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Effects of high-intensity treadmill training on timeliness and plasticity expression of irisin in mice

J. LIU[1,3], X.Y. CUI[2], Y.Q. YANG[1], W. GAO[2], L. SUN[2], Y.C. DONG[2], X.J. KOU[1]

[1]College of Health Sciences, Wuhan Sports University, Wuhan, China
[2]Graduate School, Wuhan Sports University, Wuhan, China
[3]Key Laboratory of Exercise Training Monitoring, Wuhan, China

Abstract. – OBJECTIVE: The purpose of this study was to investigate effects of high-intensity aerobic exercise training on timeliness and plasticity expression of irisin in mice and change of FNDC5, ACCβ expression, and to explore possible ways to influence its mechanism of fatty acid metabolism.

MATERIALS AND METHODS: Adult male mice of specific pathogen-free grade [Kunming mice, (20 ± 2) g] are randomly divided into 4 groups. Wherein the first group is immediately after one-time exercise groups: including control group (CN group 1), 0.5 h exercise group (group 2), 1 h exercise group (group 3), 1.5 h exercise group (group 4) and 2 h exercise group (group 5), each for 10. The second group is rest after one-time 60 min exercise groups: including control group (CN group 1), rest 20 min groups (groups 2), rest 40 min group (group 3), rest 60 min group (groups 4), rest 80 min group (group 5), each for 10. Third group is immediately after long-term exercise groups: including the control group (CN group 1), 0.5 h exercise group (group 2), 1 h exercise group (group 3), 1.5 h exercise group (group 4) and 2 h exercise group (group 5), each for 10. The fourth group is rest after long-term 60 min exercise group: including control group (CN group 1), rest 20 min group (group 2), rest 40 min group (group 3), rest 60 min group (4 groups) and rest 80 min groups (5 groups), each for 10.

RESULTS: With the extension of a one-time high-intensity exercise time, the mouse FNDC5 protein, P-ACCβ / ACCβ ratio showed fluctuations, and opposite trends between the two, its turning points are 1.5 h; FNDC5 protein and P-ACCβ / ACCβ ratio with long-term exercise in mice at different time produce adaptability; the regulation of exercise induced irisin timeliness and plasticity reflected after a long-term exercise irisin expression in serum showed a steady decline in trend and return to normal levels, compared to a one-time exercise, expression of irisin is more stable.

CONCLUSIONS: With the high-intensity exercise a one-time extension of time, the mouse FNDC5 proteins, P-ACCβ / ACCβ ratio showed fluctuations, and both changes in the opposite trend, its turning points are 1.5 h; the long-term exercise can produce FNDC5 proteins, P-ACCβ / ACCβ ratios adaptable, more stable expression of the irisin curve after long-term exercise compared to a one-time exercise.

Key Words:
High-intensity exercise, Irisin, Acetyl-coenzyme A carboxylaseβ, Endocrine, Metabolism.

Introduction

Skeletal muscle is one of the most important organs of the body and plays an important role in the exercise to promote health. Skeletal muscle mass is closely related to the human body, sports ability and the prevention of chronic diseases. However, the mechanism of exercise to health promotion has been for people to explore. Irisin is a recently discovered 112-amino acid protein and an endocrine hormone[1-2]. First, exercise can induce expression of PGC-1α (peroxisome proliferator-activated receptor γ auxiliary activator 1α) in skeletal muscle, which stimulates FNDC5 gene in muscle tissues. FNDC5 then became embedded in the muscle cell membrane, and membrane proteins FNDC5 can be released into the bloodstream as a new hormone after proteolysis which is nominated irisin[3-5]. The present work demonstrated that the expression of irisin[6-8] has relationship with exercise intensity, exercise time and exercise forms. However, on the one hand, effects of high-intensity treadmill exercise on timeliness and plasticity of irisin in mice is unknown; on the other hand, possible pathways and mechanisms of this new factor on muscle fatty acid metabolism in muscle cells also has not been determined. Therefore, we need to study the expression of irisin timeliness and plasticity and its impact on metabolism pathways.

