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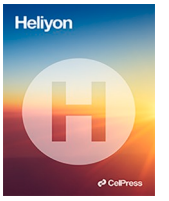

Research article

miR-122/PPARβ axis is involved in hypoxic exercise and modulates fatty acid metabolism in skeletal muscle of obese rats

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ABSTRACT

Hypoxic exercise is an effective intervention for obesity, because it promotes weight loss by regulating fatty acid (FA) metabolism. The regulation of peroxisome proliferator-activated receptor β (PPARβ) by miR-122 may be involved in this process, but the detailed mechanisms are unknown. In order to address this issue, we probed how miR-122 affected the expression of factors associated with FA metabolism in skeletal muscle of obese rats undergoing hypoxic training. By injecting adeno-associated virus 9 containing miR-122 overexpression vector or miR-122 inhibitor into skeletal muscles of rats with a 4-week hypoxic exercise regimen, the miR-122 expression level can be regulated. Body composition and blood lipid levels were analyzed, and PPARβ, carnitine palmitoyltransferase 1b (CPT1b), acetylCoA carboxylase 2 (ACC2), and FA synthase (FAS) mRNA and protein levels were evaluated using quantitative reverse transcription quantitative PCR(RT-qPCR) and Western blot analysis. We found that miR-122 overexpression increased low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels and decreased PPARβ, ACC2, and FAS expression. Conversely, miR-122 inhibition decreased TG level, increased high-density lipoprotein cholesterol (HDL-C) level, and upregulated PPARβ, ACC2, FAS, and CPT1b. These data indicated that the negative regulation of PPARβ by miR-122 promotes FA metabolism by altering the levels of the factors related to FA metabolism in skeletal muscle of obese rat under hypoxic training, thus providing molecular-level insight into the beneficial effects of this intervention.

1. Introduction

Obesity is a major global health issue, and interventions for obesity mainly focus on reducing energy intake and increasing physical activity. Compared with normal oxygen training, hypoxic exercise is a more effective weight loss intervention because it can suppress appetite and rapidly reduce body weight [1,2] via enhancement of lipid metabolism [3,4]; unfortunately, the detailed mechanisms are not yet fully understood.

Overexpression of miR-122, a lipid metabolism-related micro RNA, leads to the upregulation of the FAS and ACC, whereas miR-122 inhibition expression leads to a downregulation of FAS and ACC, reducing the rate of fatty acid synthesis [5]. This indicates that miR-122 may have an effect in regulating the expression of FAS and ACC. Additionally, when miR-122 is underexpressed, its target gene PPARβ is upregulated, activating the downstream fatty acid oxidation gene CPT1 [6]. Interestingly, the expression of miR-122

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and PPARβ is influenced by hypoxia and exercise. Our previous study showed that after four weeks of hypoxic training, miR-122 expression was downregulated, whereas PPARβ, CPT1, FAS, and ACC expressions were upregulated [7].

Most research on the lipid metabolism regulation in obesity has focused on the liver. However, obesity is also associated with dysregulation of FA metabolism in skeletal muscles [8]. Long term aerobic normoxia and hypoxic exercise can induce the FA oxidation factors expression and promote the utilization of FAs in skeletal muscle, and reports showed that the effectiveness of hypoxic training was greater compared with normoxic training [8,9]. As skeletal muscle is not a fat-storing tissue and FAs are mainly derived from the breakdown of adipose and other extramuscular tissues, an increase in FA utilization in skeletal muscle could influence lipid metabolism [10]. As such, elucidating the regulatory mechanism of skeletal muscle FA metabolism in hypoxic exercise is of great significance in determining how this intervention benefits obesity. The present study offers the scientific basis for weight reduction, improvement of lipid metabolism, and prevention and treatment of obesity-related diseases through hypoxic training.

The present study investigated the mechanism that miR-122/PPARβ regulates FA metabolism by miR-122 overexpressing or inhibition of the skeletal muscle in obese rats with hypoxic training. The expression of factors related to FA metabolism and physiological indices of obesity were then evaluated.

2. Materials and methods

2.1. Establishment of the obese rat model

3-week-old male Sprague–Dawley rats (N = 120) were bought (Beijing Vital River Laboratory Animal Technology Co., Ltd) and placed in cages with a light/dark cycle of 12/12 h at 22 °C–23 °C and 40%–60% humidity. These rats were fed weekly common chow diet and then divided into two groups. One group (N = 20) was fed common chow diet, and the other group (N = 100) was fed high-fat diet of 45% energy (D12451; Research Diets, New Brunswick, NJ, USA). Both groups obtained free access to the food and water for 10 weeks. HFD rats with body weight more than 20 % of the average body weight of the control group were obese [2,7]. Rats that were overweight compared with their average body weight were excluded. Afterward, 50 obese rats were selected for horizontal running training. During two weeks of adaptive training, by recording the performance of the obese rats including whether rats can finish the scheduled training plan, 40 obese rats were picked and randomly divided into 4 groups: hypoxic sedentary (H, n = 10), placebo control (CE, n = 10), miR-122 inhibition (IE, n = 10), and miR-122 overexpression (OE, n = 10) (Fig. 1).

2.2. Cloning and production of AAV

The miR-122 overexpression vector (adeno-associated virus 9 [AAV9]-ZsGreen-rno-miR-122) and miR-122 inhibition vector (AAV9-ZsGreen-rno-miR-122-5p inhibitor) were constructed and packaged by Beijing Xibei Hongcheng Biotechnology (Beijing, China) (Tables 1 and 2). The miR-122 cDNA and miR-122-5p sequence UGGAGUGUGUGACAAUGGUGUUUG was used for designing the miR-122-5p inhibitor sequence. The control virus (empty vector) AAV9-ZsGreen expressed only ZsGreen green fluorescent protein. AAV particles were prepared by transfecting 293 AAV cells with the plasmid. Cells were collected after three days and repeatedly freeze-thawed at −80 °C and 37 °C to obtain recombinant rAAV9. ViraBind AAV9 Purification Mega Kit was used to purify the recombinant rAAV9 (VPK-141; CellBiolabs, San Diego, CA, USA) [11]. Viral titer was determined using quantitative PCR and adjusted to 1×10^{12} vg/ml.

