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Aerobic Exercise Activates the Signaling Pathway of IGF-1/PI3K/Akt to Inhibit Skeletal Muscle Inflammation in Obese Mice

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Abstract: *This study aimed at discussing the effect of aerobic exercise on expression of insulin-like growth factor 1 (IGF-1) and its receptor Insulin like growth factor-1 receptor (IGF-1R) in skeletal muscle of obese mice, downstream signaling pathway of PI3K/Akt, skeletal muscle inflammation and atrophy. 35 healthy male C57BL/6 mice were randomly divided into normal control group (C, 10 mice) and high-fat diet group (25 mice). After 8 weeks, 20 obese mice were randomly divided into obesity control group (OS) and obesity exercise group (OE), 10 mice in each group. The OE group underwent treadmill aerobic exercise for 8 weeks. After training, the protein expression of IGF-1/IGF-1R, pPI3K/PI3K, pAkt/Akt, TNF-α, IL-1β, IL-10, Atrogin-1 and MuRF1 in skeletal muscle were measured by Western blotting. The cross-sectional area of skeletal muscle was observed and calculated by HE staining. The results showed that compared with C group, body weight in OS group was significantly increased, skeletal muscle IGF-1/IGF-1R, pPI3K/PI3K, pAkt/Akt and IL-10 protein expression were significantly downregulated, while TNF-α, IL-1β, Atrogin-1 and MuRF1 protein expression were significantly upregulated, the skeletal muscle cross-sectional area and gastrocnemius/body weight ratio were significantly decreased. Compared with OS group, body weight in OE group was significantly decreased, skeletal muscle IGF-1/IGF-1R, pPI3K/PI3K, pAkt/Akt and IL-10 protein expression were significantly upregulated, while TNF-α, IL-1β, Atrogin-1 and MuRF1 protein expression were significantly downregulated, the skeletal muscle cross-sectional area and gastrocnemius/body weight ratio were significantly increased. Conclusions are as follows, Aerobic exercise may via upregulate the expression of IGF-1 and its receptor IGF-1R protein in skeletal muscle of obese mice, activates the signaling pathway of PI3K/Akt, and inhibit inflammatory response. It shows that the signaling pathway of IGF-1/PI3K/Akt plays an important role in aerobic exercise inhibits the skeletal muscle inflammation of obese mice and improves skeletal muscle atrophy.*

Keywords: *aerobic exercise,obesity, IGF-1/PI3K/Akt signaling pathway, inflammation, skeletal muscle atrophy*

1. Introduction

Obesity is a global public health concern and can increase the risk of type 2 diabetes, it can increase insulin resistance and lead to a range of metabolic disorders such as dyslipidemia, coronary heart hypertension and stroke[1][2], which has adversely affected the quality of life. A high-fat diet can lead to obesity in animals and promote muscle atrophy and dysfunction[3]. Skeletal muscle inflammation plays an important role in skeletal muscle atrophy, inflammation and muscle atrophy can exacerbate insulin resistance caused by obesity[4], Therefore, inhibiting obesity-induced skeletal muscle inflammation and atrophy may help prevent obesity-related metabolic disorders. As we all know, exercise is an important means to reduce obesity and delay skeletal muscle atrophy, but its target and mechanism still need to further discuss.

Insulin-like growth factor-1 (IGF-1) can be secreted by skeletal muscle, heart, liver and other participate in the regulation of metabolism, and play a protective role in multiple tissues and organs. Studies have shown that aerobic exercise can up-regulate the expression of IGF-1and its receptor in skeletal muscle of MI mice, activate PI3K/Akt signaling pathway, inhibit oxidative stress, protein degradation and cell apoptosis in skeletal muscle, and improve myocardial infarction induced in mice[5]; resistance exercise can improve skeletal muscle atrophy in rats with chronic inflammation induced by peptidoglycan polysaccharides by activating protein synthesis signaling pathway[6]. whether exercise can activate skeletal muscle IGF-1/IGF-1R-PI3K/Akt signaling pathway, inhibit

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inflammatory response and improve skeletal muscle atrophy in obese mice is rarely reported in the literature. In this study, treadmill aerobic exercise was used to intervene obese mice to explore the above problems, and to provide theoretical and experimental basis for improving obesity skeletal muscle atrophy.

2. Materials and methods

2.1 Main instruments and reagents

Main instruments: animal test bench, Bio-Rad electrophoresis apparatus, tissue embedding machine, paraffin microtome, optical microscope.

