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Acute Interval Walking with and without Vascular Occlusion has a Different Effect on 4EBP1 Phosphorylation and Stimulation of the mTOR Signaling Pathway in the Skeletal Muscle of Inactive Men

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Abstract

Background: Low-intensity aerobic exercise can cause muscle hypertrophy if accompanied with vascular occlusion.

Aims: The purpose of the study was to compare of effect of acute interval walking with and without vascular occlusion on phosphorylation of 4EBP1 and stimulate the mTOR signaling pathway of skeletal muscle in inactive men.

Materials and Methods: Five healthy inactive men participated in this study on 2 separate days 2 weeks apart. The first session of the research protocol consisted of acute interval walking with vascular occlusion and the second session consisted of acute interval walking without vascular occlusion. Session one was including vascular occlusion by 5 intervals 2-min walking at 60% Maximum heart rate (MHR) and 1 min at rest. The second session was similar to the first but without vascular occlusion. All samples were collected 30 minutes before the start and 3 hours after training. Concentration of 4EBP1 skeletal muscle were evaluated by Western blotting. Dependent t-test and Independent t-test was used to analyze the data after subtracting the post-test score from the pre-test.

Results: There was a significant difference between pre and post-test of 4EBP1 in the group with vascular occlusion and the mTOR signaling pathway was stimulated ($P=0.001$). There was no significant difference in the second group ($P=0.064$). Also, significant differences were reported between the two training methods for 4EBP1 phosphorylation and stimulation of the mTOR signaling pathway ($P = 0.01$), ($P \geq 0.05$).

Conclusion: If acute interval walking is associated with vascular occlusion, it can increase 4EBP1 phosphorylation and stimulate the mTOR signaling pathway, leading to muscle hypertrophy.

Keywords: Hypertrophy; Vascular Occlusion; Interval Walking

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Introduction

Resistance training is a powerful stimulant to increase muscle protein synthesis and consequently increase muscle size which leads to increased maximal strength and muscle hypertrophy [1]. The American School of Sports Medicine has recommended to achieve muscular hypertrophy during resistance training, the intensity of training should be at least 65% Maximum one maximum repetition and any intensity less than this rarely causes hypertrophy and gaining strength [2]. But it has recently been shown that Muscular hypertrophy also occurs during low-intensity resistance training (with 20% of one maximum repetition) along with moderate vascular occlusion [1].

Vascular occlusion exercises or Kaatsu are relatively new exercises that are performed in conditions of restricting blood flow to the muscle. Initially, this exercise was performed in combination with resistance exercises with the aim of increasing hypertrophy and muscle strength. In this regard, several studies have investigated the effect of resistance training with vascular occlusion on skeletal muscle hypertrophy, increased strength as well as neurological, endocrine and cardiovascular responses. It was further found that Kaatsu training is not specific to resistance training. Several studies have been performed on the effect of aerobic exercise with vascular occlusion on aerobic capacity, strength and skeletal muscle volume. It has been shown that low-intensity exercise such as walking when associated with vascular occlusion can significantly improve thigh muscle cross-section and knee joint strength in young and old subjects [3]. Amani in a study of 28 young football players showed that Periodic exercise with vascular occlusion improves aerobic capacity and prevents a decrease in VO2max during the transition period [4].

In this regard, Naserkhani F, et al. (2015) [5], examined the effect of a training session on a treadmill with vascular occlusion on serum levels of growth hormones (GH), insulin-like growth factor-1 (Igf1) and cortisol in inactive female students. Their results showed that a treadmill exercise session with vascular occlusion could further increase catabolic-anabolic hormones in inactive young girls [5]. Bahreinipour MA, et al. (2016) [6], also showed that low-intensity aerobic exercise with vascular occlusion can have a positive effect on the structure of nerve and muscle junctions and reduce the effects of aging in rats [6].