Corresponding Author: Jun Liu, MD; e-mail: liujun@wipe.edu.cn

Materials and Methods

Animals and Grouping

Adult male mice of specific pathogen-free grade [Kunming mice, (20 ± 2) g] were obtained from Hubei Experimental Animal Research Center [Certificate of quality No. SCXK-(E) 2008-0005]. Animals were housed 5/cages and maintained under controlled conditions of temperature (22-25)°C and light (6 AM on, 6 PM off) and fed ad libitum with standard rodent chow and tap water. All protocols were approved by the Institutional Animal Care and Use Committee of China Academy of Chinese Medical Sciences. After the mice were purchased back for one week of accommodation, then, they were randomly divided into four groups. The first group was immediately set after one-time exercise groups which include: control group (CN 1 group), 0.5 h exercise group (group 2), 1 h exercise group (group 3), 1.5 h exercise group (group 4) and 2 h exercise group (group 5), each for 10. The second group was rest for different time after one-time 60 min exercise groups which include: control group (CN 1 group), rest 20 min group (group 2), rest 40 min group (group 3), rest 60 min group (group 4), rest 80 min group (group 5), each for 10. The third group was immediately set after long-term exercise groups which include: control group (CN 1 group), 0.5 h exercise group (group 2), 1 h exercise group (group 3), 1.5 h exercise group (group 4) and 2 h exercise group (group 5), each for 10. The fourth group was rest for different time after long-term 60 min exercise groups which include: control group (CN 1 group), rest 20 min group (group 2), rest 40 min group (group 3), rest 60 min group (group 4), rest 80 min group (group 5), each for 10.

Intervention

The mice were all in the adaptability treadmill training for five days (speed 18 m / min, incline 0°). They were given exercise training for different times, treadmill times were 0 min, 30 min, 60 min, 90 min, 120 min as follows, incline are 0°, a one-time exercise intensity was of 18 m / min. Long-term exercise intensity exercise group of mice was 18 m / min, training once a day, every training session was 1 h, 5 days per week, a total of four weeks of training. Use a brush to stimulate the process of training to maintain the 1/3 runway for the movement of mouse treadmill. We need to check after each animal experiment if they were injured or not. Timely treatment was given to the injured. CN group did not undergo exercise training. After the end of the one-time exercise, they were sacrificed by cervical dislocation and orbital blood was collected and separation of the rectus femoris and myocardium were standby in ultra clean cabinet. Long-term exercise group after the last movement, mouse orbital blood and sacrificed by cervical dislocation and separation of the rectus femoris and myocardium standby in clean cabinet.

Determination of FNDC5 and ACCβ Protein Expression in Skeletal and Cardiac Muscle in Mice

Rectus femoris muscle and cardiac tissue protein extraction according to total protein extraction kit instructions; protein concentration was detected by BCA protein quantification method, and to develop a standard curve; all proteins were detected by western-blotting method (results shown in Figures 1 and 2).

