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Mild aerobic training with blood flow restriction increases the hypertrophy index and MuSK in both slow and fast muscles of old rats: Role of PGC-1α

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A R T I C L E I N F O

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A B S T R A C T

Aims: Existing evidence emphasize the role of mitochondrial dysfunction in sarcopenia which is revealed as loss of skeletal muscle mass and neuromuscular junction remodeling. We assessed the effect of low-intensity aerobic training along with blood flow restriction on muscle hypertrophy index, muscle-specific kinase (MuSK), a pivotal protein of the neuromuscular junction and Peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) in aged male rats.

Main methods: Animals groups were control (CTL), sham (Sh), leg blood flow restriction (BFR), exercise (Ex), sham + exercise (Sh + Ex), and BFR plus exercise (BFR + Ex) groups. The exercise groups were trained with low intensity exercise for 10 weeks. 48 h after the last training session, animals were sacrificed under anesthesia. Soleus and EDL muscles were isolated, hypertrophy index was estimated and MuSK and PGC-1α were measured by western blot method.

Key findings: Hypertrophy index enhanced in soleus and Extensor digitorum longus (EDL) muscles of BFR + Ex group ($P < 0.01$ versus CTL and Sh groups, and $P < 0.001$ versus other groups). The MuSK protein of soleus and EDL muscles increased in BFR + Ex group ($P < 0.01$ and $P < 0.001$, respectively) in comparison with CTL and Sh groups. In BFR + Ex group, the PGC-1α protein increased in both soleus and EDL ($P < 0.001$ compared to other groups). Also the PGC-1α of soleus muscle was higher in Ex and Sh + Ex groups versus CTL and Sh groups ($P < 0.05$).

Significance: Findings suggest that low endurance exercise plus BFR improves the MuSK and hypertrophy index of both slow and fast muscles of elderly rats probably through the rise of PGC-1α expression.

1. Introduction

One of the outcomes of aging is sarcopenia, a loss of skeletal muscle mass [1] which associated with limitation in basic activities of daily living independency including working, walking, dressing, using the toilet and so forth in older adult. While it seems that sarcopenia is a multi-factorial phenomenon, several evidence emphasizes the role of mitochondrial dysfunction in its creation [2,3]. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a key protein with a role in metabolism, controlling the mitochondrial function and regulating mitochondrial biogenesis [4] and also recruits factors involved in skeletal muscle hypertrophy [5]. Previous studies revealed that PGC-1α is a key protein involved in protection and integrity of neuromuscular junction (NMJ) [6,7], deceleration of the protein degradation [8], up-regulation of the mitochondrial biogenesis [4], regulation of the autophagy and prevention of the muscle wasting [9]. The level of PGC-1α protein in skeletal muscles decreases during of aging process [10]. Accordingly, it raised this hypothesis that instability of the NMJ and consequently aging-related alterations of fiber innervations in older adult may come from the reduction of PGC-1α level [11].

Muscle-specific kinase (MuSK) is a receptor tyrosine kinase which

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requires for the formation and maintenance of the NMJ. When MuSK is activated by agrin, its signals via the proteins called Casein kinase 2 (CK2), Dok-7 and Rapsyn, induces the "clustering" of acetylcholine receptors (AChRs) in endplate of NMJ [12–14]. It has been shown that PGC-1α can activate MuSK and leads to the stabilization of NMJ [11].

On the other hand, endurance exercise can activate the PGC-1α gene in skeletal muscle and PGC-1α can regulate the mitochondrial biogenesis and muscle fiber type determination [15,16] and also can coordinates exercise-induced muscle hypertrophy [17]. In addition, the effectiveness of endurance exercise is related to its intensity so that low intensity exercise is effective on slow fibers and high intensity exercise is effective on fast fibers [18,19].

With regard to motor weakness and limitation of the cardiovascular system of elderly people, it is essential to design new models of exercise that both have the same effects as intense traditional exercises and having executable functionality in older people with minimal risk of damage. An innovative new model is the Kaatsu's exercise training as using combination of blood flow restriction (BFR) and low-load resistance exercise [20,21]. In most studies the Kaatsu model has been used in order to increase the strength and size of muscles [22–25]. However, less has been paid to the combination of BFR with low intensity aerobic exercise as an appropriate training on molecular levels of NMJ compartments in elderly [22–25]. Therefore, the aim of present study firstly was to determine the effect of BFR and a ten-week low intensity aerobic exercise on the concentration of myofibril proteins, and secondly to identify their combination effect on the expression of MuSK and PGC-1α in both soleus and extensor digitorum longus (EDL) muscles of old rats. We hypothesized that BFR plus low intensity aerobic exercise may increase the expression of PGC-1α which in turn likely improves NMJ structure and its mandatory proteins such as MuSK.

2. Materials and methods

2.1. Animals

48 male Sprague Dawley aged rats (23–24 months) weighing between 400 and 480 g were used. They were kept at temperature between 21 and 24 °C and the cycle of 12 h of darkness/brightness. The exercise was conducted during the light cycle, and they had free access to food and water. All experimental protocols were based on laboratory animal guideline of Ethics Committee of Kerman University of Medical Sciences (Ethical code: 9414).