2.3. Intramuscular injection of overexpression/inhibitor constructs into obese rats

Before initiating hypoxic exercise, AAV9-ZsGreen, AAV9-ZsGreen-rno-miR-122-5p, or AAV9-ZsGreen-rno-122 was injected using a sterilized microinjector at five sites of the right gastrocnemius muscle in the CE, IE, and OE rats, respectively [12]. The rAAV9 volume was 10 μl of virus per site (1×10^{12} vg/mL). Rats in H group did not receive any injection and was used as a control of the spread of the virus.

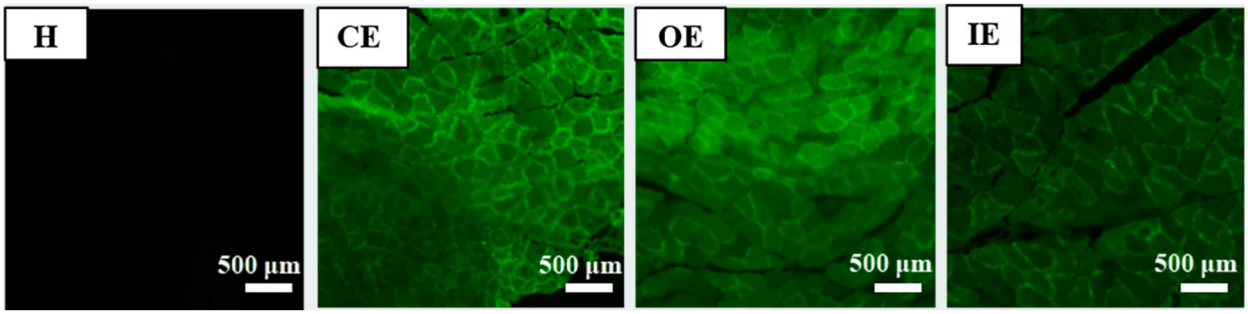

Fig. 1. Representative fluorescence images of the gastrocnemius muscle of rats injected with AAV9-ZsGreen-miR-122 (group OE), AAV9-ZsGreen-miR-122-5p inhibitor (group IE), or AAV9-ZsGreen (group CE), and not injected with AAV9 (group H). The gastrocnemius muscle of rats in groups OE, IE, and CE show ZsGreen fluorescence. Scale bar, 500 μm.

Table 1
Plasmid construction information of pAAV-ZsGreen-rno-miR-122.

Gene name	>rno-miR-122 MI0000891
Cloning vector	pAAV-ZsGreen-miR
Cloning strategy	*Bam*HI + *Eco*RI
Synthesized rno-miR-122 (5′→3′)	GGATCCCCTTAGCAGAGCTCTGGAGTGTGACAATGGTGTTTGTGTCCAAAACATCAAACGCCATCATCACACTAAACAGCTACTGCTAGGCGAATTC

Table 2
Plasmid construction information of pAAV-ZsGreen-rno-miR-122-5p inhibitor.

Gene name	>rno-miR-122-5p MIMAT0000827
Cloning vector	pAAV-ZsGreen-shRNA
Cloning strategy	*Bam*HI + *Hin*dIII
miR-122-5p-inhibitor-F (5'→3')	GATCCGCAAACACCATCAGACACTCCACGCGCAAACACCATCAGACACTCCATTTTTTA
miR-122-5p-inhibitor-R (5'→3')	AGCTTAAAAAATGGAGTGTCTGATGGTGTTTGCGCGTGGAGTGTCTGATGGTGTTTGCG

2.4. Hypoxic exercise program

Following virus injection, the 40 rats were placed in a hypoxic environment (13.6% O_2). Group H received no exercise training and groups CE, IE, and OE were trained to run on a treadmill for 4 weeks (20 m/min,1 h/d, 5 d/wk). After the last exercise bout, the rats belonging to training groups recovered for 24 h. Next, all of the rats were fasted for 12 h and measured the body weight and length. Lee's index was calculated according to body weight $(g)^{1/3}$/body length (cm) × 1000. Then, the rats were anesthetized by intraperitoneal injection of trichloroacetaldehyde hydrate (10%, 0.3 ml/100 g) and blood was extracted from the abdominal aorta. Serum was gained by centrifuging at 3000 rpm/10 min, and frozen at −20 °C. The right gastrocnemius muscle was frozen in liquid nitrogen and stored at −80 °C.

2.5. Blood lipid level measurement

The levels of serum total cholesterol (TC), TG, LDL-C, HDL-C, and free FA (FFA) were measured after thawing the frozen serum samples using commercial kits (Prodia Diagnostics, Germany; Nanjing Jiancheng Bioengineering Institute, China) [13].

2.6. RT-qPCR

RT-qPCR was to measure miR-122 expression level and the FA metabolism related genes mRNA level. Firstly, Trizol reagent lysed skeletal muscle to extract total RNA (Invitrogen, USA). Afterward, the isolated RNA was transcribed using a reverse transcription kit (dNTPs and M-MLV reverse transcriptase, Takara, Dalian, China; RNase Inhibitor, Fermentas, Canada) to obtain cDNA.

After designing and synthesizing primers for the RNA (miR-122 and U6) and cDNA (β-actin, PPARβ, CPT-1b, FAS, and ACC2) (Table 3 and Table 4), qPCR was carried out using SYBR Premium Ex Taq (Takara). One sample was amplified in triplicate. U6 and β-Actin are served as the internal control for miR-122 and other genes, respectively. The mRNA levels were calculated by using the ΔΔCT method [14].

2.7. Western blot

The protein levels of PPARβ, CPT-1b, FAS, and ACC2 in gastrocnemius muscle were measured via Western blot. After extracting the total protein from the samples and determining the protein concentration by a bicinchoninic acid assay kit (Cwbiotech, China),

Table 3
Primers used for quantitative reverse transcription PCR analysis of miR-122.

Gene	Primers (5′→3′)	Length (nt)
miR-122	RT: CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAAACA	42
	F: GCCCTGGAGTGTGACAATGG	21
	R: CTCAACTGGTGTCGTGGAGTC	21
U6	RT: CGCTTCACGAATTTGCGT	18
	F: CTCGCTTCGGCAGCACA	17
	R: CGCTTCACGAATTTGCGT	18

F, forward primer; nt, nucleotide; R, reverse primer; RT, primer for reverse transcription.