The mechanism of Kaatsu exercises is not specified, but there is a possible mechanism for low-intensity resistance training associated with vascular occlusion includes increased growth hormone secretion due to the accumulation of intramuscular metabolites such as lactate and hydrogen ions in active muscle, local growth factors and intracellular signaling pathways, and more rapid contraction fibers in hypoxic conditions [7]. Evidence suggests that exercise with vascular occlusion, despite low-intensity physical activity (10 to 30% of maximum work capacity), creates a positive training fit and can be a unique method in medicine and sports. In this regard, Abe et al. suggested that vascular occlusion training can be used as an effective exercise method for simultaneous improvement in cardiovascular fitness and muscle fitness [8]. Restriction of active muscle blood flow during low-intensity resistance training (20% -15% of RM1) results in a similar improvement in muscle hypertrophy compared with intense resistance training [9,10]. It has also been shown that low-intensity walking with vascular occlusion can significantly improve strength and hypertrophy [11]. In addition, improvements in endurance capacity (increased oxidative enzymes, capillary density, stroke volume, VO2max, glycogen stores, and decreased heart rate) and an increase in muscle size and strength have been reported with exercise combined with vascular occlusion [12]. For example, Abe et al Combined with 8 weeks of pedaling exercise with vascular occlusion compared with the group without vascular occlusion as a control group, a significant improvement in VO2max (6.4%) as well as a significant increase in the cross-sectional area of the quadriceps muscle. They also reported an increase in the strength of these muscles (3.4, 4.6 and 7.7%, respectively) compared to the control group (0.1, 0.6 and 1.4%, respectively). The results of these studies suggest that obstructive training can be used as an effective training method to simultaneously improve cardiorespiratory endurance and strength and hypertrophy in healthy individuals and even athletes [11].

The process of morphological and metabolic adaptation in skeletal muscle with obstructive training involves a number of signaling mechanisms that are associated with a temporary increase in the number of messenger RNAs for the formation of various genes and the production of encoded proteins. These changes peak 3 to 12 hours after exercise and return to baseline levels after 24 hours [13]. Therefore, the study of cellular responses to various types of obstruction exercises, which ultimately leads to increased synthesis of specific proteins and improved performance, is of interest to sports researchers. The role of obstructive training in improving muscle strength and size has been well documented [8]. And possible molecular mechanisms for this type of adaptation along with resistance obstruction training have been investigated by several studies [14,15].

However, the exact mechanisms of this training method have not been revealed, but some possible mechanisms include; Mechanical stress, metabolic stress, muscle damage, local and systemic hormones, heat shock proteins, IGF-1 / PI3K / Akt / mTOR signal pathway and activity of satellite cells to increase muscle strength and hypertrophy with resistance obstruction training [16]. Fujita S, et al. (2007) [14], also showed that resistance obstruction training, which causes hypertrophy, increases the phosphorylation of Akt / mTOR / S6K1 signal pathway proteins and increases protein synthesis [14]. The authors suggested that the mTORC1 signal pathway may play an important cellular mechanism in hypertrophy associated with resistance obstruction training. In confirmation of these findings, Gundermann DM, et al. (2012) [15], Gundermann DM, et al. (2014) [18] and Fry CS, et al. (2010) [17] in 2010 in separate studies showed that muscle protein synthesis increased even after a period of resistance obstruction training The Akt / mTOR / S6k1 signal pathway is involved in this process [15,17, and 18].

As noted, several studies have reported an increase in muscle size and strength associated with walking with vascular occlusion [8]. For example, Abe T, et al. (2006) [19] reported for the first time a 4% -7% increase in quadriceps muscle volume and an 8% -10% increase in isometric quadriceps muscle strength with 3 weeks of walking training with vascular occlusion. Several studies have since confirmed the initial findings of Abe T, et al. (2006) [19]. The mechanism of muscular hypertrophy with low-intensity obstructive aerobic training is not well understood. However, previous studies have examined several possible scenarios, such as an increase in growth hormone (GH), an increase in insulin-like growth factor (IGF-1), and other myogenic regulatory factors. For example, a very low-intensity obstructive aerobic training (50 m / min) increases blood GH levels [11]. However, these mechanisms are associated with increased muscle strength and volume along with obstructive aerobic training. Other mechanisms may also be involved. One of these possible mechanisms is the key pathway of mTOR cell growth, which is one of the main pathways for protein synthesis and muscle hypertrophy [20,21]. mTOR activity has been shown to be essential for stimulating muscle protein synthesis. Because the use of mTOR inhibitor before resistance activity has prevented protein synthesis along with resistance exercise [22]. The mTOR signal pathway for muscle hypertrophy involves phosphorylation and dephosphorylating of upstream and downstream proteins in muscle cells [21].

