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Original Article

Effects of resistance/aerobic exercise on skeletal muscle AMPK/Sirt1 signaling pathway in rats with muscular attenuation syndrome

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Abstract: Objective: To study the effects of resistance/aerobic exercise on the signaling pathway of adenosine monophosphate-activated protein kinase (AMPK)/silencing information regulator 2 related enzyme 1 (Sirt1) in skeletal muscle in rats with muscular attenuating syndrome. Methods: Ten out of 50 healthy SD rats were randomly selected as normal group and the rest of the rats were used to establish the muscle attenuation syndrome model. The successfully modelled rats were randomly divided into the model group, aerobic exercise group, resistance training group, and combined group (aerobic exercise + resistance training), with 10 rats in each group. Rats in the aerobic exercise group received treadmill training and those in the resistance training group received loaded stair climbing exercise, and those in the combined group received all these exercises at the same time. They were trained for 6 weeks, while rats in the normal group and the model group received no intervention. The body weight and skeletal muscle mass index of rats in each group were measured, and the contents of reactive oxygen species (ROS), ryanodine receptor (RYR), nitric oxide synthetase (NOS), Sirt1, adenosine monophosphate (AMP), adenine nucleoside triphosphate (ATP) and AMPK/Sirt1 signaling pathway related proteins expression levels in skeletal muscle of rats were detected. Results: The body weight, muscle mass index, and the levels of ROS, RYR and NOS, Sirt1 activity, ATP and AMP contents, and AMPK and Sirt1 protein expression levels in rat skeletal muscle of the aerobic exercise group, resistance training group and combined group were higher than those in model group respectively ($P<0.05$). The combination intervention had the best improvement effects on the above indexes and the aerobic exercise was more effective than resistance training (all $P<0.05$). Conclusion: Aerobic exercise can more significantly improve the body weight and improve the muscle mass index, enzyme activity, the expression level of AMPK/Sirt1 signaling pathway and energy metabolism in skeletal muscle of rats with muscle attenuation syndrome than resistance training. The combination of aerobic exercise and resistance training has synergistic effects on promoting energy metabolism of rats with muscle attenuation syndrome.

Keywords: Resistance training, aerobic exercise, muscle attenuation syndrome, adenylate activated protein kinase, silencing information regulator 2 related enzyme 1

Introduction

Muscle attenuation syndrome is a kind of chronic disease presenting as involuntary loss of muscle with age. Its clinical features include decreased skeletal muscle volume and strength, increased fat and connective tissue, etc., which may lead to body function degeneration or different degrees of disability [1]. At present, the pathogenesis of muscle attenuation syndrome is still unclear, but researchers regard that the occurrence of muscle attenuation syndrome is related to oxidative stress, mitochondrial dysfunction, quality control of skeletal muscle, disuse of skeletal muscle, apoptosis and so on [2, 3].

Adenosine monophosphate-activated protein kinase (AMPK) and silencing information regulator 2 related enzyme 1 (Sirt1) are important

energy metabolism effectors in the body. AMPK is widely expressed in eukaryotic organisms and belongs to the serine/threonine protein kinases, which can sense cell energy metabolism changes and maintain the balance of cell energy supply and demand by affecting cell metabolism. Sirt1 is a conserved nicotinyl adenine nucleotide dependent histone/non-histone acetylase. It is a key factor in epigenetic regulation and plays an important role in energy metabolism, mitochondrial protective function, inflammatory response, lipid and glucose homeostasis, cell cycle regulation, gene transcription, reducing oxidative stress and prolonging cell life [4, 5].

Studies have shown that exercise promotes skeletal muscle type conversion involving multiple signaling pathways, including AMPK, Sirt1, MAPKs, CaMK, and PKCs, etc. [6-8]. It has been found in clinical studies that the body can activate the activity of AMPK and Sirt1 under the stimulation of energy restriction, exercise and so on, which affects the energy metabolism of the whole body, and long-term aerobic exercise intervention can increase the secretion of ROS, enhance the oxidative stress effects, and has an impact on the energy metabolism of the body [9, 10]. Clinical research has found that, after resistance exercise intervention, the amount of AMPK and Sirt1 increased to a certain extent, and under the long-term resistance exercise intervention, the activity of AMPK and Sirt1 increased. However, compared with those that received aerobic exercise, the activity was lower [11].