Table 4
Primers used for RT-qPCR analysis.

Gene	Primer (5′→3′)	Amplicon size (bp)
β-Actin	F: GAAGTGTGACGTTGACATCCG R: GCCTAGAAGCATTTGCGGTG	282
PPARβ	F: TTCATTGCCGCCATCATTCTGTG R: AGGTCTCACTCTCCGTCTTCTTCA	232
CPT1b	F: GAGACTGTGCGTTCCTGTACTAGC R: TGGAGACGATGTAGAGGCAGAAGA	187
FAS	F: CGTGACTGCTACTGTGGAGAAGAC R: CGCTGAACGCTACTGGGTTTGT	212
ACC2	F: AGGCACAGGTGAAGCAGGAGATT R: TCGGATAGTGGAACGCAGGTTGT	194

F, forward primer; R, reverse primer.

proteins (30 μg/well) were separated via electrophoresis. The voltage of electrophoresis for concentration gel was 90 V, and the voltage of electrophoresis for separation gel was 150V. The protein samples were then transferred to a PVDF membrane (0.45-μm pore size) at a constant pressure of 100 V for 10 min, followed by blocked overnight in Tris-buffered saline with 0.1% Tween-20 (TBST), which contained 5% bovine serum albumin, and then probed overnight at 4 °C with primary antibodies against PPARβ (1:2000; GTX113250) and FAS (1:1000; GTX13550) (both from GeneTex, Irvine, CA, USA) and ACC2 (1:500; A03668-2) and CPT1b (1:500; PB9491) (both from Boster Bio, Pleasanton, CA, USA). After washing three times with TBST, the membrane was probed with the secondary antibody (1:10,000) for 1 h at room temperature, followed by another TBST wash for three times. Adding enhanced chemiluminescence substrate for 3 min detected the protein bands. Then, the membrane was fixed for 2 min and Image-Pro Plus 6.0 software was used to analyze signal intensity.

2.8. Histologic analysis

To confirm whether rAAV9 successfully entered the skeletal muscle, we assessed whether ZsGreen in the CE, IE, and OE groups of rat skeletal muscle was expressed alone or co-expressed with miR-122 compared to the H group. Firstly, the frozen skeletal muscle was embedded with OCT (# 4583, Sakura), refrozen and solidified at - 70 °C. Thereafter, continuous slices were gained by the cryoslicer (Thermo, CRYOSTAR NX50). Lastly, the sections were observed and photographed using a fluorescence microscope (MS23).

2.9. Statistical analysis

Statistical analyses were conducted with SPSS v18.0 (IBM, Armonk, NY, USA). First, the data was tested for normal distribution. If not following a normal distribution, data were transformed using an exponential transformation to achieve normal distribution. Results were analyzed by one-way ANOVA. Significance was set at $p < 0.05$.

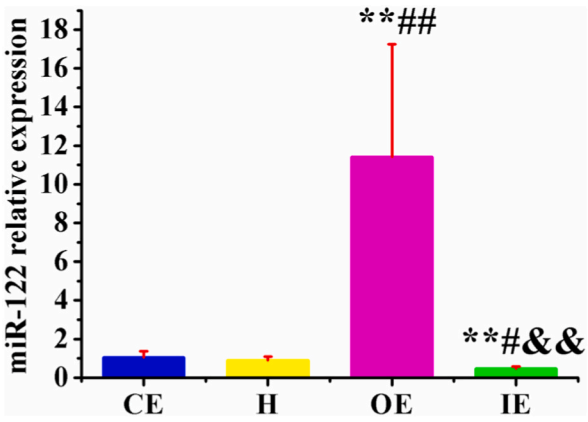

Fig. 2. Expression levels of miR-122 in obese rats from groups OE, IE, CE, and H measured using qRT-PCR. Data are presented as means ± SD. *p < 0.05, **p < 0.01; $^{\#\#}$p < 0.01 vs group CE; $^{\&}$p < 0.05, $^{\&\&}$p < 0.01 vs group OE (1-way analysis of variance).

3. Results

3.1. AAV9 transfection efficiency and miR-122 level in the skeletal muscle of obese rats

After hypoxic training for 4 weeks, the AAV9 transfection efficiency and miR-122 level in the skeletal muscle of rats were rated. Green fluorescence was detected in skeletal muscle tissue samples from the OE, IE, and CE groups but not in those from the H group (Fig. 1). OE group had the highest miR-122 level and IE group had the lowest as determined using RT-qPCR (Fig. 2). This indicates that AAV9 transduction was successful and miR-122 expression was continuously expressed during the 4-week exercise period.

3.2. Effects of hypoxic training on physiologic indices of obese rats

After hypoxic training for 4 weeks, body weight and Lee's index of rats in the training groups (CE, OE, and IE) were significantly reduced compared with the H group (all $p < 0.01$); nevertheless, there were no obvious differences among the three training groups (Fig. 3). Moreover, miR-122 overexpression and inhibition had no significant effect on body weight and Lee's index. Hence, 4-week hypoxic training can significantly decrease the body composition of obese rats independently of miR-122.

Compared with the H group, the IE, OE, and CE groups had lower serum TG, LDL-C, and FFA levels (all $p < 0.01$; Fig. 4). Furthermore, compared with the CE group, the OE group had higher TG ($p < 0.05$) and LDL-C ($p < 0.01$) levels, whereas the IE group had lower TG ($p < 0.05$) and higher HDL-C ($p < 0.01$) levels. Compared with those in the OE group, TC, TG, LDL-C, and FFA levels were lower in the IE group (TC, TG, LDL-C: $p < 0.01$; FFA: $p < 0.05$) except for HDL-C level, which increased ($p < 0.01$) in the IE group (Fig. 4). Therefore, changes in miR-122 can significantly affect the blood lipid levels of obese rats undergoing hypoxic training.