mTOR signaling pathway activity controls translation process through eukaryotic initiation factor (eIF2B) [23]. Researchers report that phosphorylation of 4EBP1 is associated with a muscle protein translation machine that is associated with muscle hypertrophy [24]. The mTOR signal pathway through phosphorylation of 4EBP1 prevents the binding of this protein to eIF2B, and allows the copying start factor (eIF2B) to form its own complex to start translating [25]. Therefore, an increase in phosphorylation of 4EBP1 results in an increase in skeletal muscle protein synthesis. Assuming that 4EBP1 protein phosphorylation indicates mTOR signal pathway activity and stimulation of protein synthesis, the present study seeks to determine the role of this protein and its effect on the activity of the mTOR signal pathway as mechanisms of stimulation of increasing muscle volume by measuring the phosphorylation of 4EBP1 protein along with acute obstructive Interval walking. Examining the research background in obstructive training, it is clear that this training method can have beneficial effects on muscle growth, but in this field, less research has been done on cellular and molecular studies in muscle tissue, especially in human specimens.

Materials and Methods

Five health inactive men were selected after qualifying to participate in this study. The research protocol was performed in two separate sessions with an interval of 14 days. First session was including acute interval walking with vascular occlusion and second session was performed without vascular occlusion. Additionally, the nutrition of the subjects was controlled 48 hours before the study. Before the study, each subject familiarized with the testing procedures including exercise protocol, muscle biopsy Samplings. All patients completed the consent before participating in the study.

Blood flow restriction of participate was undertaken by pressure belts. Before training, the seat belts were fastened on both legs of the experimenters and they sat on a chair. In this position the belt was adjusted repeatedly for 30 seconds and then released for 10 seconds to create consistency in the subjects. The pressure was increase from 120 mmHg to 160 mmHg [19]. After warming, subjects walking on treadmill (4 km/h with inclement 5%). The acute interval walking program including 5 intervals-2 min at 60% Maximum heart rate (MHR) which separate by 1 min rest [12,19]. The blood flow restriction of leg muscle was maintained for the entire session training including the interval walking and 1- min rests period (19 min BFR). For recovery, 5 minutes of walking without blood flow restriction was performed. Blood pressure and heart rate was monitoring for safety. Second session was quite similar to first session but without blood flow restriction. No adverse effects Such as excessive fatigue or pain were reported by subjects due to blood flow restriction.

Muscle sample was obtained from the lateral of the vastus lateralis (15-25 cm from mid-patella) of the subject's dominant leg using the biopsy technique (TSK ACECUT Biopsy Needle, TSK CO, Japanese). Biopsy technique is a less invasive method for tissue sampling which has been used by several studies. In this study, a 14×11 gauge needle was used for biopsy. First, local anesthesia was performed with 2 ml of 2% lidocaine And then the skin was hold by CO-axial guide needle and inserted to muscle tissue [26]. Immediately after surgery, the muscle sample was frozen in liquid nitrogen and stored at -80°C. Biopsy was performed immediately before the training protocol (30 minutes after rest) and 3 hours after the training protocol [17].

A complete protease and phosphatase inhibitor was used to homogenize muscle samples in cell lysis buffer. Nanoparticles were used to measure protein concentration. Examples of tests were denaturized at 95°C for 5 min, after that 30 ug of total protein was separated by 8% to 10% SDS-PA GE gel and transferred to polyvinylidene fluoride (PVDF) membranes to block nonspecific sites. Membranes were immersed in 5% non-fat milk in TBS containing 0.1%Tween 20 for 1 h to block non-proprietary sites. In the following, membranes were incubated with primary antibodies (dilution 1:1,000) for 2 h and then washing in PBS, they were exposed to a secondary antibody conjugated by peroxidase for 1h and finally were visualized by ECL reagent.

