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Swimming intervention mitigates HFD-induced obesity of rats through PGC-1α-irisin pathway

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Abstract. – OBJECTIVE: Irisin, a newly discovered myokine, can drive the browning of white adipocytes to control body weight or mitigate obesity progression through regulating energy metabolism. However, the underlying mechanisms or specific signal pathways of exercise-induced irisin on the management of obesity are still unclear.

MATERIALS AND METHODS: Totally 30 rats were subjected to high fat diet (HFD) feeding for 8 weeks to establish the rat model with obesity successfully. HFD-induced obese model rats were provided with 8 weeks swimming intervention at moderate intensity for exploring the treatment of obesity through exercise intervention. In addition, another 15 rats were subjected to HFD feeding coupled with total 16 weeks swimming intervention at a moderate intensity from the beginning of the experiment, which was used for exploring the prevention of obesity through exercise intervention. Blood and gastrocnemius samples were harvested from obese rats after swimming intervention to explore its specific signal pathways through ELISA analysis and Western blotting.

RESULTS: HFD feeding of rats for 8 weeks could lead to the obesity due to the disorders of lipid metabolism. Totally 8 weeks swimming intervention at moderate intensity for rats with obesity could obviously alleviate the progression of obesity and 16 weeks swimming intervention from the beginning of the experiment could significantly inhibit the development of obesity. Meanwhile, swimming intervention could result in an increased phosphorylation of AMPK and up-regulation of irisin and PGC-1α as the biomarkers of energy metabolism.

CONCLUSION: Exercise intervention can activate PGC-1α-dependent irisin to induce the browning of white adipocytes, thus inhibiting or alleviating the occurrence and development of obesity.

Key Words:
Exercise intervention, Obesity, Myokine, Irisin.

Introduction

Currently, due to the improvement of the quality of life, the changes in diet habits result in the increasing occurrence of obesity. Meanwhile, obesity is also associated with a wide range of diseases such as diabetes, insulin resistance, cardiovascular disease, chronic kidney disease, cancer and other metabolic syndromes[1,2], which leads to the serious threat to human health and the quality of life.

As a novel myokine generated and secreted by skeletal muscle, irisin has been discovered and first reported in 2012 as a hydrolyzed fragment of fibronectin type III domain-containing protein 5 (FNDC5) by proteolytic enzymes[3]. The involvement of irisin in the browning of white adipocytes and energy metabolism plays critical roles in improving insulin sensitivity, inhibiting the occurrence and development of obesity, mitigating or intervening the progression of diabetes mellitus, promoting bone metabolism and enhancing cognitive capacity during the regulation of obesity and other metabolic diseases[4]. Irisin involved in the regulation of obesity is mainly generated by skeletal muscle and secreted into the circulatory system to regulate energy metabolism in adipose tissue. Its regulation in adipose tissue is mainly completed by overexpressed uncoupling protein-1 (UCP1) to promote the formation of adipose droplets, thus resulting in the browning of white adipocytes and achieving the prevention and treatment of obesity[5]. In addition, the administration of irisin or the regulation of

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endogenous irisin-associated signaling pathway can complete the control of body weight gain in high fat diet (HFD)-induced obese mice, suggesting its intervention efficiency on obesity or corresponding metabolic diseases[6]. Similarly, irisin in obese populations has demonstrated its negative correlation with body mass index (BMI)[7], glucose[8], insulin[9], and cholesterol[10].

Regular physical activity or appropriate exercise training is an economical and convenient intervention strategy for health promotion, and prevention or treatment of many chronic diseases. Currently, many authors have confirmed that exercise can significantly reduce the levels of total cholesterol (TC) and low-density lipoprotein (LDL)[11]; in contrast, significantly increase the levels of high-density lipoprotein (HDL) and adiponectin, thus correspondingly improving lipid metabolism[12]. According to previous experimental reports, four-week swimming intervention can alleviate the increase in body weight of obese rats, optimize blood lipid compositions, and improve serum adiponectin level[13]. Also, exercise can enhance the expression of peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α)-dependent irisin in skeletal muscle, stimulate the proliferation of pancreatic β cells, increase glucose tolerance, accelerate adaptive thermogenesis in the body, and promote glucose transportation and fat decomposition[14]. AMPK, as an important regulatory molecule for fatty acid oxidation in skeletal muscle, plays a very important role in maintaining cellular energy balance. The phosphorylation of AMPK can improve glucose absorption and energy metabolism for the control of body weight[15]. Similarly, a previous report has also demonstrated that exercise can activate AMPK pathway to regulate irisin-dependent energy metabolism mainly through the activation of PGC-1α in skeletal muscle[16]. Moreover, exercise-induced autophagy in skeletal muscle also can complete the regulation of cellular homeostasis to improve glucose metabolism and insulin sensitivity, thereby realizing the prevention and treatment of metabolic disorders[17]. In the present study, we systematically investigated the preventive role of 16 weeks swimming intervention for the obesity of rats from the initial HFD induction and the treatment efficacy of 8 weeks swimming intervention on HFD-induced obese rats. Meanwhile, we also explored and confirmed PGC-1α-irisin pathway as the potential target for the prevention and treatment of obesity in the presence of exercise intervention.

