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Aerobic exercise attenuates high-fat diet-induced glycometabolism impairments in skeletal muscle of rat: role of EGR-1/PTP1B signaling pathway

Liangzhi Zhang[1†], Xiaojie Liu[1†], Jing Hu[2], Helong Quan[3], Sang Ki Lee[4], Mallikarjuna Korivi[1], Lifeng Wang[1], Ting Li[1*] and Wei Li[1*]

Abstract

Objective Impaired skeletal muscle glycogen synthesis contributes to insulin resistance (IR). Aerobic exercise reported to ameliorate IR by augmenting insulin signaling, however the detailed mechanism behind this improvement remains unclear. This study investigated whether aerobic exercise enhances glycogen anabolism and insulin sensitivity via EGR-1/PTP1B signaling pathway in skeletal muscle of rats.

Methods Sprague-Dawley rats fed a high-fat diet (HFD), and performed treadmill exercise training for 6-week. Oral glucose tolerance test was conducted to confirm the IR. Periodic Acid-Schiff (PAS) staining and anthrone colorimetry were used to assess the skeletal muscle glycogen. RT-qPCR, western blot, and immunofluorescence were used to detect the EGR-1/PTP1B pathway and associated signaling molecules.

Results We found that exercise training significantly decreased blood glucose, insulin, and homeostasis model assessment for IR (HOMA-IR) against HFD-induced elevation. Decreased muscle glycogen content due to HFD was significantly restored after exercise training. Exercise training promoted mRNA expressions of *Irs1*, *Akt*, and *Glut4*, while inhibited *Gsk-3*β expression against HFD. Next, the decreased IRS1 (phosphorylated/total), AKT (phosphorylated/total), and GLUT4, and increased GSK-3β proteins with HFD were significantly reversed by exercise. Furthermore, HFD-induced overexpression of EGR-1 and PTP1B evidenced by mRNA, protein, and immunofluorescence intensity, were substantially inhibited by exercise, which may contribute to promote insulin sensitivity and glycogen anabolism.

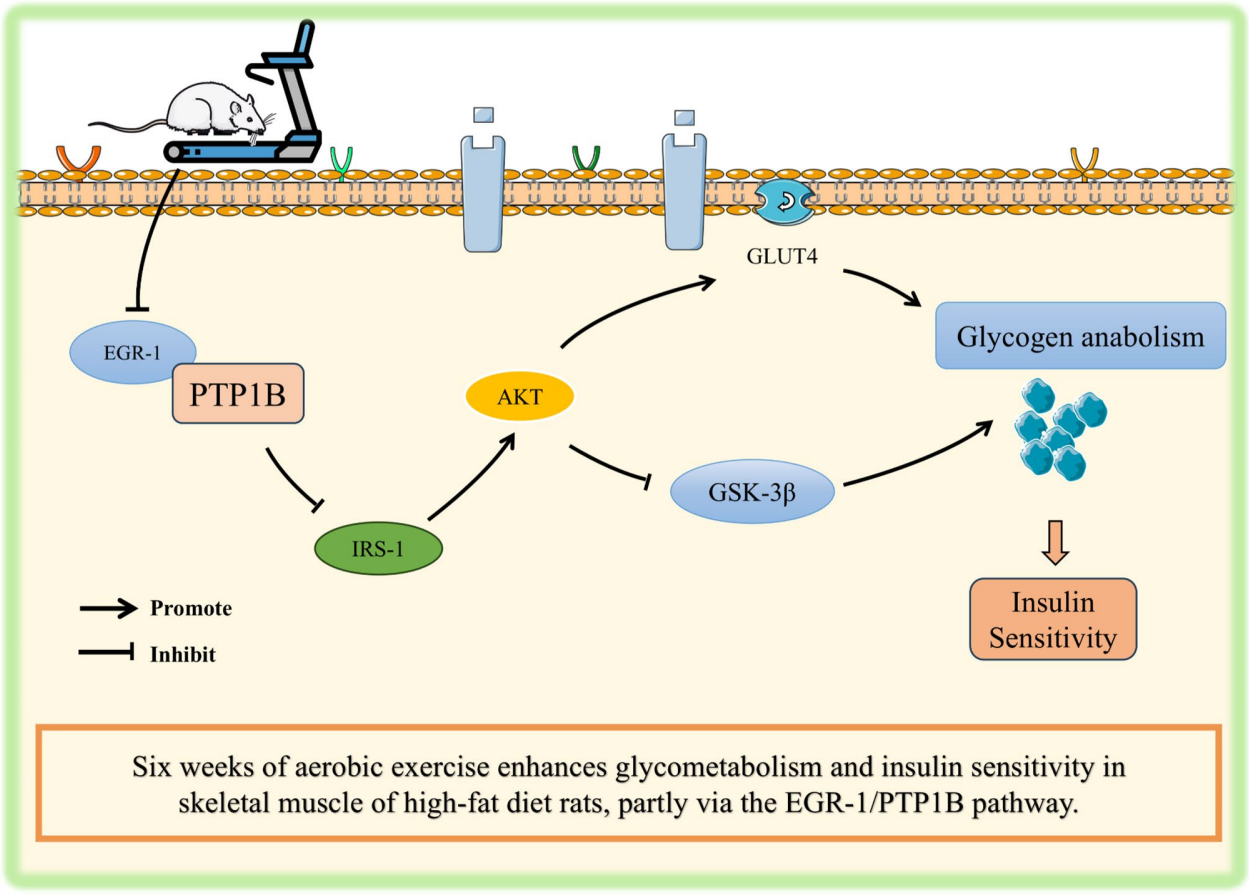
Conclusions Aerobic exercise training promotes insulin sensitivity and skeletal muscle glycogen synthesis in HFD-fed rats. The beneficial effects of exercise might be mediated by EGR-1/PTP1B signaling pathway in skeletal muscle, however further studies are necessary to confirm this mechanism.

†Liangzhi Zhang and Xiaojie Liu contributed equally to this work.

*Correspondence:
Ting Li
tingli@zjnu.edu.cn
Wei Li
ty1986@zjnu.edu.cn

Full list of author information is available at the end of the article

Graphical Abstract



Keywords Glycometabolism, Insulin sensitivity, Treadmill running, Glycogen synthesis, GLUT4, IRS1

Introduction

Chronic high-fat diet (HFD) consumption is associated with increased risk of developing obesity and insulin resistance (IR) [1], and subsequent triggering of type 2 diabetes mellitus (T2DM) [2]. Skeletal muscle, the largest energy-metabolizing organ of the body, plays a crucial role in preventing or alleviating the IR through its ability to uptake and utilize the glucose. Skeletal muscle also involved in regulation of insulin signaling pathways, and glycogen synthesis capacity [3–5].

