression with high-fat-diet supplementation, we also observed an increased expression in HFD rats, while those were significantly downregulated in the HFD-Ex group. The results indicated that the rats on the HFD that received swimming training had significantly reduced TG levels via the downregulation of fatty acid uptake.

In addition to the fatty acid uptake, regulation of lipogenesis is an important mechanism for controlling liver steatosis. ELOVL6, FAS, DGAT2, and SCD1, known as lipogenic enzymes, are involved in lipid accumulation and induce NAFLD by promoting oxidative stress and inflammation [17,18]. In the present study, we observed that the increased expression levels of ELOVL6, FAS, and SCD1 by HFD was also suppressed by swimming training. These findings suggest that aerobic swimming exercise inhibited the accumulation of hepatic lipid droplets via the downregulation of lipogenesis.

Disturbances in fatty acid oxidation-related enzymes also lead to excess lipid accumulation in the liver. Among these enzymes, AOX initiates β-oxidation and KT is involved in the final step of fatty acid oxidation, both of which have been reported to be activated by a HFD [19]. Moreover, studies that investigated the regulators of fatty acid oxidation have shown that PPARα plays crucial roles in fatty acid oxidation. PPARα-defective mice reportedly failed to induce fatty acid oxidation in the liver and developed severe steatohepatitis immediately after birth [20]. In NAFLD, the expression of hepatic PPARα was significantly decreased, while activation of PPARα expression was shown to prevent the development of steatosis [21,22], and PPARα expression was downregulated by 50% in human NAFLD [23]. In the present study, we observed similar results in the expressions of AOX and KT in rats fed with a HFD. However, the expression of PPARα was also increased with HFD supplementation. It is well known that β-oxidation is the process by which fatty acid molecules are broken down. Therefore, the increased PPARα might have been associated with the lipid overload state to consume excess fat, which would decrease after the liver becomes accustomed to the high-calorie

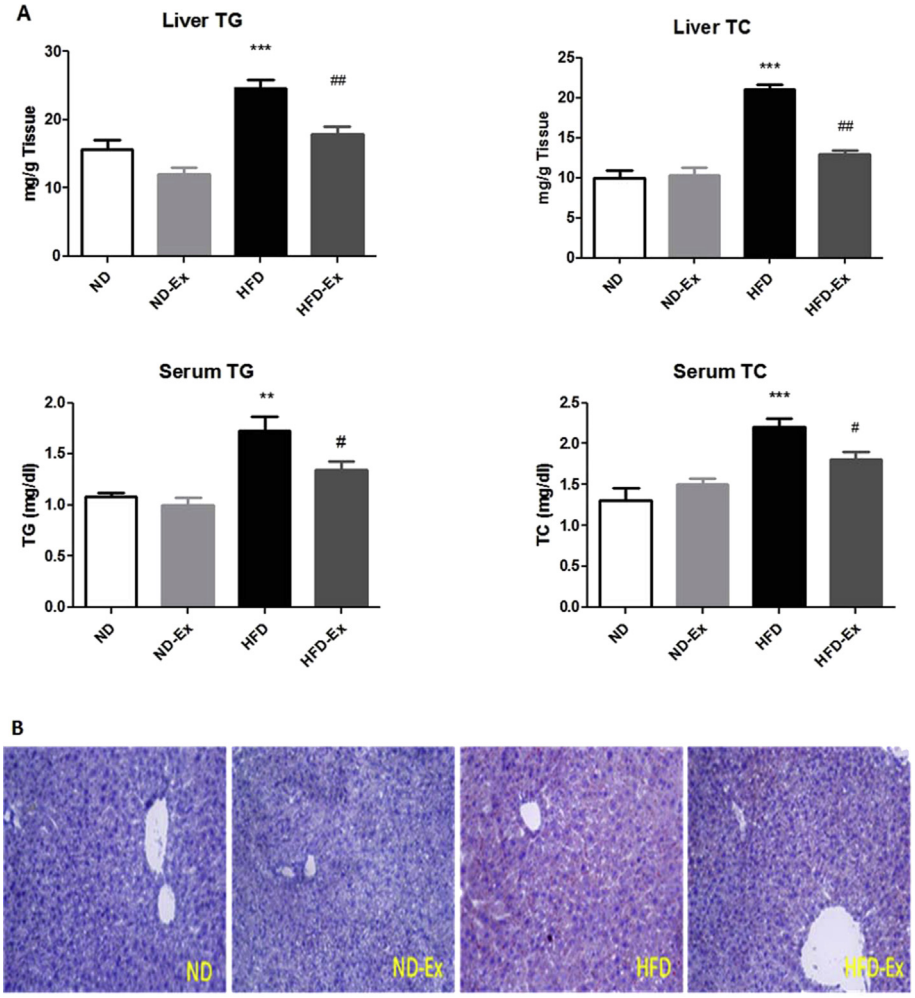

Fig. 3. (A) Total triglyceride (TG) and total cholesterol (TC) levels in the liver and serum. The results are expressed as means ± standard error (n = 8 for each group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. normal diet group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. high-fat diet group. (B) Oil red O staining of the liver (200×).

