A Study on the Protective Function on Skeletal Muscle Mitochondria due to Aerobic Endurance Training

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Abstract — In this paper we study the function of PI3K-Akt signaling pathway which develops the skeletal muscle mitochondrial protective function in aerobic endurance training. We dividing rats into 3 groups: endurance training group, non-endurance training group and control group. Rats for endurance training group are required to receive a continuous 8-week (w) and 5d/w swimming training. After that, rats for both this group and the non-endurance training group all receive a continuous downhill running exhausting exercise, but, the control group has no treatment. After grouping interference, rat heads of all groups are immediately cut and middle parts of left hind limb quadriceps are rapidly taken out in order to test the level of mitochondrial membrane potential and the activity level of succinate dehydrogenase and cytochrome C oxidase by using the Western blot method to determine the phosphorylation level of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB or Akt) in tissues. Results compared with the control group, the level of mitochondrial membrane potential, the activity level of succinate dehydrogenase and cytochrome C oxidase and the level of phosphor-PI3K and phosphor-Akt all clearly decrease for rats in non-endurance training group; yet, the above indices for rats in endurance training group are remarkably higher than that of non-endurance training group. We conclude the aerobic endurance training is likely to develop a protective function to skeletal muscle mitochondria by activating the PI3K-Akt signaling pathway.

Keywords - Aerobic endurance training; skeletal muscle; mitochondria; PI3K-Akt signaling pathway

I. INTRODUCTION

There are abundant mitochondria in the skeletal muscle cell in order to meet energy need for skeletal muscle shrink. And the aerobic metabolism ability of skeletal muscle mitochondria has a direct impact on the quality of organism endurance. The aerobic endurance training, as a common exercise mode, can remarkably improve endurance quality and sports levels of athlete. The present study has confirmed that the developed regularly aerobic endurance training can promote aerobic endurance of organism by increasing factors such as aerobic metabolism ability of skeletal muscle, amount of effective gas exchange in lung tissue, ability of cardiac ejection and oxygen carrying ability of hemoglobin[1-5]. However, the concrete mechanism of aerobic endurance training to develop its protective function of mitochondria is still not clear, and there is no study to testify if the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB/Akt) signaling pathway participates in the mechanism. In order to explore the meaning of PI3K-Akt signaling pathway which develops a protective function of mitochondria in aerobic endurance training, this paper is intended to utilize an animal experiment to test the influence of aerobic endurance training on anomaly of skeletal muscle mitochondria function guided by an exhausting exercise and the activation condition of PI3K-Akt signaling pathway. By this experiment process, the protective function of signaling pathway to mitochondria can be illustrated.

II. MATERIALS AND METHODS

A. Materials

36 healthy and clean male SD rats (160–200 g in weight) at an age of 6 weeks and relevant feed selected and purchased by the experimental animal center of Xi'an Jiaotong University; BW-ZH-PT animal treadmill purchased from Shanghai Bio-will Co., Ltd.; anti-rat p-PI3K antibody and anti-rat p-Akt antibody purchased from Cell Signaling Technology, Inc. (U.S.A.); anti-rat β-actin antibody purchased from Santa Cruz Biotechnology, Inc.; total protein extraction kit of RIPA tissue purchased from Xianfeng Biotech Co., Ltd. (Xi'an); conventional reagents all belong to domestic purely analytical reagents.

B. Grouping and interference

After 1w of adaptive feeding, all rats were divided randomly into 3 groups: control group, endurance training group and non-endurance training group (n=12 for all of them). Thereinto, rats in endurance training group received a swimming training in a pool with 0.6m in length and 0.5m in depth. The water temperature was stabilized at around 35°C in the pool and the exercise time was 5 day a week. In addition, for each day, the exercise time increased week by week, namely, 1h of exercise time for the 1st week, 1.5h of that for the 2nd week and 2h of that for the 3rd till 8th week. For each week, there were 5 days of exercise time. Yet, rats in control group and non-endurance training group had no treatment. After 8 weeks of endurance training, rats in endurance training group and non-endurance training group were required to do an continuous downhill running exhausting exercise and the particular exercise method sees the method used by Luo Jiwei et al[6]. Three-level exercise loads were 0 × 8.2m/min × 15min (equaling to 53% VO2max), 5 × 15m/min × 15min (equal to 64% VO2max) and 10 × 19.3m/min (equal to 74% VO2max) respectively, which continued to exhaustion. The criterion for judgment of exhaustion is that the rat stagnates at
C. Sample collection and treatment