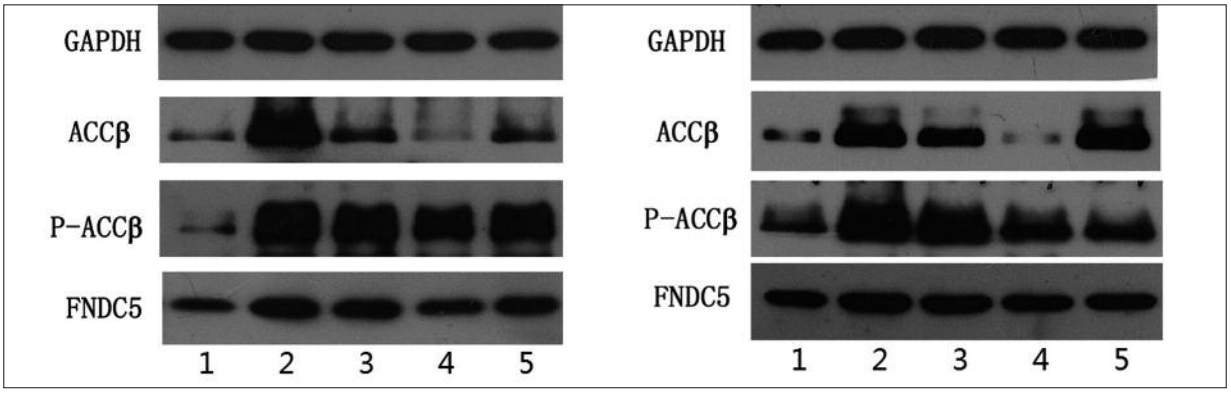

Figure 1. A one-time exercise change in skeletal muscle (*left*) and cardiac (*right*) related protein expression (1-5 :0 h, 0.5 h, 1 h, 1.5 h, 2 h after separation of the rectus femoris and myocardium which were collected to perform western blot analysis).

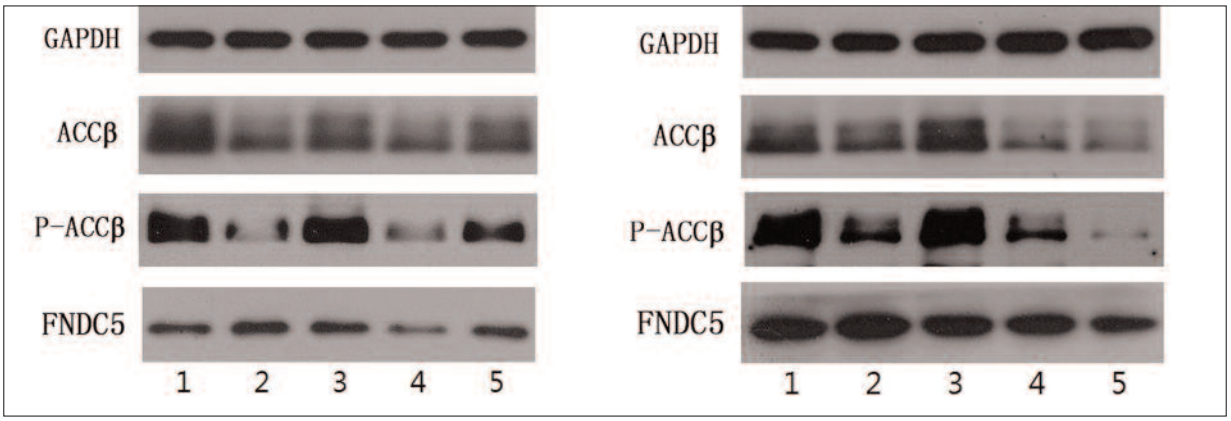

Figure 2. Long-term sports change in skeletal muscle (*left*) and cardiac (*right*) related protein expression (1-5: 0h, 0.5 h, 1 h, 1.5 h, 2 h after separation of the rectus femoris muscle, the myocardium which were collected to perform western blot analysis).

Elisa Curve of Irisin Expression

Rectus femoris muscle and cardiac tissue proteins extracted and detected according to ELISA kit instructions from AdipoGen (results shown in Figure 3).

Statistical Analysis

Data are presented as group means ± SEM. Two-way ANOVA tests were performed in all experiments using Origin 8.0 (OriginLab, Northampton, MA, USA). The Student Newman-