3.3. Hypoxic training and miR-122 regulate PPARβ and downstream FA metabolism genes

Compared with the H group, the CE group had higher *ACC2* mRNA expression ($p < 0.05$); however, ACC2 protein level was not obviously different between groups; in addition, *PPARβ* and *FAS* mRNA and ACC protein levels increased in the OE group (all $p < 0.01$), whereas PPARβ, FAS mRNA and protein (all $p < 0.01$), and CPT1b, ACC2 mRNA (both $p < 0.01$) and protein ($p > 0.05$ and $p < 0.05$) levels increased in the IE group (Figs. 5 and 6, Supplementary Figs. 1 and 2). Compared with the CE group, the OE group had the higher levels of *PPARβ,* FAS mRNA and ACC2 protein ($p < 0.01$), and IE group had the higher levels of PPARβ, FAS mRNA and protein (all $p < 0.01$), and CPT1b, ACC2 mRNA ($p < 0.05$ and $p < 0.01$) and protein ($p > 0.05$ and $p < 0.05$). Compared with the overexpressing miR-122 in the OE group, inhibition of miR-122 in IE group rats induced upregulation of *PPARβ, CPT1b* and *ACC2* mRNA (all $p < 0.01$), PPARβ and FAS protein levels (both $p < 0.01$), and downregulation of ACC2 protein level ($p < 0.01$). Thus, the comparison between the overexpression and inhibition of miR-122 suggested a significant difference in the impact on the expression of PPARβ, CPT1b, FAS, and ACC2.

4. Discussion

Exercise interventions are an effective and economical approach to managing obesity. However, weight loss through exercise occurs slowly, and exercise can increase appetite. Hypoxia and hypoxic exercise can suppress appetite and reduce body weight and fat within a short time [9,15]. It was reported that miR-122 regulates lipid metabolism in obese rodents via PPARβ signaling [7]. This present research confirmed that miR-122 effected FA metabolism of skeletal muscle in obese rodent undergoing hypoxic training as

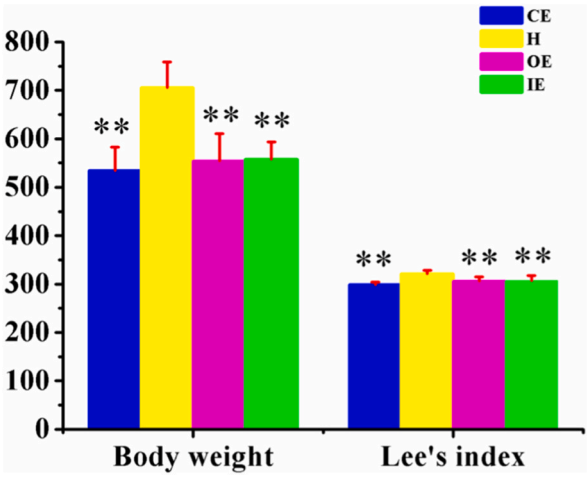

Fig. 3. Effects of hypoxic training on body composition of obese rats. Data are presented as mean ± SD. *p < 0.05, **p < 0.01, *p < 0.05, **p < 0.01 vs group H; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 vs group CE; $^{\&}$p < 0.05, $^{\&\&}$p < 0.01 vs group OE (1-way analysis of variance).