Statistical analyses were performed using the SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL, USA), and the result is expressed as mean ± SD, unless otherwise stated, with a significance level of $P<0.05$ two-tailed. The Shapiro-Wilk test was used for evaluating normality of distribution. T-dependent test was used to evaluate the difference between groups and T- independent test was used to investigate the differences in data between the two groups.

Results

The results of dependent t-test showed a significant difference between pre- and post-test 4EBP1 and stimulation of mTOR signaling pathway in acute interval walking group with vascular occlusion (P=0.001) (Figure 1). But, no significant difference was observed in 4EBP1 between pre and post-test in acute interval walking group without vascular occlusion and the mTOR signaling pathway was not stimulated (P = 0.064) (P≥0.05) (Figure 2).

The results of independent t-test showed a significant difference between the two methods of acute interval walking with and without vascular occlusion for phosphorylation of 4EBP1 and stimulation of the mTOR signaling pathway. (P = 0.01) (Figure 3).

Discussion

Few studies have examined cellular and molecular adaptation responses to acute low-intensity exercise with vascular occlusion.

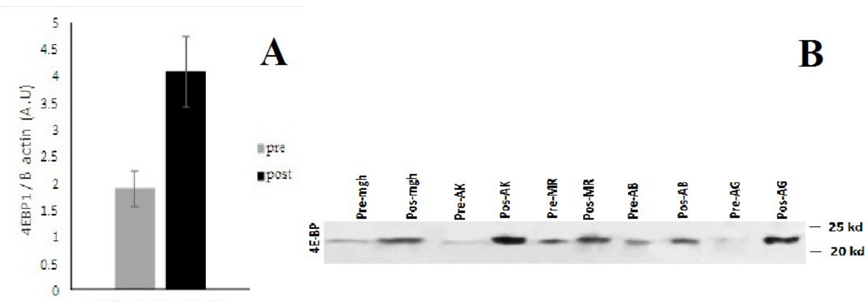

Figure 1: 4EBP1 protein level in vastus lateralis muscle in acute interval walking group with vascular occlusion (before and after activity). Changes 4EBP1 in this group were significant (n = 5). (a) Analysis histogram and (b) Representative Western blots.

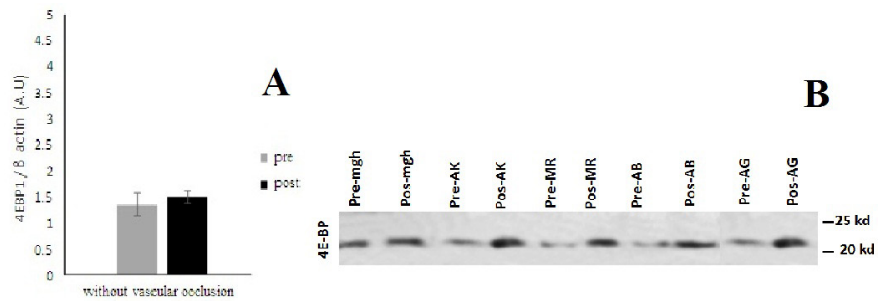

Figure 2: 4EBP1 protein level in vastus lateralis muscle in acute interval walking group without vascular occlusion (before and after activity). Changes 4EBP1 in this group were not significant (n = 5). (a) Analysis histogram and (b) Representative Western blots.