Early growth response factor-1 (EGR-1), a zinc-finger transcription factor activated by various cytokines, growth factors and hormones, plays a key role in regulation of cell proliferation, apoptosis, and cellular metabolism [6–8]. Loss of EGR-1 function has been shown to improve IR in adipose tissue and liver [9, 10]. A recent study indicated that elevated EGR-1 inhibits postprandial insulin signaling and glucose uptake in skeletal muscle, and thereby decrease insulin sensitivity [11]. Therefore, EGR-1 is considered an ideal target protein in regulation of IR. Additionally, inhibition of EGR-1/protein tyrosine phosphatase 1B (PTP1B) activity enhances skeletal muscle insulin sensitivity and postprandial glucose stability [11]. The potential mechanism underlying these beneficial effects may be associated with downregulation of PTP1B activity, phosphorylation of insulin receptor substrate (IRS), inhibition of glycogen synthase kinase-3β (GSK-3β) activity, enhancing of glycogen synthesis, and improving of insulin sensitivity [11–13]. The insulin signaling pathway plays a crucial role in regulation of glucose homeostasis and metabolism. Upon insulin binding to its receptor, IRS is activated, and initiate two major pathways, including protein kinase B (PKB/AKT)-mediated translocation of glucose transporter type 4 (GLUT4), and inhibition of GSK-3β [14, 15]. In the first pathway, activated IRS promotes the phosphorylation of phosphatidylinositol-3-kinase (PI3K), leading to the activation of AKT [16]. Activated AKT promotes translocation of GLUT4 to the cell membrane, and thereby increase glucose uptake by the cell [17]. In the second pathway, AKT

phosphorylates and inhibits GSK-3β, a negative regulator of glycogen synthesis. The inhibition of GSK-3β allows glycogen synthase to remain active, and thereby enhances glycogen storage [18]. These coordinated actions of the IRS-AKT pathway are essential for maintaining of blood glucose levels, and ensuring efficient energy storage and utilization.

Aerobic exercise, as a non-pharmacological intervention, not only improves skeletal muscle contractility in obese individuals [19], but also enhances insulin sensitivity [20]. Glycogen synthesis and its content in skeletal muscle reflect the metabolic balance between glucose availability and insulin sensitivity. Although exercise has been shown to improve IR through skeletal muscle glycogen anabolism [21], the role of EGR-1/PTP1B signaling pathway in this beneficial effect remains unclear. Therefore, the purpose of this study was to examine the effect of aerobic exercise training on skeletal muscle EGR-1/PTP1B pathway in response to a HFD. We further explored whether exercise improve skeletal muscle glycogen anabolism and insulin sensitivity in rats fed a HFD through EGR-1/PTP1B signaling pathway. Exercise-associated beneficial effects may provide new strategies for preventing and treating the IR.

Materials and methods

Experimental animals

Twenty-four male Sprague-Dawley (SD) rats (6-week-old; weighing 257±10 g) were housed in a specific pathogen-free environment. Two rats per cage were maintained in a temperature-controlled room (23±2 °C) with a 12-h light and 12-h dark cycle. Food (standard diet or high-fat diet) and water were provided ad libitum. Daily food intake was recorded, while body weights were collected for every 3-day once. All procedures complied with the National Research Council Guidelines for the Care and Use of laboratory animals. This study was conducted according to the animal management regulations of the Ministry of Health of China, and was approved by the Animal Ethics Committee of Zhejiang Normal University (No. ZSDW2022027).

Animal grouping and treatment

After a one-week adaptation period, the rats were randomly assigned into three groups, including control (CON, $n=8$), high-fat diet (HFD, $n=8$) and HFD plus aerobic exercise (HE, $n=8$), and treated for a period of 6-week. Rats in the control group fed a normal diet obtained from the Research Diets (Research Diets Inc., NJ, USA). The rats in HFD and HE groups fed a 60% high-fat diet (D12492, Research Diets Inc., NJ, USA). Following HFD feeding, the rats in HE group performed an aerobic exercise training for a period of 6-week, as described in the protocol below.

Treadmill exercise protocol

Prior to the actual exercise session, rats were familiarized with the treadmill environment. For familiarization, rats in the HE group ran on a treadmill at low running speed (16 m/min) for 20-minute on day 1. Then the running speed (2 m/min/day) and running time (10-min/day) were gradually increased to reach the target speed (24 m/min) and time (60-min) [22]. The final exercise training protocol consisted of 60-min/day, 5 days/week for a period of 6-week. The exercise load comprised a running of 15 m/min for the first 5-minute, 20–25 m/min for the next 50-minute, and 15 m/min for the last 5-minute, without incline. The exercise program was scheduled from Monday to Friday between 5:00 and 7:00 p.m. The exercise intensity ranged from approximately 60–70% of VO_2max [23]. We adopted this exercise protocol based on our previous study, which reported to be effective in producing the physiological changes in SD rats [22].

Oral glucose tolerance test (OGTT) and insulin sensitivity

OGTT was performed twice in our study following a 12-hour fasting period. The first OGTT was performed prior to the intervention (pre or baseline), and the second OGTT was performed after the intervention (post). Following the exercise training, the animals rested for 12-hour (while feeding), and then fasted for 12-hour before the OGTT. Blood samples were collected from the tail of each rat at 0-, 30-, 60-, 90-, and 120-minute after an oral administration of glucose (2 g/kg body weight). The changes in blood glucose levels were measured using a glucometer (Sinocare, Changsha, China), and recorded for three times to calculate the average value. The glucose area under the curve (AUC) was calculated and presented as histograms [24].

Serum insulin levels were measured using the rat insulin ELISA kit (Sangon Biotech, Shanghai, China), according to the manufacturer's instructions. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as follows:

HOMA-IR = ((fasting plasma insulin in μIU/mL) × (fasting plasma glucose in mg/dL))/405 [25].