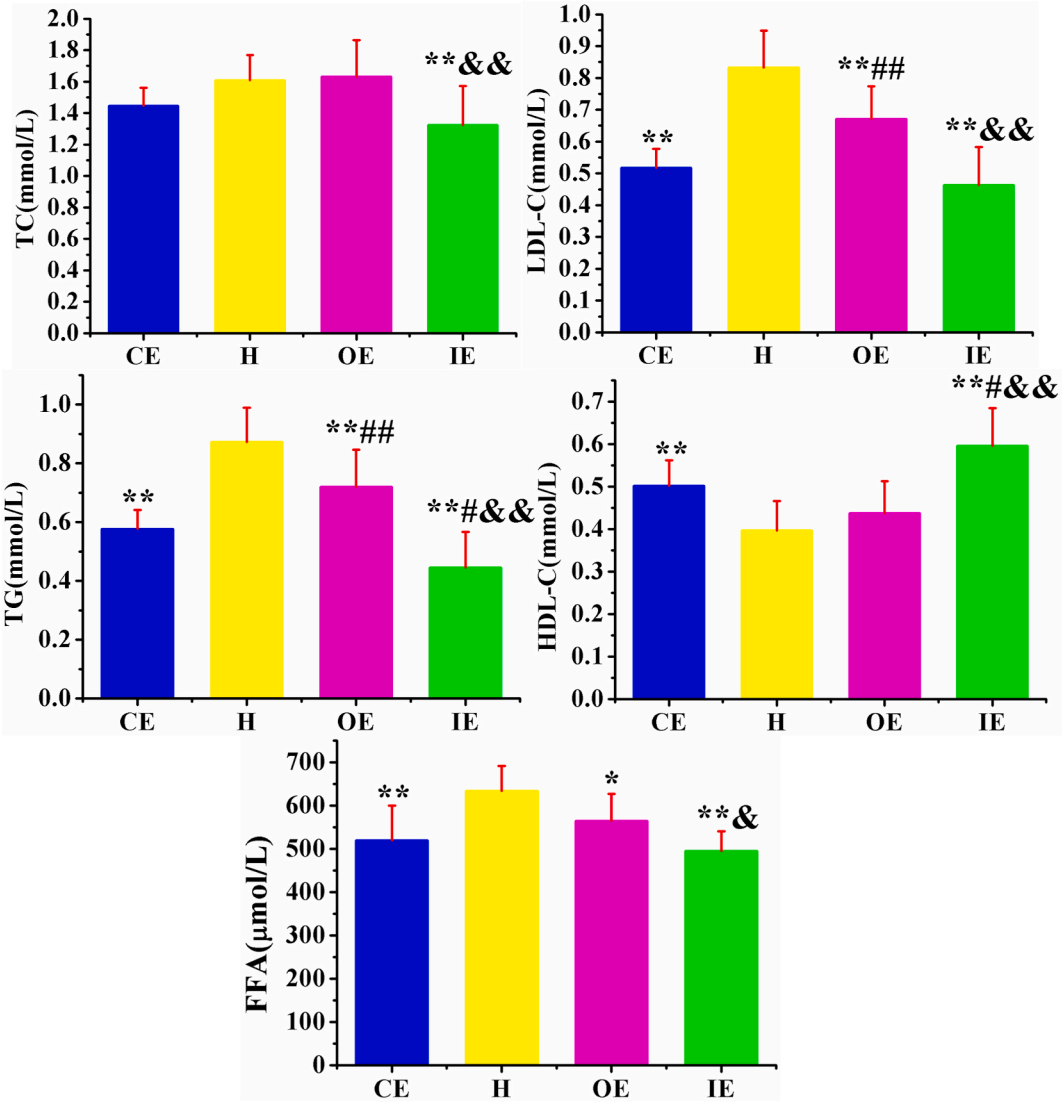

Fig. 4. Serum lipid levels of obese rats. Data are presented as mean ± SD. *p < 0.05, **p < 0.01, *p < 0.05, **p < 0.01 vs group H; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 vs group CE; $^{\&}$p < 0.05, $^{\&\&}$p < 0.01 vs group OE (1-way analysis of variance).

evidenced via decreases in body weight and Lee's index. These results may be connected to how hypoxic exercise suppresses appetite [16–19]. However, we found that the altering of miR-122 expression had no significant effect on the body composition of the obese rats under hypoxic training, suggesting that the modulation of FA metabolism in skeletal muscle does not directly lead to the control of obesity.

Hypoxic exercise has been shown to inhibit the expression of miR-122 and improve blood lipid levels [7,20]. Consistent with these results, the present study showed that after 4 weeks of hypoxic exercise, miR-122 overexpression increased serum TG and LDL-C levels, whereas miR-122 inhibition decreased TG level and increased HDL-C level in the three intervention (CE, OE, and IE) groups. Circulating miR-122 expression level was shown to be related with blood lipid levels and the occurrence of non-alcoholic fatty liver disease [21,22]. Moreover, patients with hyperlipidemia had higher plasma miR-122 level than that in healthy individuals and the miR-122 level was positively correlated with blood lipid levels [23]. Thus, miR-122 overexpression of the skeletal muscle in obese rats under hypoxic training may be related to dyslipidemia, and inhibition of miR-122 expression may improve blood lipid levels and contribute to a decrease in body weight.

PPARβ regulates FA catabolism and participates in the regulation of skeletal muscle motor adaptation [24–26]. Antisense oligonucleotide-mediated knockdown of miR-122 led to the upregulation of PPARβ through direct interaction of miR-122 with *PPARβ* transcript [27]. Another study found that a reduction in miR-122 level was related to the upregulation of PPARβ [6]. However, this study found that both the overexpression and inhibition of miR-122 increased the expression of PPARβ. This may be related to the complex regulatory mechanisms affecting the expression of PPARβ during exercise. For example, exercise may lead to an increase in

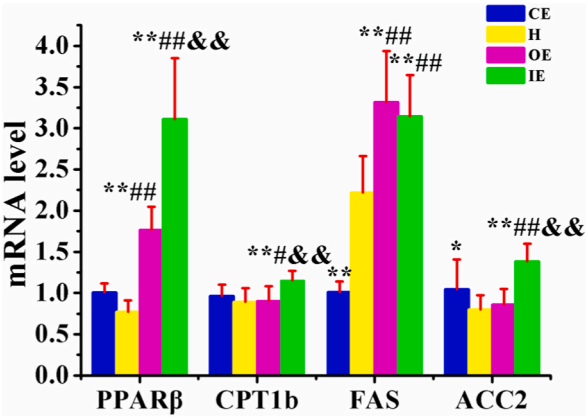

Fig. 5. Expression of the lipid metabolism regulator PPARβ and downstream effectors (CPT1b, FAS, and ACC2). mRNA levels in obese rats under hypoxic conditions, with hypoxic training, and with regulated miR-122 expression were determined using qRT-PCR. OE, obese rats with miR-122 overexpression and hypoxic training; IE, obese rats with miR-122 depletion and hypoxic training; CE, obese rats with hypoxic training only; H, obese sedentary rats without regulation of miR-122 expression. Data are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$ vs rats in group H; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs rats in group CE; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ vs rats in group OE (1-way analysis of variance).