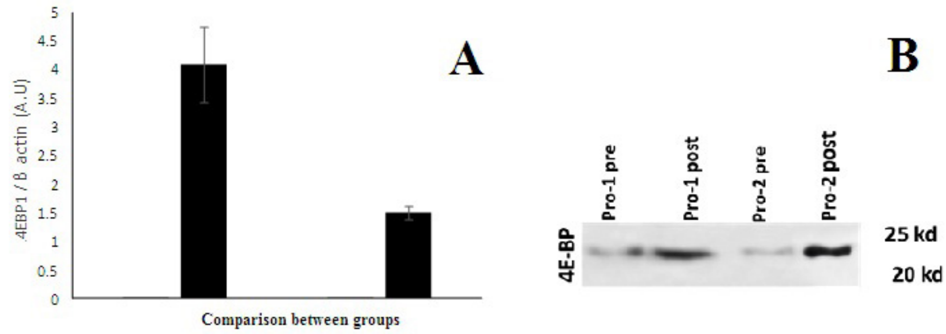

Figure 3: Comparison of 4EBP1 protein levels in vastus lateralis muscle between two groups of acute interval walking with and without vascular occlusion (before and after activity). Changes in 4EBP1 were different between the two groups. (a) Analysis histogram and (b) Representative Western blots.

Obstructive resistance training has also been studied in most previous studies. However, there is some evidence for a positive role in combining low-intensity aerobic exercise with vascular occlusion. The aim of the present study was to compare the effect of acute interval walking with and without vascular occlusion on 4EBP1 phosphorylation and mTOR signaling pathway activity in skeletal muscle of inactive healthy men. The present study shows that the phosphorylation of 4EBP1 at vastus lateralis muscle increased by acute interval walking with blood flow restriction that this is a significant increase and stimulated the mTOR signaling pathway. While 4EBP1 in the acute interval walking group without vascular occlusion did not increase significantly and did not stimulate the mTOR signaling pathway. Also in this study, a significant difference was shown between the two training methods of interval walking with vascular occlusion and interval walking without vascular occlusion for 4EBP1 phosphorylation and mTOR signaling pathway activity. Significant increase in 4EBP1 phosphorylation in the first group indicates the effect of acute obstructive interval walking on muscle protein signaling pathway and muscle stimulation for hypertrophy, which is consistent with the results of McCarthy, Regnemo and Abe research. McCarthy reported increased strength and hypertrophy combined with cycling and vascular occlusion. However, cycling training alone did not create these adaptations [27,28]. Regnemo also showed in his research that low-intensity exercise such as obstructive walking can significantly improve quadriceps muscle cross-section and knee joint strength in young and old subjects [3]. Abe also showed that short-term, low-intensity cycling, when combined with vascular occlusion, simultaneously improves hypertrophy and aerobic capacity in men [8]. All three of the above studies are consistent with the results of the present study. According to the researchers, the activity of the mTOR signaling pathway and the change in phosphorylation of the 4EBP1 protein are linked to the muscle protein translation machine which is associated with the induction of muscle hypertrophy [27]. Short-term or long-term aerobic exercise alone cannot activate the mTOR signaling pathway and alter phosphorylation of the 4EBP1 protein and cannot play a role in muscle hypertrophy [12]. However, the results of this study and previous research indicate the effect of short-term low-intensity aerobic exercise with vascular occlusion on 4EBP1 phosphorylation and activation of the mTOR signaling pathway, which stimulates muscle protein synthesis and increases hypertrophy.

Conclusions

With age, joint pain increases, one of the reasons for which can be a decrease in muscle mass due to reduced protein synthesis. The mentality of the sports medicine community is that stimulating protein synthesis and maintaining or increasing muscle mass requires the use of resistance training and the application of maximum training load. However, according to the findings of this study on the effect of acute obstructive periodic walking on phosphorylation of some factors regulating the signal pathway of protein synthesis such as 4EBP1 and stimulation of protein center and muscle growth, this training method can be recommended to people with different goals. For example, resistance training is not possible for some people, including the elderly and the young, diabetics with muscle atrophy, patients with osteoarthritis, and Injured athletes, therefore, low-intensity obstruction aerobic exercises can be introduced to these people. Even since the benefits of using obstructive training methods in improving the performance of professional athletes have been identified in several studies, Trainers can use this training method to increase the aerobic and anaerobic capacity of athletes at the same time.

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Conflicts of Interest

There are no conflicts of interest.

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