After grouping interference, rat heads of all groups were immediately cut and middle parts of left hindlimb quadriceps were rapidly taken out. Then the middle parts were washed by 4℃ phosphate buffer solution in order to extract total protein and mitochondria. The extraction method of total protein is described as follows: first, skeletal muscle tissues were cut into pieces on ice and then the RIPA lysate was added. Next, they were homogenized in a glass homogenizer on ice for about 15 minutes and then all homogenate was transferred into a centrifuge tube. In the tube, 10,000g of homogenate was centrifuged for 15 minutes. Finally, its supernate was collected, namely, the total protein, and then preserved in a refrigerator at -80℃ for reservation.

D. Extraction of mitochondria

The mitochondria of skeletal muscle tissue were extracted by animal tissue/cell activity mitochondria isolation kit (Shanghai Genmed Scientifics Inc.) and the concrete operation was carried out following instructions. Then, the mitochondria extract obtained by isolation was preserved in a refrigerator at -80℃ for reservation.

E. Determination of membrane potential

The fresh skeletal muscle tissue mitochondria obtained by isolation were resuspended in the 500μL of JC-1 working fluid (Beyotime Biotechnology Co., Ltd.) and then put into a water bath box at 37℃ to incubate for 20 min. 1,000g of them were centrifuged for 20 min and the supernate was discarded. Next, the phosphate buffer solution was used to fully wash them 2 times and then the 500μL of buffer solution was used to resuspend suspending mitochondria. After that, the level of mitochondrial membrane potential was tested by a fluorescence microplate reader. And then, two exciting lights with wavelengths of 585nm and 514nm respectively excited fluorescence in order to record red and green fluorescence intensities. Finally, the ratio of intensities exactly stood for the level of mitochondrial membrane potential.

F. Determination of enzymatic activity

The activity of succinate dehydrogenase and cytochrome C oxidase in extracts of skeletal muscle tissue mitochondria was determined by detection kit of succinate dehydrogenase activity of animal tissue mitochondria (Nanjing Jiancheng Biotechnology Co., Ltd.) and test kit of mitochondria cytochrome C oxidase activity (Shanghai Genmed Scientifics Inc.). The concrete operation was carried out following instructions. All experiments were all required to repeat 3 times and numbers of samples were all set as n=5.

G. Western blot

The BCA method was used to determine protein concentration, and total protein of skeletal muscle with equal mass had the polyacrylamide gel electrophoresis. After that, the protein was transferred to PVDF membrane by means of wet transfer method and later had closed treatment for 2h by 5% skim milk powder under room temperature. Next, the anti-p-PI3K antibody (1:500) and anti-p-Akt antibody (1:1000) or β-actin monoclonal antibody (1:4000) were respectively used to incubate protein overnight and then the phosphate buffer solution containing Tween-20 was used to wash protein. After adding the goat anti-rabbit antibody signed by biotin to incubate 2h at room temperature, the protein was fully washed again and developed by ELC method. Finally, integral absorbance values of all protein bands were analyzed in the Image-Pro Plus software and a mean obtained by 3 times of repeated detection stood for the expression level of target protein in this group with a sample number n=5.

H. Statistical analysis

The SPSS 18.0 statistical software is used for analysis and data are expressed as means ± standard deviations. In addition, the t-test is used for comparison between two groups and it is of statistical significance at the difference P<0.05 as a judgment criterion.

III. RESULTS

A. Test results of skeletal muscle mitochondria function for rats

Compared with the control group, the mitochondrial membrane potential of rats in non-endurance training group has a clear decrease trend after an exhausting exercise, while the mitochondrial membrane potential of rats in endurance training group is higher than that of non-endurance training group (P<0.05 for all, Fig. 1). In addition, compared with the control group, the activity level of succinate dehydrogenase and cytochrome C oxidase of rats in non-endurance training group reduces obviously after an exhausting exercise, yet above enzymatic activity in rats of endurance training group is higher than that of non-endurance training group (P<0.05 for all, Fig. 2). Therefore, the result hints that an exhausting exercise can cause a decrease of both mitochondrial membrane potential of skeletal muscle and enzymatic activity. However, the 8-w continuous endurance training can remarkably improve the rat resistance ability to an exhausting exercise which damages mitochondria.

Figure 1. Test results of mitochondrial membrane potential of skeletal muscle for all groups of rats.