Based on the above research, this study analyzed the effect of resistance training and aerobic exercise on AMPK/Sirt1 signaling pathway activity of skeletal muscle in rats with muscular attenuation syndrome, and explored the relationship of exercise, AMPK/Sirt1 signaling pathway and muscular attenuation syndrome, aiming to provide a new way for the prevention and treatment of muscular attenuation syndrome.

Materials and methods

Materials

Animals: 50 healthy SPF male SD rats provided by Zhongmei Guanke Biotechnology (Beijing) Co., Ltd. (animal approval No.: SYXK (Beijing 2019-0042)) were selected. The rats were 8 to 15 weeks' old, with an average age of (11.5±1.3) weeks. The weight was 210 to 260 g with an average weight of (235.6±10.2) g. All rats were fed in specific-pathogen-free cage for a week. The room temperature was kept at (22.1±1.8) C, with a relative humidity of 35%-40%. The rats received light for 12 hours a day and drank purified water. The experiment was approved by the Ethics Committee of Physical Education College of Jinggangshan University.

Main reagents: Dexamethasone sodium phosphate injection was purchased from Sinopharm Rongsheng Pharmaceutical Co., Ltd., China; chloral hydrate was purchased from Shaanxi Shengrui Pharmaceutical Technology Co., Ltd., China); RYR (ryanodine receptor), AMP (adenosine monophosphate), ATP (adenine nucleoside triphosphate) test kits were purchased from Shanghai Jianglai Biotechnology Co., Ltd., China; ROS (reactive oxygen species), NOS (nitric oxide synthetase) were purchased from Nanjing Jiancheng Bioengineering Research Institute, China; Sirtl test kit was purchased from Aimejie Technology Co., Ltd., China; anti-rat AMPK antibody and anti-rat Sirt1 antibody were purchased from Beijing Yiqiao Shenzhou Technology Co., Ltd., China; anti-rat GAPDH antibody was purchased from Shanghai BBI Life Sciences Co., Ltd., China; the secondary antibodies were purchased from Promega Co., Ltd, USA.

Methods

Modeling and grouping: Ten of the 50 rats were randomly selected as the normal group, and the rest were injected subcutaneously with 5 mg/kg of dexamethasone sodium phosphate each day, for 6 weeks in accordance with the protocol of muscle attenuation syndrome model establishment published by Chen Wei et al. [12]. One day after the last injection, all rats received the treadmill test and swimming test, and the muscle content was measured by the small animal body composition analyzer. If there were differences between the model rats and the normal rats in the frequency of falls, swimming speed and muscle content, the model was established successfully.

The model rats were randomly divided into model group, aerobic exercise group, resistance training group and combined group (aerobic exercise + resistance training), with 10 rats in each group.

Table 1. Body weight and skeletal muscle mass index of rats in each group ($\bar{X}$ ± sd)

Group (n=10)	Weight (g)	Skeletal muscle mass index
Normal group	358.26±68.59	1.64±0.15
Model group	300.25±42.01[a]	1.24±0.10[a]
Resistance training group	321.25±60.12[a,b]	1.36±0.07[a,b]
Aerobic exercise group	342.26±59.58[a,b,d]	1.43±0.08[a,b,d]
Combined group	353.25±54.32[a,b,c,d]	1.56±0.06[a,b,c,d]

Note: Compared with the normal group, [a]P<0.05; compared with the model group, [b]P<0.05; compared with the aerobic exercise group, [c]P<0.05; compared with the resistance training group, [d]P<0.05.

Intervention measures: Rats in the aerobic exercise group received treadmill training. After the adaptive training for three days, the rats received treadmill training at the speed of 9 m/min for 0.5 h, three times a week. The treadmill gradient was 0.