Materials and Methods

Establishment of Rat Models with Obesity

Totally 80 male SD rats with the age of 8 weeks old were purchased from Experimental Animal Research Center in Hubei Province, and randomly divided into three groups, including 15 rats in control group (C group), 15 rats in HFD feeding combined with exercise intervention from the beginning group (HDE group), and another 55 rats subjected to HFD feeding for the establishment of obesity model. After HFD feeding for 8 weeks in HD group, 30 rats with an significant difference in body weight before and after HFD feeding were selected and then divided into two subgroups including 15 HFD-induced obese rats as the model control group (HD group) and 15 HFD-induced obese rats coupled with exercise intervention group (OE group) for 8 weeks swimming intervention. During the swimming intervention, body weights of the rats were determined according to the designed time points in each week. Meanwhile, the rats from HDE group were subjected to continuous swimming intervention for another 8 weeks with total swimming intervention period of 16 weeks from the beginning of this experiment. All rats from HDE and OE groups were provided with 90 min swimming training within each training day during 5 training days of a week.

Determination of Serum Lipid Compositions in Obese Rats

Upon the completion of the last swimming training for these rats, all rats were immediately sacrificed for blood sampling from the eyeball. The blood samples were allowed to stand at room temperature for 2 h and then subjected to the centrifugation with the speed of 2500 × g at 4°C for 10 min. The serum was collected and stored at -20°C freezer for future use. The concentrations of serum TC, HDL and LDL were determined by using TC, HDL and LDL assays kits (Biosino Bio-Technology and Science Inc., Beijing, China) according to the manufacturer's instructions.

Serum Irisin Concentration in Obese Rats Determined by ELISA

The concentration of serum irisin from each group was determined through double sandwich ELISA kits coated with anti-rat monoclonal irisin antibody (Phoenix Pharmaceuticals, Burlingame, CA, USA). Totally 0.1 mL of diluted serum was

added to the wells coated with irisin antibody for the reaction at 37°C for 1 h. The wells after reaction were washed with washing buffer three times. Then, 0.1 mL of streptavidin-horseradish peroxidase (SA-HRP) was added to each well for titration and each well was washed with washing buffer three times. Next, 0.1 mL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added to each well for coloration at 37°C for 20 min. After the coloration, 0.05 mL of termination solution was added to each well for stopping the reaction and coloration. The absorbance at 450 nm was recorded in a microplate reader. The standard curve was established through the concentration of standard peptide as X-axis and absorbance of standard peptide as Y-axis. The concentrations of irisin in serum samples were determined and calculated according to the established standard curve.

Expression of Irisin and its Related Proteins Evaluated by Western Blotting

After the last swimming intervention, all rats from each group were sacrificed by cervical dislocation and gastrocnemius samples were harvested and then immediately frozen in liquid nitrogen. The frozen gastrocnemius samples were transferred to -80°C refrigerator for future use. The gastrocnemius samples with the addition of protease inhibitor PMSF (Beyotime Institute of Biotechnology, Wuhan, China) were homogenized for 2 times and subjected to 5 min sonication on a JY 92IIN sonicator (Ningbo Scientz Biotechnology Co., Ltd, Ningbo, China). The supernatant was collected after centrifugation at 10000 × g for 10 min. The protein concentration was determined by BCA method. Approximately 60 μg protein sample with sample buffer after boiled at 95°C water bath for 5 min was separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to PVDF membrane. The membrane with protein was blocked with 5% skim milk in TBS-T buffer for 2 h at room temperature. Then, the membrane was incubated with primary antibodies such as AMPK, p-AMPK and PGC-1α (1:1000, Cell Signaling Technology, Danvers, MA, USA), as well as irisin (1:1000, Abcam, Cambridge, MA, USA) at 4 ºC overnight, respectively. The membrane incubated with primary antibody was washed with TBS-T buffer for three times with 15 min for each time. Sequentially, HRP-labeled anti-rabbit IgG secondary antibody (1:10000, Cell Signaling Technology, USA) was added for incubation at 37 ºC for 60 min. After washing the membrane with TBS-T buffer for three times, 10 min each time, the chemiluminescence agent was incubated with the membrane in a dark room for 2 min and the protein probed by primary antibody in the membrane was imaged. The tubulin was used as an internal reference.