Main reagents: IGF-1, IGF-1R, pPI3K, PI3K, pAkt, Akt antibodies were purchased from Cell Signaling Technology, USA; Atrogin-1, MuRF-1, TNF-α, IL-1β, and IL-10 antibodies were purchased from Abcam, USA.

2.2 Experimental animals and groups

After 1 week of adaptability, 5 weeks C57BL/6 Healthy male mice was randomly divided into normal control group (group C, 10) and high -fat feeding group (25), which were fed with ordinary diet and high-fat diet, respectively. After 8 weeks, the average weight of the high-fat feeding group by the high -fat feeding group under the air stomach is 20% of the normal control group as the standard for judging the success of obese mice. 20 were selected from high-fat feeding obesity mice, randomly divided into obesity control group (OS group) and obesity exercise group (OE group), 10 in each group.

2.3 Aerobic exercise program

The obesity exercise group adopted treadmill aerobic exercise. After 1 week of adaptive exercise, then formal exercise, 14m/min, 6 days a week, 60 minutes a day, for 8 weeks.

2.4 Animal materials and sample handling

The day after the training end, mice gastrocnemius muscle was taken, and some gastrocnemius muscles are fixed in 10% of the neutral formaldehyde, making paraffin slices for HE staining; another part of the gastrocnemius muscle liquid nitrogen was placed in the refrigerator at -80℃ for 24h, and the protein was extracted for Western blotting experiment.

2.5 Western Blotting experiment

About 50 mg of obese mouse gastrocnemius tissue was clipped, protein extraction reagent was added, and the protein was extracted and quantified by BCA protein. The primary antibody IGF-1, IGF-1R, PI3K, pPI3K, Akt, pAkt, TNF-α, IL-1β, IL-10, Atrogin-1 and MuRF-1 were incubated overnight at 4℃. The next day, the second antibody was incubated.

2.6 HE staining

After the gastrocnemius was fixed with 10% neutral formaldehyde, paraffin sections were prepared, stained with hematoxylin and eosin, and neutral gum tablets were sealed. The cross-sectional area of skeletal muscle cells was analyzed and measured by Image J software under an optical microscope.

2.7 Image and data processing

Image Lab 5.2 was used to process the results of Western Blotting experiments, Image J software was used to analyze microscope images, and GraphPad Prism5.0 software was used to map. SPSS17.0 software was used to conduct one-way ANOVA for experimental data, and the significance level of differences between groups was selected as p&lt. 0.05 or p< 0.01.

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3. Experimental results

3.1 Aerobic exercise inhibits the weight growth of obesity mice

The body weight results of obese mice showed that compared with C group, the body weight of the OS group increased significantly. Compared with OS group, the body weight of the OE group was significantly reduced (Figure 1). It shows that high-fat feeding induced obesity, and aerobic exercise significantly inhibites the weight growth of obesity mice.

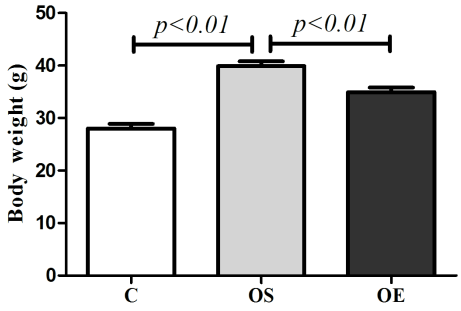

Figure 1 Effects of aerobic exercise on body weight of obese mice

3.2 Aerobic exercise up-regulates the expression of IGF-1 and its receptor IGF-1R in skeletal muscle of obese mice

Western Blotting results showed that compared with C group, the skeletal muscle protein expressions of IGF-1 and IGF-1R in OS group were significantly decreased. Compared with OS group, the expression of IGF-1 and IGF-1R protein in OE group was significantly increased (Figure 2). These results indicate that aerobic exercise can significantly up-regulate the expression of IGF-1 and its receptor in skeletal muscle of obese mice.