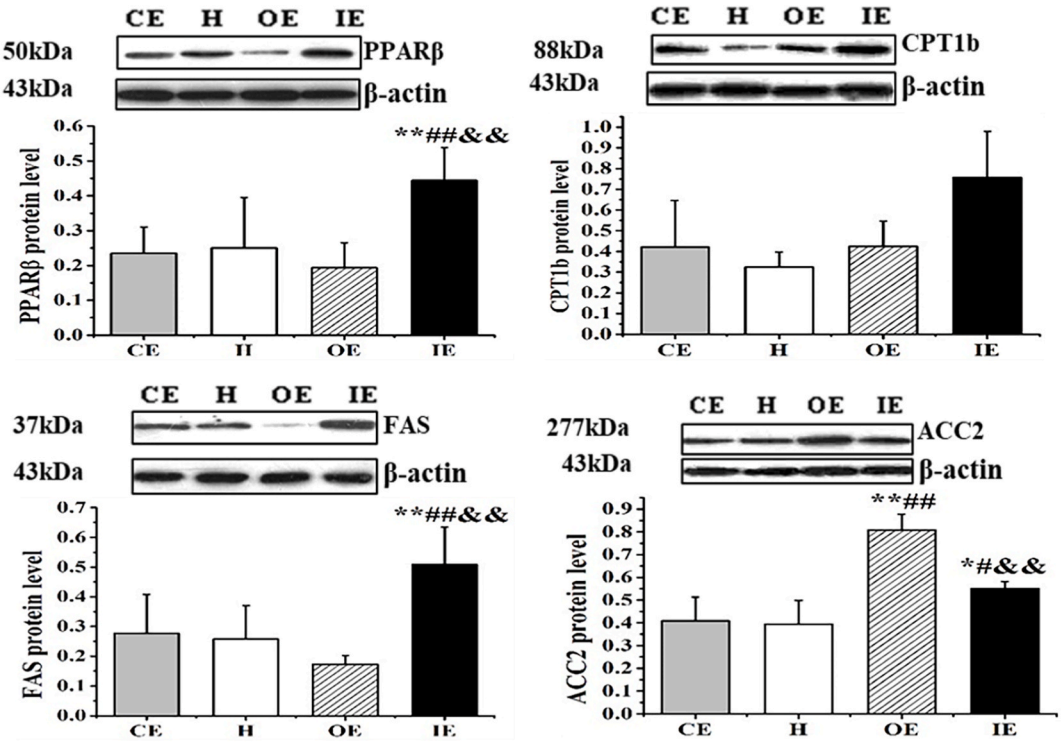

Fig. 6. Expression of the lipid metabolism regulator PPARβ and downstream effectors (CPT1b, FAS, and ACC2). Protein levels in obese rats under hypoxic conditions, with hypoxic training, and with regulated miR-122 expression were determined using Western blot analysis. OE, obese rats with miR-122 overexpression and hypoxic training; IE, obese rats with miR-122 depletion and hypoxic training; CE, obese rats with hypoxic training only; H, obese sedentary rats without regulation of miR-122 expression. Data are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$ vs rats in group H; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs rats in group CE; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ vs rats in group OE (1-way analysis of variance).

the ligand content of PPARβ in the body tissues and thus promote the differentiation and combustion of fatty acids. The increased endogenous ligand content of PPARβ and its metabolites induced by exercise can activate PPARβ to a certain extent, because exercise induces the expression of the transcription coactivator of the PPARs family, PGC-1α, thereby activating PPARβ [28]. The present study showed that the miR-122 expression inhibition contributed to a higher PPARβ expression in the skeletal muscle of rats. Therefore, an miR-122/PPARβ signaling axis in skeletal muscle exist, and it is modulated by hypoxic exercise in the physiologic environment of

obesity and can modulate FA metabolism by controlling the expression of factors associated with the lipid metabolism.

FAS is involved in cellular FA biosynthesis [29] and is downregulated upon inhibition of miR-122 expression [7,30,31]. However, the present study revealed that after hypoxic exercise, FAS level was increased in skeletal muscle of obese rats by both miR-122 overexpression and inhibition. FAS does not contain the miR-122 Seed sequence; therefore, FAS is not the target gene of miR-122 [5]. In this research, both miR-122 overexpression and inhibition in obese rats subjected to four weeks of hypoxic training were investigated, with the only difference being the different levels of miR-122 expression. Therefore, it is possible that exercise under hypoxic conditions may trigger different intermediate pathways in obese rats with miR-122 overexpression and inhibition. For example, the significant changes of miR-122 expression may affect the transcription factors of FAS or the activity of other miRNAs, leading to an increase in FAS expression [5].

ACC regulates FA metabolism; ACC2 is the main isoform in skeletal muscle [32]. In our study, after four weeks of hypoxic exercise, miR-122 overexpression upregulated the ACC2 protein expression of the skeletal muscle in obese rats, without affecting its mRNA expression. This suggests that the trend of ACC2 protein and mRNA expression is inconsistent when miR-122 is overexpressed, possibly due to other regulatory pathways leading to an extended half-life or reduced degradation of ACC2 protein [7]. Conversely, miR-122 inhibition resulted in an upregulation of both ACC2 mRNA and protein level, which is similar with the data of Zhu Lei et al. and indicates that hypoxic training suppresses miR-122 expression and upregulates ACC expression [7]. Negative regulation of ACC expression by miR-122 was also observed in high-intensity interval exercise model [33]. However, like *FAS*, *ACC2* transcript is not directly targeted by miR-122 [5]; therefore, the regulation of *ACC2* expression may occur through an intermediate pathway [5].

CPT1 is required for FA β-oxidation in mitochondria; CPT1b is the isoform that is specifically expressed in the heart and skeletal muscle [34,35]. An inverse association has been reported between miR-122 and CPT1 expression [6,36,37]. CPT1 expression may be regulated by PPARβ, a target gene of miR-122. Treatment with PPARβ agonist was shown to increase *CPT1* transcription in cardiomyocytes [38], whereas CPT1 levels were downregulated in PPARβ knockout mice. However, *CPT1* transcription was increased upon treatment with PPARβ ligand, which activated the *CPT1* promoter [39]. This study revealed that miR-122 overexpression doesn't influence CPT1b expression, whereas miR-122 inhibition significantly increased CPT1b expression, and the increase was more obvious than that in the miR-122 overexpression rats. This change trend is basically consistent with the effect of miR-122 on the PPARβ. Thus, the miR-122/PPARβ signaling axis directly regulates the expression of CPT1b to promote FA oxidation of skeletal muscle in obese rats under hypoxic exercise.

In conclusion, the effect of exercise on body weight is independent of miR-122 during hypoxic training. miR-122 inhibition improved blood lipid levels and promoted FA metabolism by altering ACC, FAS, and CPT1b expression in skeletal muscle via negative regulation of PPARβ. Overexpression of miR-122 attenuates the reduction in TG and LDL-C and the increase in HDL-C during exercise. These results provide molecular-level insight into the benefits of hypoxic exercise as an intervention for obesity.

Data availability statement

The datasets generated for this study can be obtained from the corresponding author on reasonable request.

Funding

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Ethics declarations

This study was reviewed and approved by Animal Use Committee at the China Institute of Sport Science, with the approval number: [CISSLA-2014032].

CRediT authorship contribution statement

Xuebing Wang: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Lianshi Feng:** Supervision, Funding acquisition. **Yingli Lu:** Writing – review & editing, Supervision. **Haibo Zhang:** Investigation, Data curation.

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.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26572.

References

[1] A. Camacho-Cardenosa, M. Camacho-Cardenosa, G. Olcina, R. Timón, J. Brazo-Sayavera, Detraining effect on overweight/obese women after high-intensity interval training in hypoxia, Scand. J. Med. Sci. Sports 29 (2019) 535–543, https://doi.org/10.1111/sms.13380.

[2] J. Feng, X. Wang, Y. Lu, C. Yu, X. Wang, L. Feng, BAIBA involves in hypoxic training induced browning of white adipose tissue in obese rats, Front. Physiol. 13 (2022) 882151, https://doi.org/10.3389/fphys.2022.882151.