Statistical Analysis

All statistical analyses were conducted using SPSS19.0 software (SPSS Inc., Chicago, IL; USA) and GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA) through independent *t*-test. The significant difference and very significant difference were considered at $p < 0.05$ and $p < 0.01$, respectively.

Results

Swimming Intervention Inhibits Or Alleviates the Progression of Obesity

As shown in Figure 1, after the rats were subjected to HFD feeding for 8 weeks, the body weights of the rats from HD group were higher than that from C group, which revealed a significant difference, suggesting that the HFD-induced

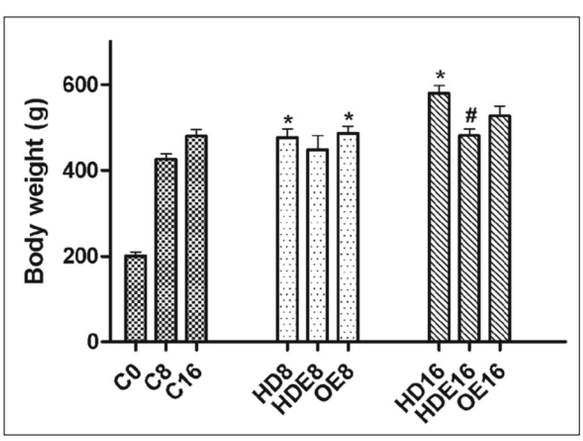

Figure 1. The body weights of rats (n = 15 rats in each group) before and after 8-week HFD feeding for the model establishment of obesity and another 8-week swimming intervention. C0, C8 and C16 were the body weights of the rats from the control group at 0, 8th and 16th week. HD8 and HD16 were the body weights of the rats subjected to HFD feeding alone at the 8th and 16th week. HDE8 and HDE16 were the body weights of the rats subjected to HFD feeding coupled with exercise at the 8th and 16th week. OE8 and OE16 were the body weights of the obese rats with exercise intervention at the 8th and 16th week. *Significant difference when compared with control group ($p < 0.05$); #significant difference when compared with HD group ($p < 0.05$).

obesity rat model was successfully established. The obese rats from OE group were provided with 8 weeks swimming intervention to reveal a slow increase in body weight when compared with that of the rats from HD group without swimming intervention. Similarly, body weights of the rats from HDE group provided with 16 weeks swimming intervention from the beginning of the experiment exhibited reduction when compared with that of the rats from HD group without swimming intervention at the 16th week. These results indicated that regular exercise at a moderate intensity such as swimming intervention could realize the prevention of obesity or alleviate the progression of obesity.

Swimming Intervention Optimizes Lipid Metabolism of Obese Rats

As shown in Figure 2, the concentration of TC in rats from HD group revealed increase when compared with that in C group ($p < 0.01$); in contrast, the TC level in rats from HDE group revealed a significant reduction when compared with that in HD group ($p < 0.01$), suggesting that HFD could induce the increase of TC level during the establishment of rat model with obesity, but the swimming intervention at moderate intensity could inhibit HFD-induced increase of TC level. On the other hand, HDL level in rats from HDE group was significantly reduced when compared with that in rats from C group ($p < 0.05$), while HDL level in rats from HDE group exhibited a very significant improvement when compared with that in rats from HD and C groups ($p < 0.01$), suggesting that HFD-induced reduction of HDL could be reversed by exercise, and exercise intervention may realize the optimization of lipid compositions in the body. In addition, LDL level in HFD-treated rats was obviously higher than that in C group ($p < 0.01$), but the LDL levels in rats from swimming intervention groups such as HDE and OE groups present a reducing trend to some extents ($p < 0.01$), which further demonstrated that exercise intervention could regulate of lipid metabolism, enhance HDL generation and reduce LDL accumulation. Therefore, exercise will be benefit for controlling body weight, mitigating the progression of obesity, preventing or treating obesity through effective optimization of lipid metabolism.