Rats in the resistance training group received loaded climbing exercise according to the method reported by Yu et al. [13]. After the adaptive training for three days, the rats received loaded climbing training with increasing load. The length of the climbing ladder was 1m, which was placed at an angle of 85° with the ground. The training was carried out three times a week. Three groups of rats received training each time, and each group of rats carried out training three times. The interval between each exercise was 0.5-1 min, and the interval between each group was 2-3 min. The rats in the combined group were given both treadmill training and loaded climbing exercise, and both kinds of exercise were carried out alternately. All the rats in the three groups were trained daily for 6 weeks and rested on Sunday. The normal group and the model group received no intervention.

Determination of body weight and skeletal muscle mass index: The rats were weighed the next day after the last training, and then anesthetized with 10% chloral hydrate (3 mL/kg). After the aorta blood was collected, the whole quadriceps femoris was removed immediately, washed with phosphate buffer solution, and then dried with filter paper. The skeletal muscle mass was measured and the skeletal muscle tissue was stored in liquid nitrogen. Skeletal muscle mass index = anterior tibial muscle weight/tibial length.

Enzyme-linked immunosorbent assay (ELISA): The activities of ROS, RYR, NOS, Sirt1, and the content of AMP and ATP in skeletal muscle of rats were detected by enzyme-linked immunosorbent assay (ELISA) according to the instruction of the kits.

Western blot

Western blot was used to measure the expression of AMPK/Sirt1 signaling pathway related proteins in rat skeletal muscle. Proteins in the collected samples were extracted and quantified by the BCA method. After 50 μg of protein sample was loaded, SDS-PAGE electrophoresis was carried out. Then protein was transferred to PVDF membrane. TBST containing 5% skim milk powder was used to block, in the dark for 1 h. After washing with TBST, the membrane was incubated with anti-rat primary antibodies, AMPK (1:500), Sirt1 (1:500) and GAPDH (1:1,000, internal reference) overnight at 4C. After washing with TBST again, the membrane was incubated with the second antibody (1:1,000) in at 37C for 1 h. The membrane was developed by ECL kit on Tanon 5200 Multi Automatic Chemiluminescence/fluorescence Image Subsystem (Shanghai Tianneng Technology Co., Ltd.). The grey areas were analyzed with ImageJ software.

Statistical analysis

SPSS 20.0 statistical software was used for analysis. The data were presented as mean ± standard deviation ($\bar{X}$ ± SD), and the comparison among multiple groups was performed by one-way ANOVA followed by post Bonferroni test. P<0.05 was considered statistically significant.

Results

The aerobic exercise and resistance training group could increase the body weight and skeletal muscle mass index of rats with muscle attenuation syndrome

As shown in **Table 1**, compared with the normal group, the body weight and skeletal muscle mass index of rats in the other groups were significantly lower (P<0.05). Compared with the model group, rats in the three treatment groups had significantly higher body weight and skeletal muscle mass index (P<0.05). The combined group showed highest levels of the body weight

Table 2. ROS, RYR, NOS and Sirt1 activity in skeletal muscle of rats in each group ($\bar{X}$ ± sd)

Group (n=10)	ROS (μ/g)	RYR (ρg/g)	NOS (U/g protein)	Sirt1 activity (U/g protein)
Normal group	845.25±98.35	1998.36±963.68	0.25±0.05	0.25±0.02
Model group	345.28±54.14[a]	1526.34±569.68[a]	0.06±0.01[a]	0.05±0.01[a]
Resistance training group	598.28±72.49[a,b]	1686.36±598.67[a,b]	0.10±0.03[a,b]	0.10±0.02[a,b]
Aerobic exercise group	649.69±70.48[a,b,d]	1759.35±856.34[a,b,d]	0.16±0.04[a,b,d]	0.13±0.01[a,b,d]
Combined group	816.25±85.46[a,b,c,d]	1896.36±987.68[a,b,c,d]	0.20±0.03[a,b,c,d]	0.20±0.03[a,b,c,d]

Note: Compared with the normal group, [a]P<0.05; compared with the model group, [b]P<0.05; compared with the aerobic exercise group, [c]P<0.05; compared with the resistance training group, [d]P<0.05. ROS: reactive oxygen species; RYR: ryanodine receptor; NOS: nitric oxide synthetase; Sirt1: silencing information regulator 2 related enzyme 1.