Sample collection and biochemical assays

Forty-eight hours after the last exercise session (6-week), rats were anesthetized with urethane (2 g/kg, intraperitoneally) following a 12-hour fasting period. The fasting time was the same in CON and HFD groups to ensure the similar conditions in all groups. Blood samples (5 mL) were collected, and centrifuged at 3000 rpm for 15-minute. Then, the portions of the quadriceps were excised for different analyses: one portion was embedded in the optimal cutting temperature compound (OCT, Sakura, Tokyo, Japan), and frozen in isopentane, while the other portion was frozen in liquid nitrogen, and stored at

-80 °C. The quadriceps muscle was selected for its balanced distribution of type I and type II muscle fibers, ease of access for reliable sampling, and its relevance to metabolic outcomes [26, 27].

In addition, the subcutaneous fat was carefully dissected beneath the dermis, ensuring minimal contamination from surrounding tissues. Following this, epididymal fat was excised from around the epididymis. Special care was taken to preserve its structural integrity. Both fat tissue samples were weighed on an analytical balance (Sartorius, BSA224S, Germany). The weight of each fat depot was recorded immediately to determine the total fat mass for subsequent analysis.

Periodic acid-schiff (PAS) staining and measurement of skeletal muscle glycogen content

For histological analysis, muscle tissue Sect. (10 μm thickness) were prepared using a cryostat (Leica CM1860, Germany). The sections were mounted on glass slides, and fixed with 4% paraformaldehyde for 10-minute at room temperature before undergoing PAS staining. The slides were immersed in periodic acid solution for 10-minute, washed three times with distilled water, and incubated with Schiff solution at 37 °C for 45-minute. Finally, the slides were washed with distilled water for 5-minute, and dehydrated using graded alcohols.

To measure the glycogen levels in the quadriceps, the glycogen assay kit (BC0345, Solarbio, China) was used, and performed according to the manufacturer's instructions. Briefly, snap-frozen quadriceps (80 mg) were homogenized, and diluted with 1 mL distilled water. The homogenate was boiled at 100℃ for 20-minute, centrifuged at 8000 g for 10-minute at 25℃, and then the supernatant was collected. Glycogen was extracted by a strong alkaline extract solution, and the glycogen content was determined using anthrone chromogen under strong acidic conditions [28].

Quantitative real-time polymerase chain reaction (qPCR)

Quadriceps muscle tissue (50 mg) was lysed with TRIzol reagent to extract total RNA. The concentration and purity of the extracted RNA were assessed with a NanoDrop spectrophotometer (Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, USA). The RNA purity was determined by measuring the absorbance ratios at 260/280 nm and 260/230 nm on a NanoDrop spectrophotometer (Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, USA), ensuring the ratios were above 1.8 and 2.0, respectively, to confirm high purity. For cDNA synthesis, 1 μg of total extracted RNA from each sample was used with the PrimeScript RT Master Mix (Takara, Shiga, Japan). The qPCR assays were performed using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, Waltham, USA) to ensure specific and sensitive detection of target gene expression. All qPCR analyses were conducted on the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, USA). The reaction conditions comprised an initial pre-denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 30 s. Gene expression levels were quantified using the $2-^{\Delta\Delta Ct}$ method with GAPDH serving as the internal reference [22]. The primer sequences are provided in Table 1.

Western blot

Total protein was extracted from rat quadriceps muscle using the commercially available RIPA lysis buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP40, 1% sodium deoxycholate, and 0.1% SDS, Thermo Fisher Scientific, 89901USA) with protease and phosphatase inhibitors (Thermo Fisher Scientific, 78440, USA). The protein concentration was determined using the BCA protein assay kit (Thermo Fisher Scientific, 23235, USA). Equal amounts of protein along with a protein ladder (Bio-Rad, 1610374, USA), were subjected to SDS-PAGE for electrophoresis. Electrophoresis was conducted at a constant voltage of 70–100 V until the dye front reached an appropriate distance. Following separation, proteins were transferred to polyvinylidene fluoride (PVDF) membranes using a wet transfer system (Bio-Rad) at 80 V for 2 h. The transfer buffer consisted of 25 mM Tris, 192 mM glycine, and 20% methanol. the PVDF membranes were then blocked in Tris-buffered saline with Tween® 20 (TBST, 50 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.4) containing 5% skimmed milk at room temperature for 1 h. The membranes were then incubated overnight at 4 °C with primary antibodies specific for EGR-1 (Proteintech, 22008-1-AP), PTP1B (Santa Cruz, sc-133259),

Table 1 Primers for qPCR used in this study

Gene	Forward	Reverse
Egr-1	ATTTCCTATTCAAAGTCCGAGAGTCAG	TCCATCAGTAAGAGGCAGGTGTC
Ptp1b	CGGACCACCTCAACTCAACCAATC	CAACACCACCTTGTCGTACTCGTC
Glut4	GCTGGGCGACGGACACTC	GGACACATAACTCATGGATGGAACC
Gsk-3β	CCACCATCCTTATCCCTCCTCAC	TGTCCACGGTCTCCAGCATTAG
Irs1	CAAGCCTGTCCTCTCCTACTACTC	GCAGTTGCGGTATAGCGAAGG
Akt	TGTGGCAAGATGTGTATGAGAAGAAG	AGGCGGCGTGATGGTGATC
Gapdh	AAGTTCAACGGCACAGTCAAGG	GACATACTCAGCACCAGCATCAC

GSK-3β (Proteintech, 67329-1-Ig), phospho-AKT Ser473 (p-AKT, Proteintech, 66444-1-Ig), AKT (CST, 9272), phospho-IRS1 Ser307 (p-IRS1, CST, 2381), IRS1 (Proteintech, 17509-1-AP) and GLUT4 (Proteintech, 66846-1-Ig), all diluted at a ratio of 1:2000. After washing the membranes three times with TBST, they were incubated at room temperature for 2 h with appropriate secondary antibodies: goat anti-mouse (Proteintech, SA00001-1) or goat anti-rabbit (CST, #7074), also diluted at 1:2000. Detection of protein bands was performed using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Waltham, USA) and visualized with a gel imaging system (Bio-Rad, Hercules, USA). The optical density values of the target proteins were quantified relative to that of GAPDH using Fiji software [29].