MMP: mitochondrial membrane potential; ET: endurance training group; NET: non-endurance training; Control: control group; compared with the Control group, *P<0.05; and compared with the NET group, †P<0.05.
SDH: succinate dehydrogenase; COX: cytochrome C oxidase; ET: endurance training group; NET: non-endurance training; Control: control group; compared with the Control group, \(^*P<0.05\); and compared with the NET group, \(^#P<0.05\)

**Fig. 2** Test results of mitochondrial respiratory chain enzyme activity of skeletal muscle for all groups of rats

**Activation state of PI3K-Akt signaling pathway of skeletal muscle for rats**

Compared with the contrast group, levels of p-PI3K and p-Akt in skeletal muscle tissue go down clearly for rats in non-endurance exercise group. However, above protein levels in endurance training group are notably higher than that of non-endurance training group (\(P<0.05\) for all). Thus, it illustrates that an exhausting exercise can restrain activation of PI3K-Akt signaling pathway, yet the endurance training can remarkably improve the resistance function of rats to an exhausting exercise, but it restrains activation of PI2K-Akt signaling pathway.

**Fig. 3** Relevant protein expression levels of PI3K-Akt signaling pathway of skeletal muscle for all groups of rats

**IV. DISCUSSION**

The aerobic endurance training is capable of effectively improving skeletal muscle mitochondria function and overall metabolic activity of cells, yet the concrete mechanism therein is not clear[7-8]. And the PI3K-Ark signaling pathway possesses a biological function to promote cell multiplication and differentiation, restrain cell apoptosis and adjust substance metabolism within cells[9]. Moreover, it also widely participates in occurrence and develops processes of cancer, neurodegenerative diseases and infectious diseases[10-12]. Recently, when exploring the function of endoplasmic reticulum stress regulation of myocardial cell mitochondria, Zhang et al. found that while Thapsigargin induces endoplasmic reticulum stress of myocardial cell, it restrains indirect induction to damage mitochondria during the phosphor-Akt process. Nevertheless, Apocynin can eliminate reduction of mitochondrial membrane potential and transition pore opening of mitochondrial membrane permeability both induced by Thapsigargin by means of continuously activating the PI3K-Akt signaling pathway[13]. In addition, Ai Chunyu et al. also discovered the protective effect of mitochondrial structure and function by controlled low perfusion pressure which activates the PI3K-Akt signaling pathway and is against spinal ischemia/reperfusion in rabbits[14]. Besides, in a further study, it is also found that glycogen synthase kinase 3β, a downstream molecule of PI3K-Ark signaling pathway, has its activation which belongs to the main mechanism for this pathway to develop complete protecting mitochondria structure[15].

The experiment utilizes the rat swimming exercise to imitate aerobic endurance training and evaluates the influence of regular 8-w aerobic endurance trainingon organism’s resistance to an exhausting exercise which induces mitochondrial damage ability. From results, it can be found that the aerobic endurance training is able to remarkably reduce mitochondrial membrane potential of skeletal muscle and respiratory chain enzyme activity level both caused by an exhausting exercise. Rats in non-endurance training show obvious mitochondrial functional obstacles and PI3K-Akt signaling pathway activity restraint after an exhausting exercise. However, while the aerobic endurance training activates the PI3K-Akt signaling pathway, it partly reverses the skeletal muscle mitochondria damage function induced by an exhausting exercise. This fully indicates that activation of PI3K-Akt signaling pathway is possible to become a main mechanism for aerobic endurance training to protect the skeletal muscle mitochondria function. After phosphor-Akt for activation induced by PI3K, initiation of factor 4E-BP1 and promotion of increasing mitochondrial respiratory chain activity can be done via eucaryote cells translation[16]. Besides, the mitochondrial oxygen uptake capacity can be
raised by stimulating insulin’s mitochondrial fusion effect. Based on results of the experiment, the aerobic endurance training is likely to activate the PI3K-Akt signaling pathway and then initiate above two kinds of stated mechanism to develop the mitochondrial protective function.

V. CONCLUSIONS

In summary, the aerobic endurance training is possible to obviously improve inducement of the mitochondrial damage ability of organism against an exhausting exercise, and activation of PI3K-Akt signaling pathway may be one main mechanism for aerobic endurance training to protect the skeletal muscle mitochondria function. This provides a scientific evidence to clearly define mitochondrial protective mechanism of aerobic endurance training. Nevertheless, a further study, the research group’s next study direction, is still required to testify if Akt of mitochondrial part activation increases relevantly and what meanings of Akt are in the mitochondrial protective function aerobic endurance training.

REFERENCES