Table 3. Skeletal muscle energy in each group [($\bar{X}$ ± sd), nmol/L]

Group (n=10)	ATP	AMP
Normal group	3.56±0.19	14.28±3.25
Model group	2.15±0.32[a]	8.32±1.08[a]
Resistance training group	2.65±0.86[a,b]	10.96±1.30[a,b]
Aerobic exercise group	3.05±0.69[a,b,d]	12.25±2.00[a,b,d]
Combined group	3.42±0.21[a,b,c,d]	13.64±3.01[a,b,c,d]

Note: Compared with the normal group, [a]P<0.05; compared with the model group, [b]P<0.05; compared with the aerobic exercise group, [c]P<0.05; compared with the resistance training group, [d]P<0.05. ATP: adenine nucleoside triphosphate; AMP: adenosine monophosphate.

and skeletal muscle mass index, while aerobic exercise group took second place and resistance training group had the weakest effect (P<0.05).

The aerobic exercise and resistance training group had increased ROS, RYR and NOS levels and Sirtl activity in skeletal muscle of rats with muscle attenuation syndrome

As shown in **Table 2**, compared with the normal group, the levels of ROS, RYR, NOS and Sirt1 activity in skeletal muscle of rats in other groups were significantly lower (P<0.05). Compared with the model group, rats in the three treatment groups had significantly higher levels of ROS, RYR, NOS and Sirt1 activity in skeletal muscle (P<0.05). The combined group showed highest levels of ROS, RYR, NOS and Sirt1 activity in skeletal muscle of rats, while the aerobic exercise group took second place and resistance training group had the weakest effect (P<0.05).

The aerobic exercise and resistance training group had increased skeletal muscle energy in rats with muscle attenuation syndrome

As shown in **Table 3**, compared with the normal group, the content of ATP and AMP in the skeletal muscle of rats in other groups were significantly lower (P<0.05). Compared with the model group, rats in the three treatment groups had significantly higher content of ATP and AMP in their skeletal muscle (P<0.05). The combined group showed the highest content of ATP and AMP in skeletal muscle of rats, while the aerobic exercise group took second place and the resistance training group had the weakest effect (P<0.05).

The aerobic exercise and resistance training group had increased expression of AMPK/Sirt1 signal pathway related proteins in skeletal muscle of rats with muscle attenuation syndrome

As shown in **Figures 1** and **2**, compared with the normal group, the expression of AMPK and Sirt1 protein in skeletal muscle of rats in the other groups were significantly lower (P<0.05). Compared with the model group, rats in the three treatment groups had significantly higher expression of AMPK and Sirt1 proteins in skeletal muscle (P<0.05). The combined group showed highest the expression of AMPK and Sirt1 proteins in skeletal muscle of rats, while the aerobic exercise group took second place and the resistance training group had the weakest effect (P<0.05).

Discussion

Muscle attenuation syndrome is a geriatric syndrome with the loss of skeletal muscle mass and strength due to aging. The occurrence of this disease is related to the attenuation of growth hormone signal systems, the hyperactivity of systemic inflammation, the increase of oxidative damage, the disorder of mitochondrial function and the decrease of muscle specific protein synthesis [14]. Previous studies reported that reasonable exercise and diet could