Swimming Intervention Rescues the Down-Regulation of Irisin In Obese Rats

The irisin content in serum from rats in HD group was significantly lower than that in serum from rats in C group ($p < 0.05$), as shown in Figure 3, suggesting that the accumulation of adipose tissue in obese rats could result in the declined generation of irisin, and the limited secretion and distribution of irisin in blood, thus resulting in the lower irisin level in serum from obese population. On the other hand, the irisin level in serum of the rats from HDE group revealed a significant increase when compared with that in HD group ($p < 0.05$), which is probably due to the significant activation of irisin in skeletal muscle and secretion of irisin to circulation system in the presence of swimming intervention, finally resulting in the significant improvement of irisin in serum of the rats from exercise intervention groups. All of these results suggest that the inhibition or mitigation of obesity is highly associated with the generation and secretion of irisin.

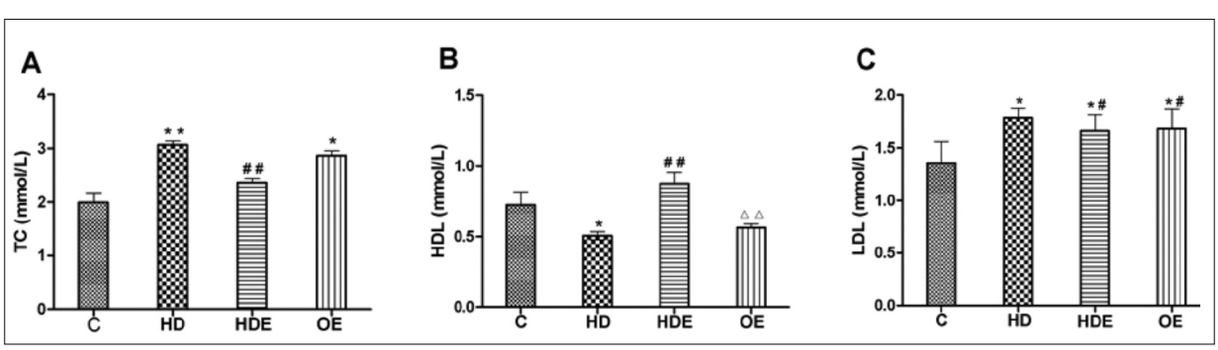

Figure 2. Effect of swimming intervention on TC *(A)*, HDL *(B)* and LDL *(C)* in obese rats. *Significant difference when compared with control group ($p < 0.05$); *very significant difference when compared with control group ($p < 0.01$); ##significant difference when compared with HD group ($p < 0.01$); ΔΔvery significant difference when compared with HDE group ($p < 0.01$).

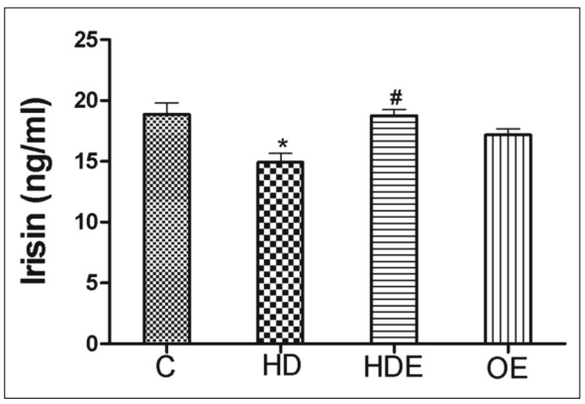

Figure 3. Effect of swimming intervention on the level of irisin in serum from obese rats. *Significant difference when compared with control group ($p < 0.05$); #significant difference when compared with HD group ($p < 0.05$).