[3] J. Elmén, M. Lindow, A. Silahtaroglu, M. Bak, M. Christensen, A. Lind-Thomsen, M. Hedtjärn, J.B. Hansen, H.F. Hansen, E.M. Straarup, K. McCullagh, P. Kearney, S. Kauppinen, Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver, Nucleic Acids Res. 36 (2008) 1153–1162, https://doi.org/10.1093/nar/gkm1113.

[4] C. Workman, F.A. Basset, Post-metabolic response to passive normobaric hypoxic exposure in sedendary overweight males: a pilot study, Nutr. Metab. 9 (2012) 103, https://doi.org/10.1186/1743-7075-9-103.

[5] C. Esau, S. Davis, S.F. Murray, X.X. Yu, S.K. Pandey, M. Pear, L. Watts, S.L. Booten, M. Graham, R. McKay, A. Subramaniam, S. Propp, B.A. Lollo, S. Freier, C. F. Bennett, S. Bhanot, B.P. Monia, miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting, Cell Metabol. 3 (2006) 87–98, https://doi.org/10.1016/j.cmet.2006.01.005.

[6] Y. Dai, S. Ghosh, B.C. Shin, S.U. Devaskar, Role of microRNA-122 in hepatic lipid metabolism of the weanling female rat offspring exposed to prenatal and postnatal caloric restriction, J. Nutr. Biochem. 73 (2019) 108220, https://doi.org/10.1016/j.jnutbio.2019.108220.

[7] Z. Lei, The Study on Hypoxia Exercise Inducing miR-27/PPARY, miR-122/PPARB to Regulate Lipid Metabolism in Obese Rat's Liver, Ph.D Thesis, Shanghai University of Sport, Shanghai, 2016.

[8] A.M. Mengeste, A.C. Rustan, J. Lund, Skeletal muscle energy metabolism in obesity, Obesity 29 (2021) 1582–1595, https://doi.org/10.1002/oby.23227.

[9] L. Yingli, X. Minhao, F. Lianshi, Z. Li, X. Jianfang, H. Zihong, et al., Promotion of fatty acid oxidation in gastrocnemius of obese rats through 4-week hypoxic living and exercise, Chin. J. Sports Med. 33 (2014) 1060–1068.

[10] A. Chabowski, J. Górski, J.J. Luiken, J.F. Glatz, A. Bonen, Evidence for concerted action of FAT/CD36 and FABPpm to increase fatty acid transport across the plasma membrane, Prostaglandins Leukot. Essent. Fatty Acids 77 (2007) 345–353, https://doi.org/10.1016/j.plefa.2007.10.017.

[11] Y. Li, H. Li, J. Han, Sphingosine-1-phosphate receptor 2 modulates pain sensitivity by suppressing the ROS-RUNX3 pathway in a rat model of neuropathy, J. Cell. Physiol. 235 (2020) 3864–3873, https://doi.org/10.1002/jcp.29280.

[12] C. Le Guiner, K. Stieger, A. Toromanoff, M. Guilbaud, A. Mendes-Madeira, M. Devaux, L. Guigand, Y. Cherel, P. Moullier, F. Rolling, O. Adjali, Transgene regulation using the tetracycline-inducible TetR-KRAB system after AAV-mediated gene transfer in rodents and nonhuman primates, PLoS One 9 (2014) e102538, https://doi.org/10.1371/journal.pone.0102538.

[13] C. Zhou, J. Cao, L. Shang, C. Tong, H. Hu, H. Wang, D. Fan, H. Yu, Reduced paraoxonase 1 activity as a marker for severe coronary artery disease, Dis. Markers 35 (2013) 97–103, https://doi.org/10.1155/2013/816189.

[14] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.

[15] Z. Lei, L. Yingli, F. Lianshi, Z. Suxian, Hypoxia exercise induced miR-27/PPARγ to regulate fatty acid metabolism in obese rat gastrocnemius, China Sport Sci 39 (2019) 55–61.

[16] M. Nishiwaki, R. Kawakami, K. Saito, H. Tamaki, F. Ogita, The effects of exercise training under mild hypoxic conditions on body composition and circulating adiponectin in postmenopausal women, Clin. Physiol. Funct. Imag. 36 (2016) 468–475, https://doi.org/10.1111/cpf.12252.

[17] W. Ji, L. Gong, J. Wang, H. He, Y. Zhang, Hypoxic exercise training promotes apelin/APJ expression in skeletal muscles of high fat diet-induced obese mice, Protein Pept. Lett. 24 (2017) 64–70, https://doi.org/10.2174/0929866524666161111111726.

[18] S. Shin, T. Matsuoka, W.Y. So, Influences of short-term normobaric hypoxic training on metabolic syndrome-related markers in overweight and normal-weight men, J. Mens Health 14 (2018) e44–e52, https://doi.org/10.22374/1875-6859.14.1.5.

[19] I.M. Guardado, B.S. Ureña, A.C. Cardenosa, M.C. Cardenosa, G.O. Camacho, R.T. Andrada, Effects of strength training under hypoxic conditions on muscle performance, body composition and haematological variables, Biol. Sport 37 (2020) 121–129, https://doi.org/10.5114/biolsport.2020.93037.

[20] W. Qining, H. Yang, G. Yuling, Z. Hua, Effects of 4-week hypoxic exercise combined with diet on body weight, blood lipid and insulin resistance of obese adults, Chin. J. Sports Med. 31 (2012) 289–294, https://doi.org/10.16038/j.1000-6710.2012.04.013.

[21] H. Yamada, K. Ohashi, K. Suzuki, E. Munetsuna, Y. Ando, M. Yamazaki, H. Ishikawa, N. Ichino, R. Teradaira, S. Hashimoto, Longitudinal study of circulating miR-122 in a rat model of non-alcoholic fatty liver disease, Clin. Chim. Acta 446 (2015) 267–271, https://doi.org/10.1016/j.cca.2015.05.002.

[22] A.R. Abdel-Hamed, N.M. Mesbah, M.H. Ghattas, D.M. Abo-Elmatty, S.M. Saleh, Serum miRNA-122 expression in non-alcoholic fatty liver disease among Egyptian patients and its correlation with interleukin-1a gene polymorphism, Meta Gene 14 (2017) 19–23, https://doi.org/10.1016/j.mgene.2017.07.012.