Immunofluorescence

Muscle samples were collected, and immediately frozen in OCT. Using a cryostat (Leica CM1860, Germany), 10 μm thick sections were cut, and mounted on glass slides. The slides were fixed with 4% paraformaldehyde for 10-minute at room temperature. After three washes with PBS (5-minute each), slides were permeabilized with 0.5% Triton X-100 in PBS for 20-minute, followed by three additional washes with PBS. Subsequently, the slides were blocked with 1% BSA in PBST (PBS+22.52 mg/mL Glycine+0.1% Tween-20) for 30-minute at room temperature. Primary antibodies, EGR-1 and PTP1B, were incubated overnight at 4℃. Slides were rinsed three times with PBS, and incubated with fluorescent secondary antibodies (Alexa Fluor 488-conjugated goat anti-mouse, Abcam, AB150113 or Alexa Fluor 594-conjugated goat anti-rabbit, AB150080) at a ratio of 1:500 in antibody dilution buffer for one-hour at room temperature in the dark. Finally, the cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Beyotime Institute of Biotechnology, China). The stained tissue was observed, and photographed using a fluorescence microscope (Leica, Wetzlar, Germany) [29].

Statistical analysis

Data were statistically analyzed using IBM SPSS Statistics 20 (Chicago, USA), and image analysis and mapping were performed using Fiji software and GraphPad Prism 9 (San Diego, USA). The Pearson correlation coefficient of the selected images was analyzed using Fiji software. All data were summarized as mean±SEM. Comparisons between multiple groups were conducted using a one-way analysis of variance (ANOVA), with post hoc analysis by Tukey's multiple comparisons test. A significance level of $P<0.05$ was considered statistically significant.

Results

Exercise decreases body weight and fat weight in rats fed a high-fat diet

During the intervention, we recorded the body weight changes in all groups. We found significantly increased body weights in the HFD group compared with that of in control group after 6-week (Fig. 1a-b). However, the increased body weight was effectively decreased by exercise training in the HE group (Fig. 1a-b). Our analysis further revealed a significant reduction in subcutaneous fat depot in the HE group compared to that of in HFD group (Fig. 1c). Conversely, no significant difference was observed in epididymal fat weight between HFD and HE groups (Fig. 1d). These results suggest that aerobic exercise training preferentially mobilizes the subcutaneous fat rather than the visceral fat for energy.

Aerobic exercise restores insulin sensitivity in rats fed a high-fat diet

To evaluate the glucose homeostasis and insulin sensitivity in response to a HFD, we conducted pre- and post-intervention OGTT. There were no significant differences in pre-intervention OGTT results among the groups (Fig. 2a-b). Post-intervention OGTT results suggesting the impaired glucose tolerance in rats fed a HFD, as we found higher glucose AUC for the HFD group compared with that of the control (Fig. 2c-d). However, the

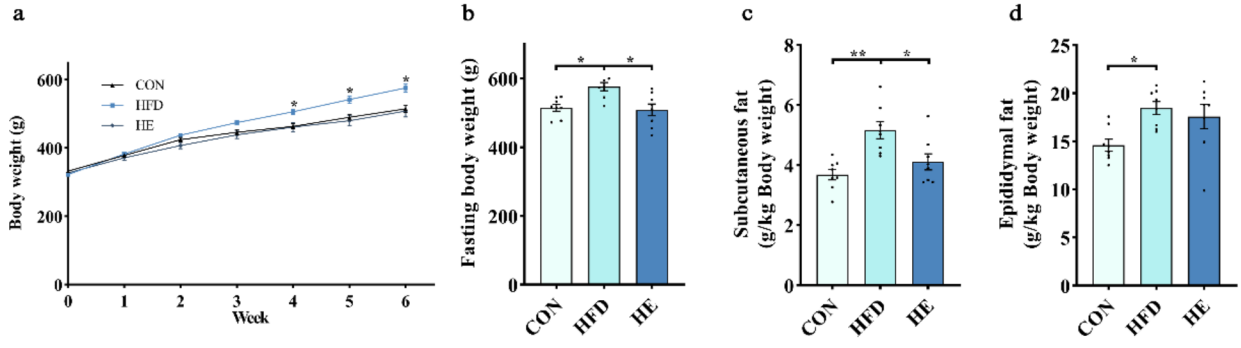


Fig. 1 Aerobic exercise decreases body weight and fat weight in rats fed a high-fat diet. (**a**) Body weight changes during the intervention. (**b**) Body weight at the end of the intervention. (**c**, **d**) Subcutaneous fat and epididymal fat weight. All data are presented as mean ± SEM ($n=8$ per group); *$P<0.05$, ** $P<0.01$, *** $P<0.001$