condition. Interestingly, accumulating studies have demonstrated that PPARα contributes to the regulation of genes involved in fatty acid uptake [24,25], lipid synthesis [26,27], and fatty acid oxidation [28]. These findings suggest that PPARα could have been involved in upregulation of fatty acid uptake, lipogenesis, and fatty acid β-oxidation directly or indirectly. Moreover, the fatty acid uptake and lipogenesis stimulated by PPARα expression were much more predominant than fatty acid β-oxidation, and eventually resulted in TG storage in HFD condition. However, we also observed that swimming exercise enhanced the expressions of PPARα and AOX in the mice in the HFD-Ex group compared with those in the HFD group. Recently, Hsw et al., [29] has reported that PPARα agonists attenuate NAFLD by upregulating farnesoid X receptor (FXR), peroxisome proliferator-activated receptor gamma co-activator (PGC)-1α to induce fatty acid oxidation in the liver. Activation of PPARα ameliorates hepatic steatosis in high Fructose–fed mice despite the marked upregulation of lipogenesis-related genes (SREBP1c, ACC, FAS, and SCD1) [30]. Another study investigating the effects of swimming training on disease has reported that it can protect ovariectomy-induced obesity though activation of PPARα and uncoupling protein 3 (UCP3) in skeletal muscle [31]. These results imply that swimming training can prevent NAFLD via fatty acid β-oxidation, and the pathways might be different from that in HFD condition. Meanwhile, these findings also suggest that PPARα can play different roles by activating various pathways under different conditions.

4.2. Swimming training regulates lipid metabolism-associated genes by affecting Pander-AKT-FOXO1 pathway

Although multiple mechanisms were reported to be involved in the regulation of liver lipid homeostasis [32], the exact mechanism of NAFLD still remains unknown. Recently, accumulating evidence has indicated the crucial roles of PANDER on lipogenesis. Pander was demonstrated to induce fatty acid accumulation in the liver by blocking AKT and AMPK [10,11,33], both of which can regulate FOXO1.FOXO1 has been reported to be associated with NAFLD by regulating fatty acid uptake, lipogenesis, and oxidation [34–36]. In the present study, we observed that PANDER and FOXO1 were significantly downregulated and that AKT was increased in the HFD-EX group compared with the HFD group. These findings suggest that swimming prevents the development of NAFLD through, at least in part, a PANDER-Akt-FOXO1-dependent mechanism.

Taken together, aerobic swimming training prevents the development of HFD-induced NAFLD via the downgulation of fatty acid uptake (CD36 and LFABP) and fatty acid synthesis (ELOVL6, FAS, and SCD1), and upregulaltion of fatty acid oxidation (PPARα

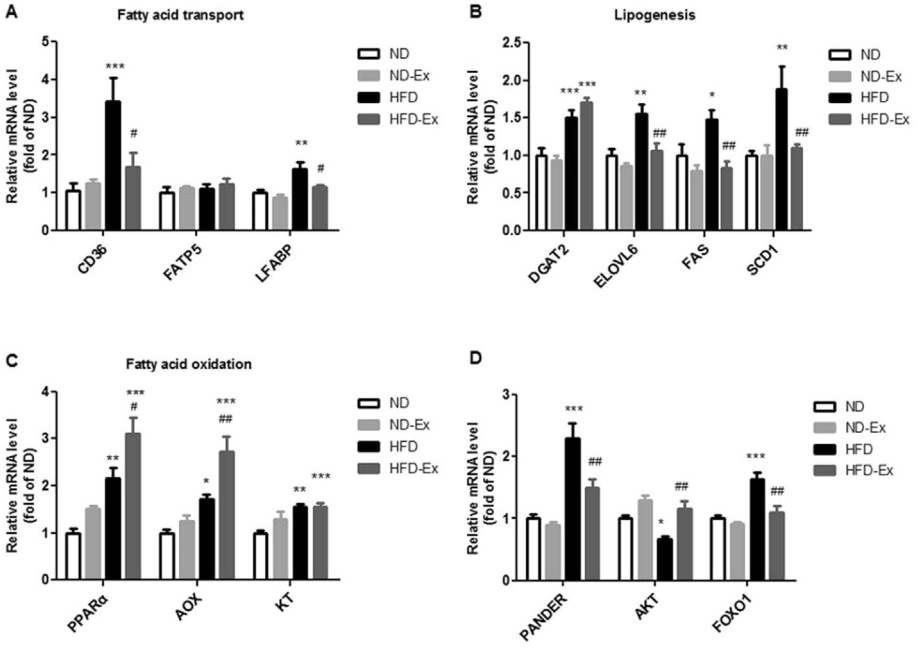

Fig. 4. Relative mRNA levels (fold change relative to the normal diet): (A) Fatty acid transport-associated gene expression. (B) Lipogenesis-associated gene expression. (C) Oxidation-associated gene expression. (D) Expression of gene regulators. Results are expressed as means ± standard error (n = 8 for each group). *p < 0.05, **p < 0.01, ***p < 0.001 vs. normal diet group; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. high-fat diet group.

and AOX). Meanwhile, aerobic swimming training attenuates HFD-induced liver steatosis by contributing to the suppression of hepatic PANDER and FOXO1 expressions via an increase in AKT expression. Further studies should be focused on investigating the effects of swimming on the other pathways (e.g., AMPK-FOXO1) to clarify the exact mechanism of physical exercise on HFD-induced NAFLD.

Conflict of interest

None.

Acknowledgments

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Transparency document

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