Swimming Intervention Improves the Expression of Irisin and its Related Proteins

The expression of irisin and its related proteins in skeletal muscle of the rats in the presence of swimming intervention was evaluated by western blotting. As shown in Figure 4, the significantly down-regulated expression of irisin, p-AMPK and PGC-1a was observed in skeletal muscle of the rats from HD group when compared with C group ($p < 0.05$), suggesting that HFD could induce the accumulation of adipose tissue and result in the lower energy metabolism in obese rats; in contrast, swimming intervention at moderate intensity could significantly up-regulate the expression of irisin, p-AMPK and PGC-1α in skeletal muscle of the rats from HDE and OE groups ($p < 0.05$). Moreover, the relative expression of PGC-1α and irisin exhibited a significant increase, indicating that exercise intervention can significantly improve energy metabolism in the body, thus inhibiting the accumulation of adipose tissue, inhibiting or delaying the occurrence of obesity, and realizing the prevention or treatment of obesity through exercise intervention.

Discussion

The HFD feeding of rats for 8 weeks can result in a significant increase in body weights of the rats when compared with that from control rats and realize the successful establishment of a rat model with obesity. In the present study, the body weights of the rats from HDE group revealed a significant reduction when compared with that from HD group, suggesting that swimming intervention at moderate intensity for 16 weeks can prevent or alleviate the occurrence and development of obesity, which is good agreement with previous reports that regular exercise or physical activity can increase energy metabolism in body to contribute the control of body weights and the mitigation of obesity[18]. Although body weights of the rats from OE group were lower than that in HD group, no significant difference was observed, which is possibly due to the relatively short term of exercise intervention (only 8 weeks swimming intervention) after obesity model is established. However, 8-week swimming intervention significantly suppressed the gain of body weight in rats. Therefore, the exercise could alleviate the progression of the obesity, and the long-term exercise intervention will be useful for the prevention and treatment of obesity.

A previous study[19] has demonstrated that HFD can result in a significant increase in TC and LDL levels and a significant reduction in HDL level; in contrast, exercise intervention can cause the significant reverse of HDL, and the significant decrease in TC and LDL levels, which suggests that HFD-induced disorders of lipid metabolism can be recovered through appropriate exercise intervention, which is consistent with our experimental results. After exercise intervention, TC and HDL levels between HD group and OE group did not reveal an obvious difference, but a gradually increasing trend of HDL level and a declining trend of LDL level were observed in OE group, whose possible reason may be the relatively shorter exercise intervention duration (only 8 weeks swimming intervention). Conversely, after 16 weeks swimming intervention at moderate intensity, TC and LDL present significantly lower levels in both HDE and OE groups when compared with HD group, and HDL was significantly improved in both HDE and OE groups compared with HD group. These results further suggest that the term of exercise intervention may be a positive correlation with the recovery of HFD-induced metabolic disorders.

Based on the analysis of irisin level in serum and skeletal muscle in rats from OE group and C group, irisin in serum revealed lower level than that in skeletal muscle, which may be due to the secretion of irisin to blood after the generation of irisin in skeletal muscle. Exercise intervention

can improve the expression of PGC-1α in skeletal muscle and enhance mitochondrial biosynthesis, and accelerate glucose transportation and lipid decomposition. Irisin, as a PGC-1α-dependent myokine, has the capacity to stimulate the browning of white adipose tissue and to regulate lipid metabolism. Compared with HD group without exercise, serum irisin level in exercise groups was higher, indicating that exercise intervention can promote the accumulation of irisin in the blood due to its overexpression in skeletal muscle. However, no significant difference in serum irisin levels between HDE group and OE group was observed, and the short-term exercise intervention revealed an effect on the accumulation of irisin in serum when compared with the long-term exercise intervention, which is consistent with previous reports[20,21] that long-term exercise intervention may cause partial degradation of irisin or its partial involvement in metabolic process in body. In addition, serum irisin level in HD rats is lower than normal rats, indicating that serum irisin level is negatively correlated with body mass index.

AMPK in skeletal muscle is an important regulatory molecule for the regulation of lipid metabolism, and high fat diet can reduce the phosphorylation level of AMPK in the present study ($p < 0.05$), so that increased phosphorylation of AMPK can improve glucose intake and energy metabolism in the body. As shown in Figure 4, p-AMPK/AMPK ratio in exercise groups revealed a higher level than that in HD group ($p < 0.05$); therefore, exercise intervention has a significant effect on AMPK activation. Through the comparison of AMPK activity between OE group and HDE group, AMPK activity is positively correlated with exercise intervention time. The down-regulated PGC-1α level can result in the reduction of p-AMPK level in myoblasts, which may be correlated with the secretion of irisin in skeletal muscle[14]. From our experimental data, we can also find that irisin in skeletal muscle is correlated with the phosphorylation level of AMPK. Therefore, the up-regulated expression of irisin in skeletal muscle can activate AMPK, promote fatty acid oxidation, and alleviate the occurrence of metabolic disorders including obesity[16].