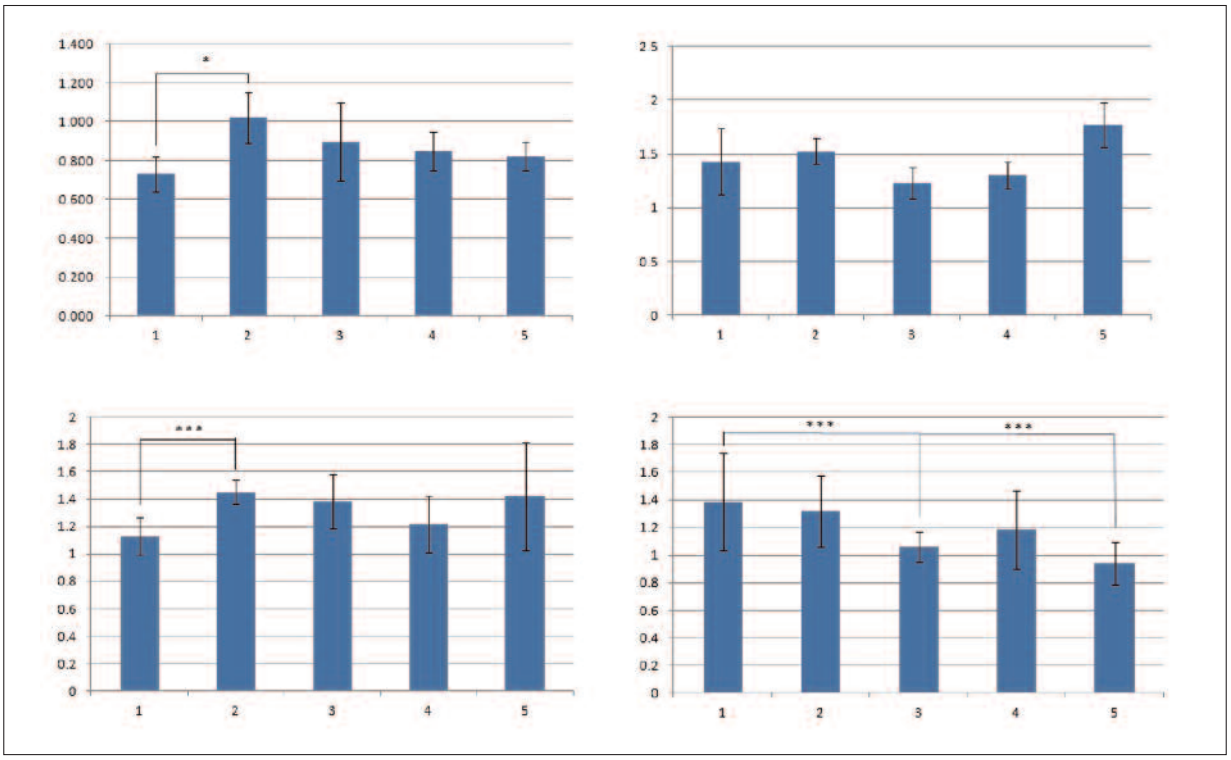

Figure 3. Hormonal regulation of timeliness and plasticity of exercise for irisin (Left and right image above 1-5 was a one-time exercise 0 h, 0.5 h, 1 h, 1.5 h, 2 h and one-time exercise for 1 h with disposable stop for 0 min, 20 min, 40 min, 60 min, 80 min after serum hormone irisin were collected to perform Elisa; left and right image below 1-5, respectively, was for the long term exercise 0 h, 0.5 h, 1 h, 1.5 h, 2 h and 1 h long campaign stop 0 min, 20 min, 40 min, 60 min, 80 min after serum hormone irisin were collected to perform Elisa, unit μg/ml). $p < 0.05$ = *, $p < 0.01$ = ***.

Keuls test was used for post-hoc analyses, or the Student's *t*-test was utilized when an experiment had only two groups to compare. $p < 0.05$ was considered significant.

Results

A One-Time Exercise Change in Skeletal Muscle and Cardiac Related Protein Expression

The results showed that although there was no significant difference in the results, but still there were certain trends. With the extension of a one-time high-intensity exercise time, the expression of the mouse rectus femoris and myocardium FNDC5 and P-ACCβ / ACCβ protein ratio showed a fluctuation, and the two trends change in the opposite, its turning points are both 1.5 h (results shown in Figure 1). Rectus femoris and myocardium FNDC5 protein expression in mice with a one-time exercise relative to CN 1 group have all increased. Rectus femoris and myocardium P-ACCβ / ACCβ protein ratio with a one-time exercise relative to CN 1 group only in 1.5 h has significantly increased. The expression of these proteins show that exercise can promote the expression of a general increase in the amount of mice FNDC5, but don not increase the amount of them obviously; at the same time, to promote a substantial increase in P-ACCβ / ACCβ protein ratio, and its turning points are 1.5 h.