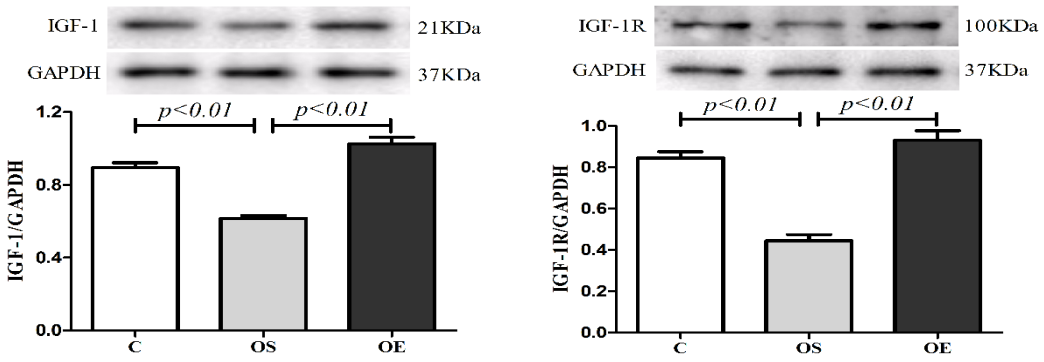

Figure 2 The protein expression result of IGF-1 and its receptor IGF-1R in skeletal muscle of obese mice

3.3 Aerobic exercise activates PI3K/Akt signaling pathway in skeletal muscle of obese mice

Western Blotting results showed that compared with C group, the pPI3K/PI3K and pAkt/Akt protein expression in the group were significantly decreased. Compared with OS group, pPI3K/PI3K and pAkt/Akt protein expression in OE group were significantly increased (Figure 3). It shows that aerobic exercise can effectively activate PI3K/Akt signaling pathway in skeletal muscle of obese mice.

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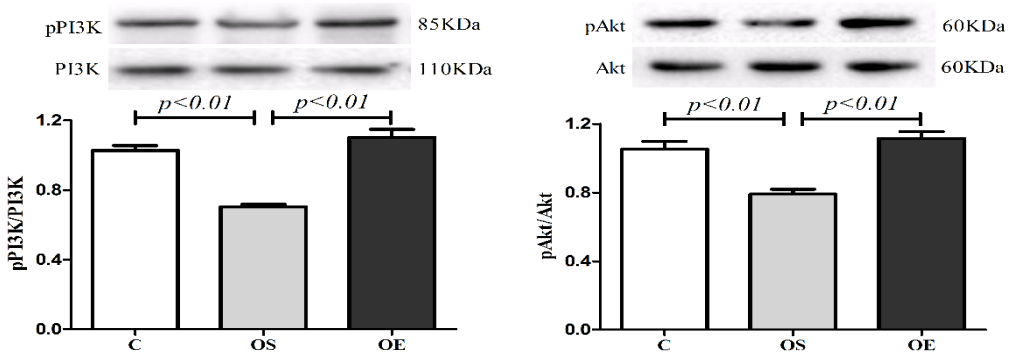


Figure 3 The protein expression result of pPI3K/PI3K, pAkt/Akt in skeletal muscle of obese mice

3.4 Aerobic exercise inhibits skeletal muscle inflammation in obese mice

Western Blotting results showed that compared with C group, the protein expressions of TNF-α and IL-1β were significantly increased in OS group, while the protein expression of IL-10 was significantly decreased. Compared with OS group, the protein expressions of TNF-α and IL-1β in OE group were significantly decreased, while the protein expression of IL-10 was significantly increased (Figure 4). The results showed that the skeletal muscle inflammation of obese mice was significantly increased, and aerobic exercise could significantly inhibit the skeletal muscle inflammation of obese mice.

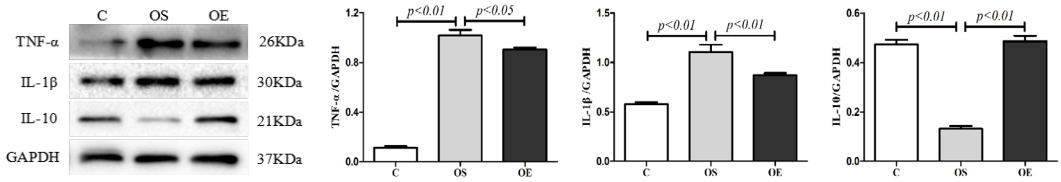
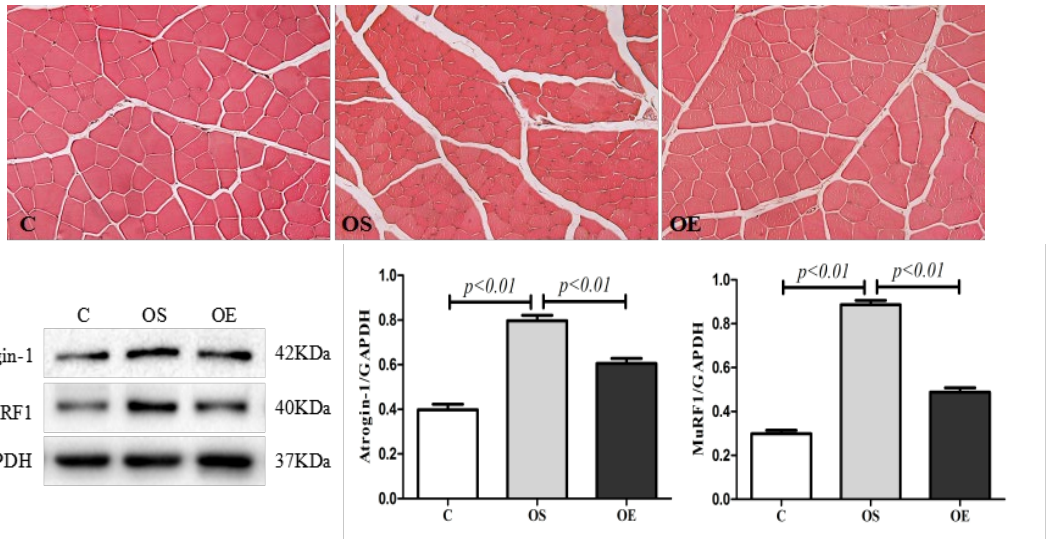