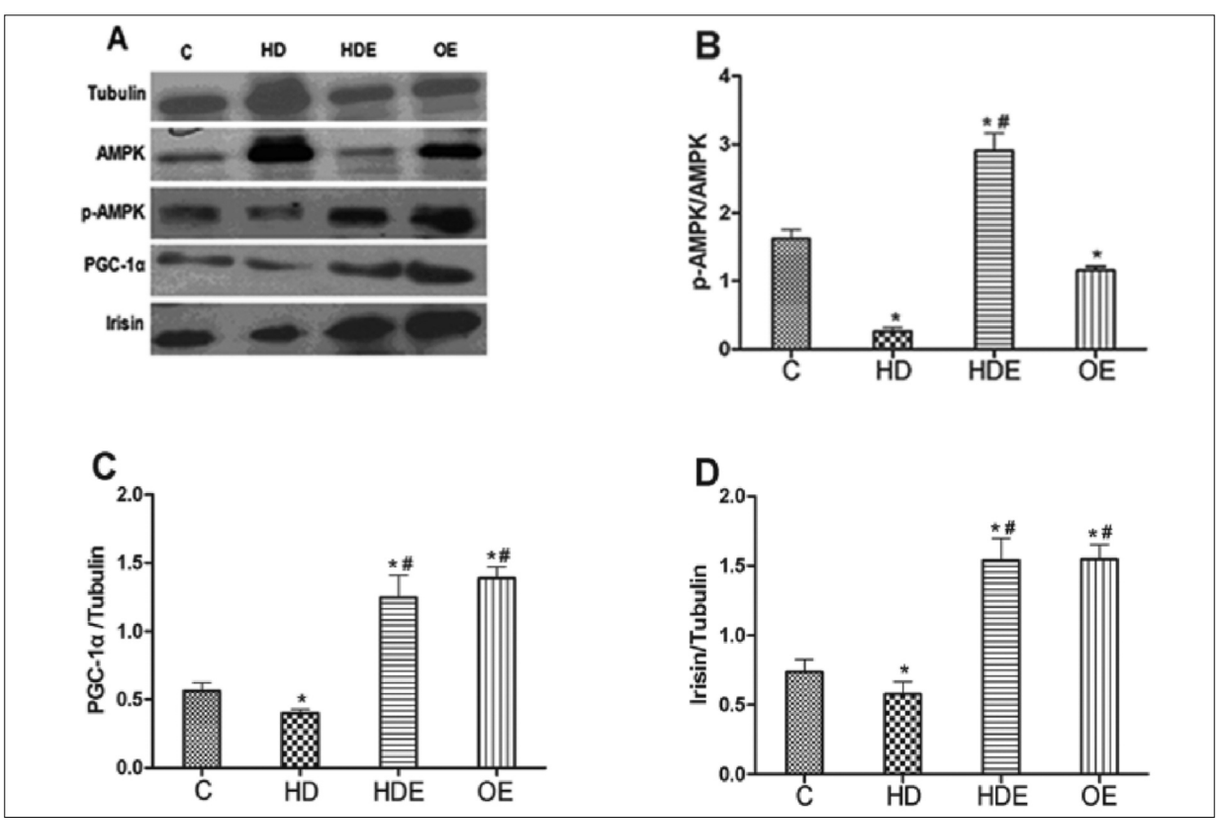

Figure 4. *A,* Effect of swimming intervention on the expression of irisin and its related proteins. *Significant difference when compared with control group ($p < 0.05$); #significant difference when compared with HD group ($p < 0.05$).

Conclusions

Long-term high fat diet can lead to metabolic disorders including obesity. Exercise intervention can activate PGC-1α-dependent irisin to induce the browning of white adipose tissue, thus inhibiting or alleviating the occurrence and development of obesity. Irisin is negatively correlated with body mass index and cholesterol level, but the activation of irisin can activate the phosphorylation of AMPK to improve energy metabolism in the body. Therefore, exercise intervention can improve the expression of irisin to realize the browning of white adipose tissue, increase thermogenesis and reduce body weight as a hot research field of obesity treatment. However, the further exploration of accurate and specific mechanisms of irisin to regulate obesity in animals and human is still highly necessary.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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