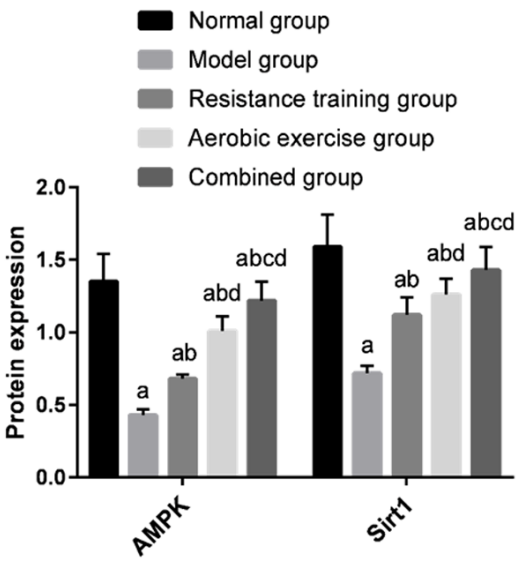

Figure 1. AMPK/Sirt1 signal pathway related protein expressions in skeletal muscle of rats in each group ($\bar{X}$ ± sd). Compared with the normal group, [a]P<0.05; compared with the model group, [b]P<0.05; compared with the aerobic exercise group, [c]P<0.05; compared with the resistance training group, [d]P<0.05. AMPK: activities of adenosine monophosphate-activated protein kinase; Sirt1: silencing information regulator 2 related enzyme 1.

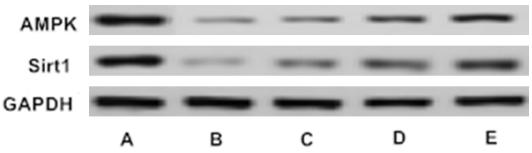

Figure 2. Representative bands of AMPK/Sirt1 signaling pathway related proteins. A: the normal group; B: the model group; C: the resistance training group; D: the aerobic exercise group; E: the combined group. AMPK: activities of adenosine monophosphate-activated protein kinase; Sirt1: silencing information regulator 2 related enzyme 1; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

effectively prevent and treat muscle attenuation syndrome [15, 16]. During exercise, the skeletal muscle tissue will release polypeptide and cytokines and enhance the contraction ability and exercise ability of skeletal muscle, so as to improve the health level of the body. Exercise plays an important role in stimulating the release and transfer of energy.

AMPK and Sirt1 are important energy metabolism receptors in the body, which can sense the state of energy metabolism in the body and regulate the process of energy metabolism by changing the gene expression or activity of downstream molecules [17]. AMPK/Sirt1 signaling pathway regulates the quality and function of skeletal muscle by affecting autophagy, apoptosis, proliferation, differentiation, protein synthesis and degradation of skeletal muscle, as well as inflammatory response, which may be closely related to the occurrence, development and outcome of muscle attenuation syndrome in the process of aging [18, 19]. Based on the above research, the model of muscle attenuation syndrome was established in this research, and the AMPK/Sitr1 signaling pathway components were analyzed to clarify whether it was related to the exercise of muscle attenuation syndrome with resistance/aerobic exercise interventions.

The results of this study showed that the activity of AMPK and Sirt1 were increased after exercise intervention, but the activity degree in the aerobic exercise group was higher than that of resistance exercise group, which may be related to the effective enhancement of oxidative stress by aerobic exercise. During exercise, ROS secretion was increased in skeletal muscle, which promotes the oxidative stress reaction [20, 21]. Moreover, AMPK/Sirt1 signal pathway was activated, which could regulate the energy metabolism process of the body, inhibit the oxidative damage of the body, increase the synthesis of muscle-specific proteins, and inhibit the disuse of skeletal muscle and cell apoptosis. Our study also found that compared with the rats treated by aerobic exercise or resistance training alone, the rats received combined training had best results on all observed indicators, indicating that the effect of combination of resistance training and aerobic exercise was better than that of single exercise. It could better increase the content of ATP and AMP, enhance the effect of oxidative stress, increase the content of ROS, activate the AMPK/Sirt1 signaling pathway, and finally greatly regulate the energy metabolism in skeletal muscle.

However, we only studied the role of resistance/aerobic exercise and AMPK/Sirt1 signaling pathways in muscle attenuation syndrome, which may also affect other pathways. The mechanism needs further study. In addition, the research sample size was small, and the study interval was short. Therefore, our results

need to be further confirmed by subsequent experiments.

In conclusion, resistance training and aerobic exercise can improve the body weight and skeletal muscle quality of rats with muscle attenuation syndrome, improve the enzyme activity in skeletal muscle, regulate AMPK/Sirt1 signaling pathways related proteins, and promote energy metabolism. Therefore, combination therapy has better effects.

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Disclosure of conflict of interest

None.

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