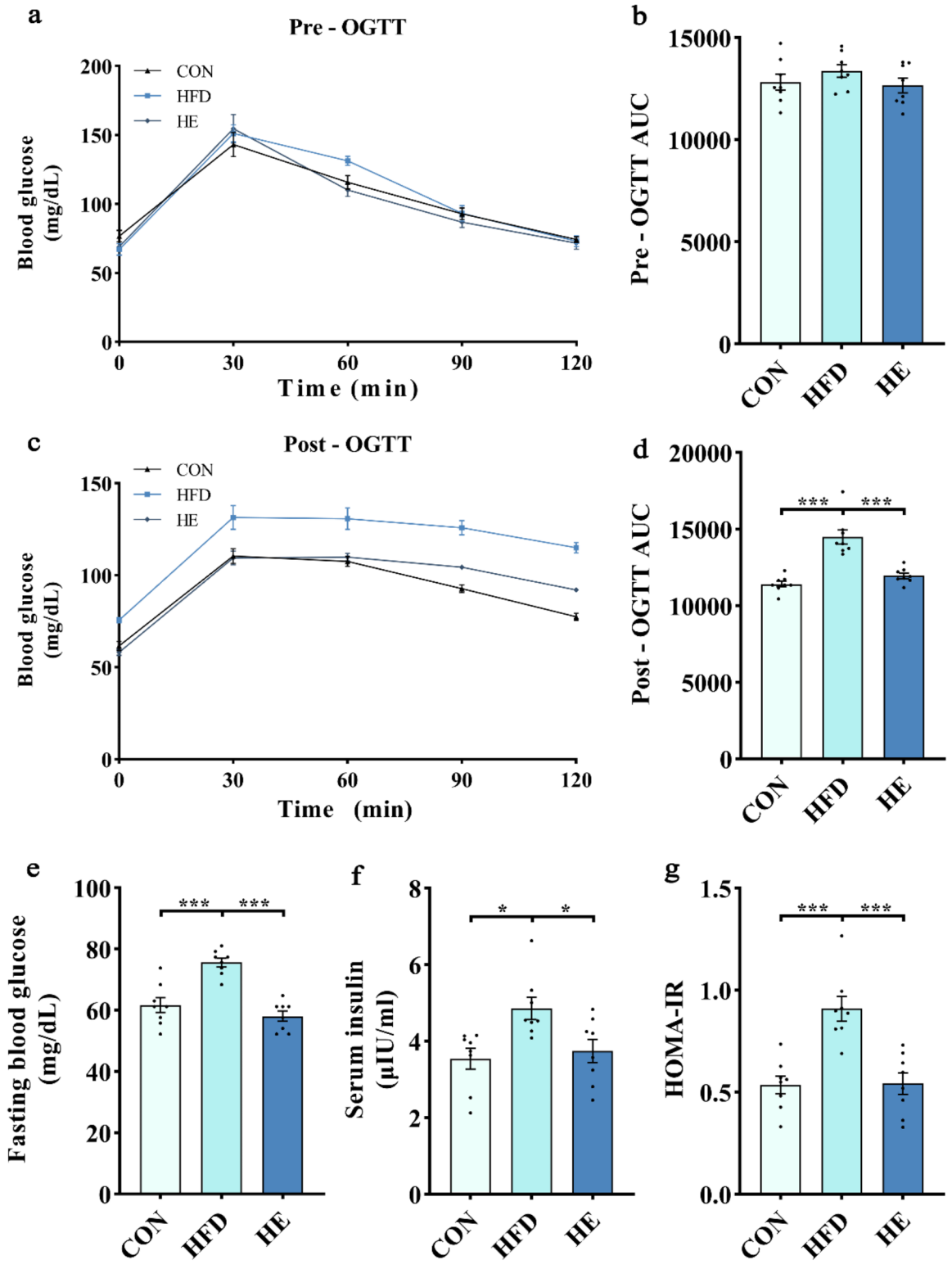


Fig. 2 Aerobic exercise restored insulin sensitivity in rats fed a high-fat diet. (**a**-**d**) Blood glucose concentrations and the area under the curve (AUC) in response to OGTT before and after intervention. Changes in (**e**) fasting blood glucose, (**f**) serum insulin, and (**g**) HOMA-IR after intervention. All data are presented as mean ± SEM ($n = 8$ per group); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

impaired glucose tolerance was significantly ameliorated by exercise training, as we noticed lower glucose AUC in HE group compared with HFD group. In addition, HFD for 6-week significantly elevated fasting blood glucose, insulin, and HOMA-IR compared to the control diet (Fig. 2e-g). These elevations were significantly decreased in HE group (Fig. 2e-g), underscoring the beneficial effects of aerobic exercise on insulin sensitivity against HFD.

Exercise restores skeletal muscle glycogen content against HFD

Skeletal muscle glycogen content was determined by PAS staining and glycogen content detection kits. Both protocols consistently showed a substantial reduction in glycogen content in HFD compared to control diet (Fig. 3a-c). The decreased glycogen content indicates potential adversities of HFD on glycogen anabolism in the skeletal muscle. This impact may be due to the reduced insulin sensitivity and/or decreased glucose uptake with chronic HFD. However, 6-week aerobic exercise restored skeletal muscle glycogen content, as evidenced by both PAS staining and glycogen content assay results (Fig. 3a-c).

Exercise restores glycogen anabolism in skeletal muscle of rats fed a high-fat diet

To elucidate the molecular mechanism underlying the improvement of glycogen anabolism through exercise, we investigated the mRNA expressions and protein levels of molecules involved in this phenomenon. We found HFD significantly suppressed the mRNA expressions of *Irs1*, *Akt*, and *Glut4* in quadriceps (Fig. 4a-c). Correspondingly, HFD decreased the ratios of p-IRS1/IRS1 and p-AKT/AKT, and protein levels of GLUT4 compared to normal diet (Fig. 4e-g). However, aerobic exercise significantly restored the mRNA expressions (Fig. 4a-c), and protein levels (Fig. 4e-g) of all signaling molecules. In addition, overexpression of *Gsk-3β* mRNA and elevation of GSK-3β protein levels with HFD were completely reversed by exercise training (Fig. 4d and h). The results showed that HFD adversely affected the skeletal muscle glycogen anabolism, and exercise training restored these adverse effects by promoting the p-IRS1/IRS1, p-AKT/AKT, GLUT4, and GSK-3β signaling molecules.

Exercise inhibits EGR-1/PTP1B mRNA and protein against HFD and restores glycogen anabolism

We further investigated the underlying mechanism on exercise-induced restoration of glycogen anabolism by assessing the mRNA expressions and protein levels of EGR-1 and PTP1B in quadriceps. Pearson correlation coefficient analysis was also performed to assess the correlation between EGR-1 and PTP1B across the experimental groups. The Pearson correlation coefficient quantifies the strength and direction of linear association between two variables, ranging from −1 (perfect negative correlation) to 1 (perfect positive correlation) [30]. We found that the mRNA expressions of *Egr-1* and *Ptp1b* (Fig. 5a and b), and protein levels of EGR-1 and PTP1B (Fig. 5c and d) were significantly elevated with HFD. Furthermore, colocalization of EGR-1 and PTP1B was also significantly higher in the HFD, as evidenced by immunofluorescence images (Fig. 5i). However, the overexpressed mRNA of *Egr-1* and *Ptp1b*, correspondingly increased protein levels, and increased immunofluorescent intensities with HFD were markedly decreased by exercise training (Fig. 5a-i). These findings suggest that aerobic exercise can enhance insulin sensitivity and skeletal muscle glycogen anabolism probably through the inhibition of EGR-1/PTP1B signaling.