[23] W. Gao, H.W. He, Z.M. Wang, H. Zhao, X.Q. Lian, Y.S. Wang, J. Zhu, J.J. Yan, D.G. Zhang, Z.J. Yang, L.S. Wang, Plasma levels of lipometabolism-related miR-122 and miR-370 are increased in patients with hyperlipidemia and associated with coronary artery disease, Lipids Health Dis. 11 (2012) 55, https://doi.org/10.1186/1476-511X-11-55.

[24] N. Bonnet, B. Desvergne, S. Ferrari, PPAR beta-deficiency impairs muscle and skeletal response to exercise, Bone 48 (2011) S269, https://doi.org/10.1016/j.bone.2011.03.665.

[25] J.G. Neels, P.A. Grimaldi, Physiological functions of peroxisome proliferator-activated receptor β, Physiol. Rev. 94 (2014) 795–858, https://doi.org/10.1152/physrev.00027.2013.

[26] R. Manickam, W. Wahli, Roles of peroxisome proliferator-activated receptor β/δ in skeletal muscle physiology, Biochimie 136 (2017) 42–48, https://doi.org/10.1016/j.biochi.2016.11.010.

[27] D. Gatfield, G. Le Martelot, C.E. Vejnar, D. Gerlach, O. Schaad, F. Fleury-Olela, A.L. Ruskeepää, M. Oresic, C.C. Esau, E.M. Zdobnov, U. Schibler, Integration of microRNA miR-122 in hepatic circadian gene expression, Genes Dev. 23 (2009) 1313–1326, https://doi.org/10.1101/gad.1781009.

[28] S. Luquet, J. Lopez-Soriano, D. Holst, A. Fredenrich, J. Melki, M. Rassoulzadegan, P.A. Grimaldi, Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability, Faseb. J. 17 (2003) 2299–2301, https://doi.org/10.1096/fj.03-0269fje.

[29] A.N. Günenc, B. Graf, H. Stark, A. Chari, Fatty acid synthase: structure, function, and regulation, Subcell. Biochem. 99 (2022) 1–33, https://doi.org/10.1007/978-3-031-00793-4_1.

[30] D. Iliopoulos, K. Drosatos, Y. Hiyama, I.J. Goldberg, V.I. Zannis, MicroRNA-370 controls the expression of microRNA-122 and Cpt1alpha and affects lipid metabolism, J. Lipid Res. 51 (2010) 1513–1523, https://doi.org/10.1194/jlr.M004812.

[31] J.A. Mennigen, C.J. Martyniuk, I. Seiliez, S. Panserat, S. Skiba-Cassy, Metabolic consequences of microRNA-122 inhibition in rainbow trout, *Oncorhynchus mykiss*, BMC Genom. 15 (2014) 70, https://doi.org/10.1186/1471-2164-15-70.

[32] H.M. O'Neill, J.S. Lally, S. Galic, T. Pulinilkunnil, R.J. Ford, J.R. Dyck, B.J. van Denderen, B.E. Kemp, G.R. Steinberg, Skeletal muscle ACC2 S212 phosphorylation is not required for the control of fatty acid oxidation during exercise, Phys. Rep. 3 (2015) e12444, https://doi.org/10.14814/phy2.12444.

[33] F. Kalaki-Jouybari, M. Shanaki, M. Delfan, S. Gorgani-Firouzjae, S. Khakdan, High-intensity interval training (HIIT) alleviated NAFLD feature via miR-122 induction in liver of high-fat high-fructose diet induced diabetic rats, Arch. Physiol. Biochem. 126 (2020) 242–249, https://doi.org/10.1080/13813455.2018.1510968.

[34] I. Miljkovic, L.M. Yerges, H. Li, C.L. Gordon, B.H. Goodpaster, L.H. Kuller, C.S. Nestlerode, C.H. Bunker, A.L. Patrick, V.W. Wheeler, J.M. Zmuda, Association of the CPT1B gene with skeletal muscle fat infiltration in Afro-Caribbean men, Obesity 17 (2009) 1396–1401, https://doi.org/10.1038/oby.2008.677.

[35] M.K. Kim, S.H. Kim, H.S. Yu, H.G. Park, U.G. Kang, Y.M. Ahn, Y.S. Kim, The effect of clozapine on the AMPK-ACC-CPT1 pathway in the rat frontal cortex, Int. J. Neuropsychopharmacol. 15 (2012) 907–917, https://doi.org/10.1017/S1461145711000976.

[36] S. Wei, M. Zhang, Y. Yu, H. Xue, X. Lan, S. Liu, G. Hatch, L. Chen, HNF-4α regulated miR-122 contributes to development of gluconeogenesis and lipid metabolism disorders in Type 2 diabetic mice and in palmitate-treated HepG2 cells, Eur. J. Pharmacol. 791 (2016) 254–263, https://doi.org/10.1016/j.ejphar.2016.08.038.

[37] J.L. Wang, R.H. Lu, J.J. Sun, D.Z. Xie, F. Yang, G.X. Nie, Differential expression of lipid metabolism-related genes and miRNA s in *Ctenopharyngodon idella* liver in relation to fatty liver induced by high non-protein energy diets, Aquacult. Res. 48 (2017) 4070–4085, https://doi.org/10.1111/are.13228.

[38] L. Cheng, G. Ding, Q. Qin, Y. Xiao, D. Woods, Y.E. Chen, Q. Yang, Peroxisome proliferator-activated receptor delta activates fatty acid oxidation in cultured neonatal and adult cardiomyocytes, Biochem. Biophys. Res. Commun. 313 (2004) 277–286, https://doi.org/10.1016/j.bbrc.2003.11.127.

[39] L. Cheng, G. Ding, Q. Qin, Y. Huang, W. Lewis, N. He, R.M. Evans, M.D. Schneider, F.A. Brako, Y. Xiao, Y.E. Chen, Q. Yang, Cardiomyocyte-restricted peroxisome proliferator-activated receptor-delta deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy, Nat. Med. 10 (2004) 1245–1250, https://doi.org/10.1038/nm1116.