Figure 4 The protein expression result of TNF-α, IL-1β, IL-10 in skeletal muscle of obese mice

3.5 Aerobic exercise improves skeletal muscle atrophy in obese mice

HE staining and gastrocnemius/body weight results showed compared with C group, a the skeletal muscle cross-sectional area and gastrocnemius/body weight ratio were significantly decreased in OS group. Compared with OS group, the skeletal muscle cross-sectional area and the gastrocnemius/body weight ratio were significantly increased in OE group. Western Blotting results showed that compared with C group, Atrogin-1 and MuRF1 protein expressions in OS group were significantly increased. Compared with OS group, Atrogin-1 and MuRF1 protein expressions were significantly decreased in OE group(Figure 5). The results show that skeletal muscle atrophy occurs in obese mice, and aerobic exercise can significantly improve skeletal muscle atrophy in obese mice.



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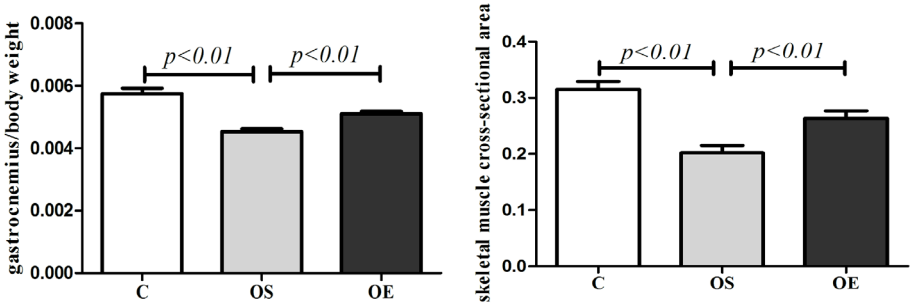

Figure 5 The result of HE staining, gastrocnemius/body weight ratio, Atrogin-1 and MuRF1 protein expression in skeletal muscle of obese mice

4. Analysis and discussion

Obesity is a chronic disease and one of the induction of muscle atrophy. Skeletal muscle atrophy can cause the decline in the quality and exercise ability of skeletal muscle, affect the quality of life, and increase the medical burden of family and society. As we all know, regular sports are one of the effective means to reduce fat accumulation and control weight, and can bring sustainable benefits to physical health. The results of this study showed that the weight of mice increased significantly after a high-fat diet, and the aerobic exercise in 8 weeks can significantly inhibit the weight growth of obese mice. The literature reports that the protein expression of MuRF1 and Atrogin-1 in skeletal muscle of obese mice and type 2 diabetic rats is significantly increased, and the cross-sectional area of skeletal muscle is significantly decreased, aerobic exercise significantly inhibits the protein expression of MuRF1 and Atrogin-1 in skeletal muscle, significantly increases the cross-sectional area of skeletal muscle, and improves muscle atrophy[7][8]. Other studies have shown that treadmill aerobic exercise can increase skeletal muscle weight and cross-sectional area of skeletal muscle cells in type 2 diabetic mice[9], inhibit oxidative stress in skeletal muscle of diabetic rats, increase skeletal muscle weight and cross-sectional area of skeletal muscle cells[10], and improve exercise ability. Whole body vibration training and high-intensity interval exercise can alleviate myocardial infarction induced sarcopenia[11]. Resistance exercise can increase the cross-sectional area of skeletal muscle cells in myocardial infarction rats[12]. The results show that exercise can inhibit the loss of skeletal muscle caused by a variety of factors and improve the atrophy of skeletal muscle. The results of this study showed that the protein expression of MuRF1 and Atrogin-1 in skeletal muscle of obese mice was significantly increased, and the skeletal muscle cross-sectional area and the ratio of gastrecnemius/body weight were significantly decreased. Aerobic exercise significantly inhibited the protein expression of MuRF1 and Atrogin-1, significantly increased the ratio of skeletal muscle cross-sectional area to gastrecnemius/body weight, and improved muscle atrophy. This is consistent with the results reported in the literature. It shows that skeletal muscle atrophy occurs in obese mice, and aerobic exercise can significantly improve obesity-induced skeletal muscle atrophy.