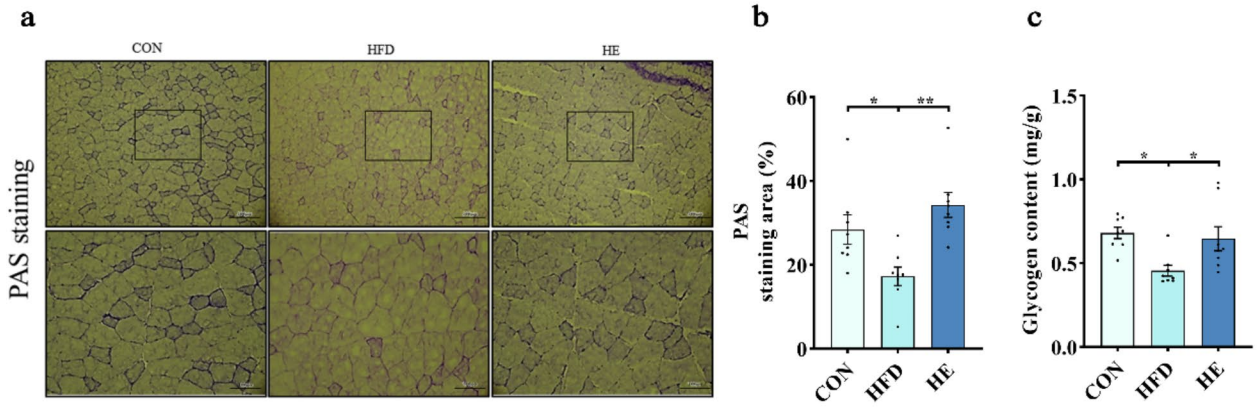


Fig. 3 Aerobic exercise restored glycogen content in skeletal muscle of rats fed a high-fat diet. (**a**-**c**) PAS staining and quadriceps glycogen content. Scale bars, 200 μm and 100 μm; Upper panel: image of PAS staining. Lower panel: zoomed-in view of the frames indicated in the upper panel. Five regions were randomly selected from each sample, and quantified ($n = 8$ per group); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

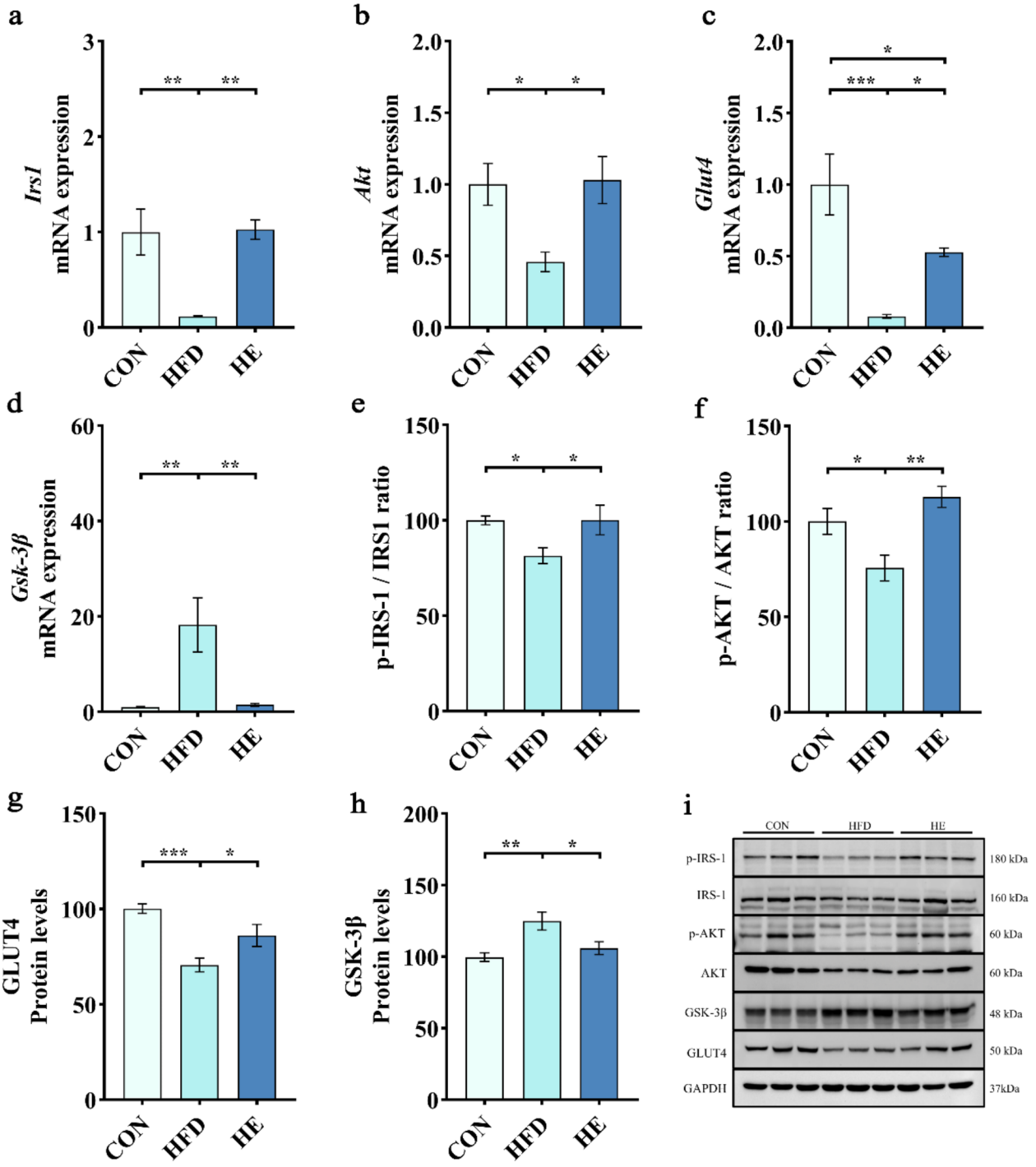


Fig. 4 Aerobic exercise restored skeletal muscle glycogen anabolism in rats fed a high-fat diet. (**a**-**d**) mRNA expression of *Irs1*, *Akt*, *Glut4*, and *Gsk-3β*. (**e**-**f**) Phosphorylated and total protein levels of IRS1 and AKT represented as p-IRS1/IRS1 and p-AKT/AKT ratios. (**g**-**h**) Protein levels of GLUT4 and GSK-3β. (**i**) Representative western blot images of p-IRS1, IRS1, p-AKT, AKT, GSK-3β, GLUT4, and internal control GAPDH in quadriceps. For western blot, we selected 3 animals from each group, and assessments were repeated for 3 times, which gives 9 protein bands per group ($n = 3$ per group). All data are presented as mean ± SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