2.2. Groups details and testing conditions

Animals randomly put into 6 groups: control (CTL), sham (Sh), blood flow restriction (BFR), exercise (Ex), sham + exercise (Sh + Ex) and blood flow restriction + exercise (BFR + Ex). CTL group received regular care under normal condition without any surgery or exercise training. In Sh group, just skin surgery and operation without blood flow restriction was done. In BFR group, animals subjected to operation and hind blood flow restriction. Ex group was trained with low-intensity treadmill exercise for ten weeks. Sh + Ex group, which operated without blood flow restriction and then trained with ten weeks low-intensity treadmill exercise. BFR + Ex group, subjected to blood flow restriction along with ten weeks low-intensity treadmill exercise [26].

2.3. Surgical and blood flow restriction technique

All animals were weighed 72 h before the onset of exercise training. In order to access the femoral artery, after anesthesia with ketamine (100 mg/kg) and xylazin (10 mg/kg) and cutting the skin of the thigh, the femoral artery was freed from surrounding tissues. Then a little penicillin was poured on the wound and the skin was sutured in Sh and Sh + Ex groups. However, as per BFR and BFR + Ex, a steel wire with diameter 0.014 in. was put on the femoral artery and was tied tightly with silk suture (4–0) at the level of bottom of inguinal ligaments. Then, the wire was carefully pulled out to prevent vessels from any harm. In the last step, penicillin was poured on wounds and the skin was sutured. This situation does not limit muscle blood flow at rest position, but in case of muscle contraction and exercise on a treadmill, the muscle is faced with blood flow restriction [26–28].

2.4. Exercise protocol

Animals in exercise groups underwent exercise for 10 weeks and 5 days per week. There were three stages of exercise including learning, overload and maintaining/stabilizing load intensity. In learning phase (first week), the animals walked at a speed of 7.5 m per minute every day for 15 min on a treadmill designed for rodents. The treadmill speed and duration of exercise sessions gradually increased. At the last week, treadmill speed was 15 m per minute and its duration was 60 min. In all of the above steps, incline was zero degrees. The first 5 min of the exercise was used to warm up, and the last 5 min was employed to cool down with the speed of 7 m per minute. In addition to be familiar with the treadmill during the 10 weeks, the animals of control and sham groups without exercise were put at least twice a week on the device [26,29].

2.5. Sampling

At the end of 10 weeks, 48 h after the last exercise session, all animals in all groups were weighed and then killed under deep anesthesia. Immediately after the killing, soleus and EDL muscles of the hind limbs were isolated, and after cleaning them of their connective tissues and fat, the muscles were weighed by digital scales and then frozen with liquid nitrogen. Then the samples were kept at a temperature of −80° until MuSK and PGC-1α were measured. The reason for choosing these muscles was that soleus has a lot of slow-twitch motor units, and EDL muscle has more fast-twitch motor units and less often used in ordinary gestures [30,31].

2.6. Western blot protocol

To measure the amount of MuSK and PGC-1α of muscle samples, Western blot method was used. First, each muscle was crushed by liquid nitrogen and then homogenized in lysis buffer (Ripa buffer) by cold protease inhibitors. To homogenize the texture, lysis buffer was casted four to five times more than the weight of the samples. The homogenization was done on ice by Tommy homogenizer (Micro Smash model) at 3000 rpm for three two-minute times with an interval of 5 min. The homogenized tissue was centrifuged for 10 min, at 4 °C and at around 3600 rpm. After that, the supernatant was isolated and stored at −20 °C. Bradford method was used to determine the protein concentration [32]. The protein samples were mixed at a ratio of 1 to 0.5 with sample buffer and boiled for 5 min at 100 °C. Then, the amount of 10 μl of each sample was casted into the gel wells SDS PAGE (10%). A Voltage of 100 was used to load protein electrophoresis solutions for 90 min. Then the proteins were transferred to PVDF paper through the machine for 70 min. The PVDF paper was blocked by blocking solution containing nonfat milk in Tris-buffered saline with Tween 20 (TBS-T) (blocking buffer, TBS-T, 150 mM NaCl, 20 mM Tris-HCl [pH 7.5], 0.1% Tween 20) for 2 h. Then, it was incubated with the primary antibody (PGC-1α Sc-13,067 and MuSK SC-6010 Santa Cruz Biotechnology, USA, 1: 1000) on a nightly basis. Thereafter, the PVDF paper was washed three times (each time 10 min) by TBST solution. Then, the PVDF paper was incubated after 2 h with secondary antibody (goat anti-rabbit IgG-HRP, Sc-2004 and MuSK: rabbit anti-goat IgG-HRP: SC-2768, Santa Cruz Biotechnology, 1: 10,000), and finally, after three times of being washed by TBST solution, the paper was covered by Keith ECL (5 min) and the film was processed. Following that, the paper was rinsed in

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