Long-Term Sports Change in Skeletal Muscle and Cardiac Related Protein Expression

The results showed that although there was no significant difference in the results, but still there are certain trends. The expression of rectus femoris and myocardium FNDC5 protein and P-ACCβ / ACCβ ratio have adaptation to long-term exercise at different times in mice (results shown in Figure 2). The expression of myocardium FNDC5 protein and P-ACCβ / ACCβ ratio are stable in long-term exercise and the expression is no significant correlation with the long-term exercise.

Hormonal Regulation of Timeliness and Plasticity of Exercise for Irisin

The results show that exercise on irisin hormone regulation reflects the timeliness and plasticity after a rest after long-term exercise irisin hormone expression was steadily declining and return to normal levels. With respect to a rest after a one-time exercise, irisin hormone expression is more stable in long-term. The expression of serum irisin is more in immediately after different times of long-term exercise compared to that of immediately after different times of one-time exercise. The expression of serum irisin is less in different times of rest after long-term exercise compared to that of different times of rest after one-time exercise (results shown in Figure 3).

Discussion

Fatty acyl coenzyme A and carnitine with catalysis of carnitine-palmitoyl transferase 1 (CPT1) generates fatty acyl carnitine A, and malonyl CoA plays a key role in fatty acid oxidation by inhibiting CPT1[9]. Malonyl CoA is formed by acetyl CoA under the catalysis of acetyl coenzyme carboxylase ACC (this step is the limiting step of fatty acid synthesis). ACC beta is mainly distributed in the heart, muscle and liver which produced malonyl CoA that is mainly to adjust carnitine palmitic acid shuttling the body[10]. Fasting, exercise and hormone can activate adenosine monophosphate-dependent protein kinase AMPK which inhibits the enzyme through the phosphorylation of ACC and thus further inhibits fat synthesis and promotes fatty acid oxidation[7,9,11-15]. That is, the phosphorylation of ACC activation will eventually lift CPT1 inhibitory effect, thereby, increasing fatty acid oxidation. Irisin has no direct relevance with ACC beta in skeletal and cardiac muscle. Therefore, irisin are not able to influence muscular and myocardial fatty acid oxidation. It may mainly implemented beige brown in adipose tissue by influencing the ACC alpha in the fat cells[16], so as to achieve control of weight loss and metabolic diseases. Irisin expression in skeletal and myocardial muscle is more stable in long-term exercise. The expression of serum irisin is more in immediately after different times of long-term exercise compared to that of immediately after different times of one-time exercise. The expression of serum irisin is less in different times of rest after long-term exercise compared to that of different times of rest after one-time exercise. That is, immediately after the high-intensity endurance exercise, irisin can smoothly raise to above the level of the control group, and after a rest can reduce to below the level of the control group. The expression of irisin in mice

seems relative to a wider range after a high-intensity endurance exercise. This study finds that although skeletal and cardiac muscle FNDC5 has not the same level of expression, but their expression trends converge, which indicate exercise can simultaneously activate the expression of irisin in both skeletal and cardiac muscle. Existing studies have shown that serum irisin is in hippocampal neurons the target[17] and regulating the expression of irisin in other target cells in a positively controlled way by releasing irisin. Therefore, exercise may be the trigger point that triggered and adjust irisin expressed in other cells.

Conclusions

The expression of irisin in mice seems relative to a wider range after a high-intensity endurance exercise. Since the target of irisin are widely distributed in the myocardium, fat, liver, pancreas, bone, nerve, kidney, immune system, ovarian, pancreatic islet cells and other organs[18-20], and it has now been approved the effectiveness and relevance of irisin for metabolic diseases[21-26] and a lot of other diseases[15,27-29], it may provide some clues for the exercise to promote health in future research.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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