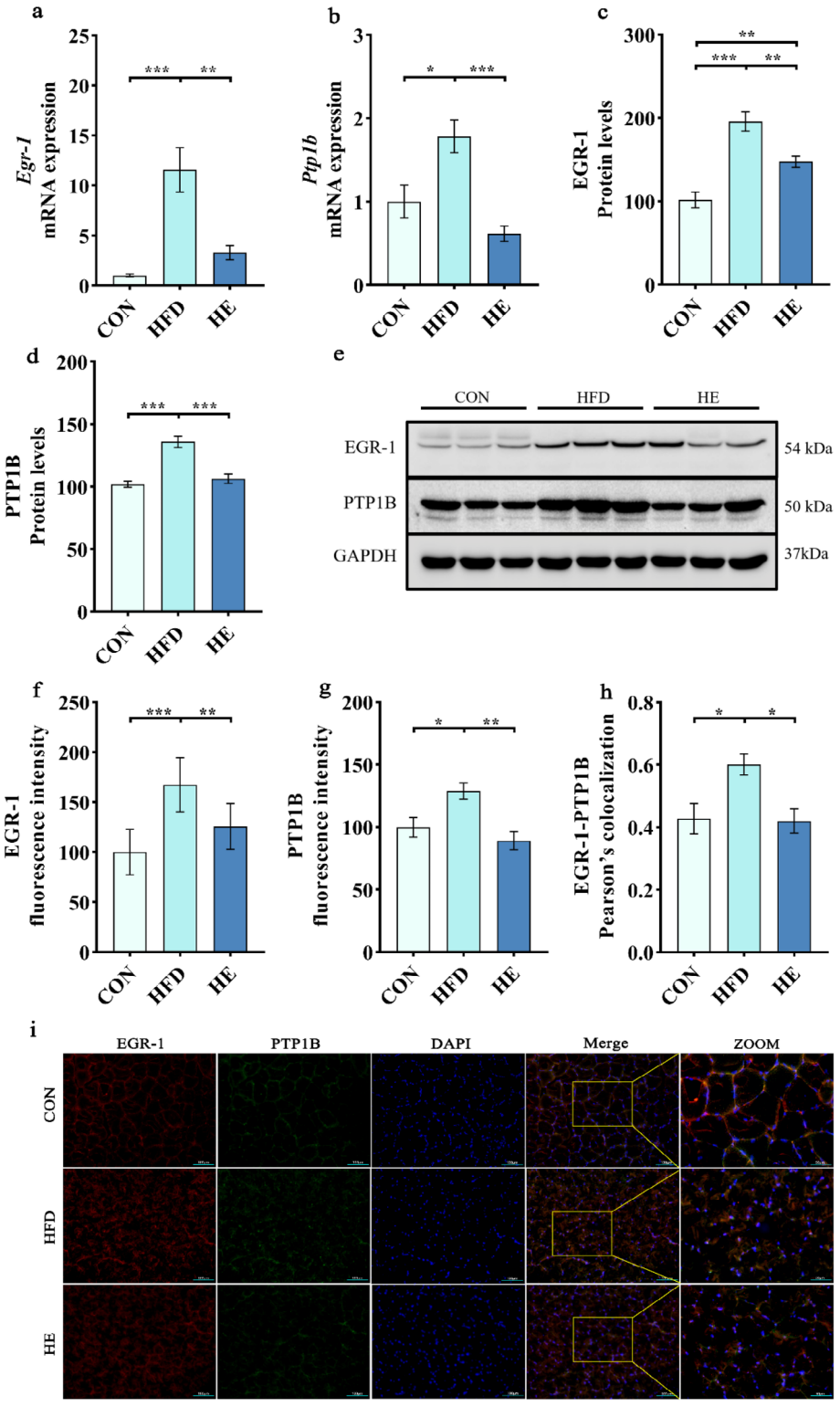


Fig. 5 Aerobic exercise inhibited EGR-1/PTP1B mRNA and protein expressions in rats fed a high-fat diet. (**a**-**b**) mRNA expression of *Egr1* and *Ptp1b*. (**c**-**d**) Protein levels of EGR-1 and PTP1B. (**e**) Representative western blot images of EGR-1 and PTP1B. (**f**, **g**) Fluorescence intensities of EGR-1 and PTP1B. (**h**) Colocalization of EGR-1-PTP1B. (**i**) Representative fluorescence images of EGR-1 and PTP1B. Scale bars, 100 μm and 50 μm. All data are presented as mean ± SEM. For western blot, we selected 3 animals from each group, and assessments were repeated 3 times, which gives 9 protein bands per group ($n = 3$ per group). Five regions were randomly selected from each sample, and quantified ($n = 8$ per group); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Discussion

In this study, we demonstrated the beneficial effects of aerobic exercise training against HFD-induced insulin resistance and glycometabolism impairments in rats. HFD-induced impaired insulin sensitivity and decreased muscle glycogen content were reversed by exercise training. These beneficial effects of exercise were supported a significant upregulation skeletal muscle IRS1, AKT, and GLUT4 mRNA expressions and protein levels against HFD-induced downregulation. Furthermore, HFD-induced overexpression of EGR-1/PTP1B mRNA and protein levels were effectively suppressed by exercise training. Our findings revealed that 6-week aerobic exercise training can improve the insulin signaling possibly by mediating and promoting the skeletal muscle glycogen anabolism through the EGR-1/PTP1B signaling pathway in HFD-fed rats.

Pancreatic beta cells play a crucial role in the regulation of glucose homeostasis by secreting the insulin, and facilitating the storage of approximately 20% and 30% of glycogen in liver and skeletal muscle, respectively [31, 32]. Previous studies have emphasized the close connection between glycogen anabolism and diabetes pathogenesis with a reduction of glycogen content in liver and skeletal muscle of patients with diabetes [33–35]. Impaired skeletal muscle glycogen anabolism and glucose transport represents early metabolic abnormalities in pathogenesis of T2DM [35–38]. Skeletal muscle glycogen anabolism, coordinated by GSK-3β, is impaired in patients with obesity or T2DM, which then lead to reduce insulin sensitivity [39–41]. GSK-3β is a key protein kinase in glycogen metabolism that phosphorylates and inhibits glycogen synthase, a key enzyme in glycogen synthesis, and thereby contribute to decrease glycogen storage in the muscle [42–44]. Elevated levels of GSK-3β in skeletal muscle result in an imbalance in glycogen metabolism, and impaired glucose tolerance [45]. Numerous studies have shown that long-term exercise training-induced physiological adaptation in skeletal muscle could favorably modulate GSK-3β activity, and that could improve insulin sensitivity and increase glycogen content [46–48]. Our findings revealing the capability of 6-week aerobic exercise training in the restoration of muscle glycogen content through the inhibition of GSK-3β mRNA expression and protein in HFD-fed rats.