Exercise can stimulate the expression and release of skeletal muscle factor or cytokines, which will affect the skeletal muscle of the body. Resistance exercise, moderate intensity continuous aerobic exercise and high intensity intermittent aerobic exercise can up-regulate the level of skeletal muscle IGF-1, reduce oxidative stress and protein degradation, increase the cross section of muscle weight and muscle fiber Skeletal muscle atrophy[12]. The Akt/mTOR signaling pathway plays an important role in the regulation of skeletal muscle hypertrophy and atrophy[13]. Resistance training can reduce cell apoptosis and inhibit the loss of skeletal muscle mass in aging rats by regulating IGF-1 and its receptor, Akt/mTOR signaling pathway[14]. Aerobic exercise can inhibit skeletal muscle atrophy induced by heart failure by activating IGF-1/Akt/mTOR signaling pathway[15], up-regulate IGF-1 and its receptor IGF-1R in skeletal muscle, activate PI3K/Akt signaling pathway, and improve sarcopenia induced by MI mice[5]. These results indicate that different exercise modes can activate IGF-1/Akt/mTOR signaling pathway to inhibit skeletal muscle atrophy induced by various factors. The results of this study show that aerobic exercise can significantly increase the protein expression of IGF-1 and its receptor IGF-1R in skeletal muscle of obese mice, and up-regulate the phosphorylation levels of PI3K and Akt. Therefore, it is suggested that aerobic exercise may inhibit skeletal muscle atrophy by up-regulating the protein expression of IGF-1 and its receptor IGF-1R in skeletal muscle of obese mice, activating PI3K/Akt signaling pathway.

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Obesity is a chronic inflammatory state, and skeletal muscle inflammation plays a very important role in skeletal muscle atrophy and systemic metabolic dysfunction. Therefore, it is believed that reducing skeletal muscle inflammation can improve the metabolic disorders caused by obesity. TNF-α and IL-1β are typical pro-inflammatory cytokines, which are involved in the occurrence and development of chronic inflammation[16]. IL-10 is a typical anti-inflammatory cytokine that inhibits inflammation and promotes tissue repair[17].It has been reported that treadmill exercise can down-regulate the expression of TNF-α and Atrogin-1 in skeletal muscle of hindlimb unloaded mice, and inhibit skeletal muscle atrophy[18]. Endurance interval exercise can inhibit the protein expression of TNF-α, IL-1β and IL-6 in obese mice, and reduce skeletal muscle inflammation[19]. Aerobic exercise can inhibit the protein expression of TNF-α and IL-1β and increase the protein expression of IL-10 in obese mice, thereby reducing the inflammatory response of skeletal muscle, improving muscle atrophy and exercise ability[7]. These results indicate that exercise can reduce the inflammatory response of skeletal muscle, improve the atrophy of skeletal muscle by inhibiting the expression of pro-inflammatory factor and up-regulating the expression of anti-inflammatory factor. The results of this study show that the protein expression of TNF-α and IL-1β in skeletal muscle of obese mice was significantly increased, and the protein expression of IL-10 was significantly decreased. Aerobic exercise significantly inhibites the protein expression of TNF-α and IL-1β, and significantly increase the protein expression of IL-10. These results suggest that aerobic exercise may inhibit skeletal muscle inflammation and improve skeletal muscle atrophy in obese mice by activating IGF-1/IGF-1R-PI3K/Akt signaling pathway.

5. Conclusions

Aerobic exercise may via upregulate the expression of IGF-1 and its receptor IGF-1R protein in skeletal muscle of obese mice, activates the signaling pathway of PI3K/Akt , and inhibit inflammatory response. It shows that the signaling pathway of IGF-1/PI3K/Akt plays an important role in aerobic exercise inhibits the skeletal muscle inflammation of obese mice and improves skeletal muscle atrophy.

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