GLUT4 plays a vital role in regulation of insulin-responsive glucose transport in skeletal muscle, which increase correlates with enhanced glucose transport and increased glycogen content [49, 50]. GLUT4 deficiency skeletal muscle is implicated in impaired insulin-stimulated glycogen synthesis in patients with diabetes [50, 51]. Evidence from a GLUT4 knockout mice model demonstrated insulin resistance and glucose intolerance that are resembling a diabetic phenotype [52, 53]. Aerobic exercise reported to upregulate the GLUT4, and further contribute to lower the fasting and postprandial blood glucose levels [54–56]. This effect possibly mediated through increased insulin sensitivity, and activation of key signaling pathways, such as AMP-activated protein kinase (AMPK) and PI3K/AKT cascade [57]. Aerobic exercise training also reported to augment GLUT4 gene expression through the transcription factors, myocyte enhancer factor 2 (MEF2) and peroxisome proliferator-activated receptor-1α (PGC-1α), facilitates glucose delivery, and thereby improves overall glucose metabolism [58, 59]. Elevated GLUT4 activity not only augments glucose uptake but also supports glycogen synthesis, which is crucial for maintaining the muscle energy reserves and functions [60]. Our findings demonstrated that increased mRNA expression and activated AKT activity, as well as decreased mRNA and protein levels of GSK-3β with exercise training might have contributed to promote anabolic pathways and glycogen storage in skeletal muscle. The role of AKT is essential in facilitating the GLUT4 translocation and glycogen synthase activity for efficient glucose utilization and storage [61]. In our study, exercise intervention effectively ameliorated the HFD-induced insulin resistance by modulating the gene expressions, phosphorylation and protein levels of key signaling molecules involved in glycogen anabolism. These insights underscore the intricate interplay of glycogen anabolism in responses suggesting the potential therapeutic avenues for the effective management of insulin resistance and blood glucose.

IRS1 is a key signaling protein in muscle, which activates intracellular signaling cascades that promote insulin response to aerobic exercise [62, 63]. Activation of IRS1 promotes AKT, and regulates various cellular processes, such as glucose metabolism, apoptosis, and cell proliferation [64, 65]. Therefore, IRS1/AKT pathway is one of the important signaling pathways regulating the glucose homeostasis, and its activation is critical for IR in skeletal muscle. Furthermore, GSK-3β which is capable of phosphorylating the IRS1, was found to decrease with exercise, and subsequently represented by restored p-IRS1/IRS ratio. It is also shown that the reduction of EGR-1 in adipose tissue can augment the IRS1 tyrosine phosphorylation, and restore insulin sensitivity through the PI3K/AKT and ERK/MAPK pathways [9]. PTP1B is widely expressed in the endoplasmic reticulum of insulin-targeted tissues, including liver, muscle, and adipose tissue [66]. PTP1B negatively regulates the insulin signaling by dephosphorylating the IRS or insulin receptor (INSR) [67–69]. A study on PTP1B-deficient mice showed improved insulin sensitivity, lower blood glucose levels, and resistance to HFD-induced obesity [70], emphasizing the pivotal regulatory role of PTP1B in metabolic disorders. Conversely, overexpression of PTP1B

in skeletal muscle impairs insulin signal transduction, diminishes glucose uptake, and induces IR [71]. Human study indicated a negative correlation between skeletal muscle PTP1B gene expression and insulin sensitivity [69]. Exercise reported to decrease PTP1B activity and expression in rats, leading to enhanced skeletal muscle insulin sensitivity through increased tyrosine phosphorylation of insulin receptors and reduced PTP1B binding [72]. Another study showed that short-term strength training can improve the hepatic insulin sensitivity by reducing the liver PTP1B in obese mice [73]. Evidence from our study revealed that 6-week aerobic exercise training can reduce the skeletal muscle EGR-1/PTP1B expressions, and contribute to restore the insulin sensitivity against HFD in rats.

Limitations

Although our findings provide valuable insights into the effects of aerobic exercise on glycometabolism and insulin sensitivity in HFD-fed rats, there are few limitations that should be acknowledged. The absence of exercise-only group in our study, constrains the capacity to demonstrate the independent effects of exercise on studied biomarkers. Next, the six-week exercise duration may not comprehensively capture the beneficial effects or adaptations that are associated with prolonged exercise. Moreover, the absence of gene knockout models perhaps imprecise to demonstrate the molecular evidence particularly the role of EGR-1 and PTP1B in mediating the beneficial effects. Future studies with a broader range of experimental conditions, extended intervention duration, and advanced genetic models could further deepen the understanding of exercise-induced beneficial effects and metabolic health.

Conclusion

Our findings demonstrated that 6-week aerobic exercise training is beneficial on enhancing the insulin sensitivity and promoting the glycogen anabolism in HFD-fed rats. These beneficial effects possibly mediated through the EGR-1/PTP1B signaling pathway in skeletal muscle. Further confirmatory studies are necessary to define the detailed molecular mechanism involved in this phenomenon.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12986-024-00888-8.

Supplementary Material 1

Author contributions

Wei Li and Ting Li: Conceptualization, Methodology, Project administration and Funding acquisition. Liangzhi Zhang and Xiaojie Liu: Investigation and Visualization. Mallikarjuna Korivi, Jing Hu, and Helong Quan: Software, Formal analysis, Data Curation and Supervision. Liangzhi Zhang and Wei Li: Writing - Original Draft and Methodology. Sang Ki Lee, Lifeng Wang and Ting Li: Validation, Resources and Writing - Review & Editing. All authors read and approved the final paper.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Author details

[1]College of Physical Education and Health Sciences, Zhejiang Normal University, 688 Yingbin Road, Jinhua 321004, Zhejiang Province, China
[2]Department of Clinical Medicine, Medical College, Jinhua University of Vocational Technology, Jinhua, Zhejiang, China
[3]Exercise Capacity Assessment and Promotion Research Center, School of Physical Education, Northeast Normal University, Changchun, Jilin, China
[4]Department of Sport Science, College of Natural Science, Chungnam National University, Deajeon, Korea

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