# Effect of blood flow restriction on serum levels of some factors of muscle atrophy in male elite athletes after anterior cruciate ligament reconstruction

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## Abstract

**Introduction:** The cellular mechanisms preventing muscle atrophy after anterior cruciate ligament (ACL) regeneration are not well understood. The aim of present study was to investigate the effect of blood flow restriction (BFR) training on serum levels of some muscle atrophy factors in male athletes after ACL reconstruction surgery.

**Materials and methods:** Twenty male athletes after ACL reconstruction surgery were selected and randomly divided into two groups of BFR training and control (10 in each group). Subjects in both groups performed resistance training (at intensity of 30 to 70 percentage of 10 repetition maximum, 2- 4 sets) for 12 weeks. Furthermore, subjects in the training group performed BFR training (resistance training combined with putting the pressure cuff in the upper thigh at a pressure of 120-180 mm Hg) during 12 weeks. Blood samples were collected before and 48 hours after the last training session and used for analyzing serum levels of atrogin 1 and muscle RING finger protein 1 (MuRF1). Data analysis was done by analysis of covariance and paired t test, and $P<0.05$ considered significant.

**Results:** Twelve weeks of BFR training significantly decreased serum levels of atrogin 1 ($P=0.01$) and MuRF1 ($P=0.035$) in the post-test compared to the pre-test. In addition, significant differences were observed between the BFR and control groups for atrogin 1 levels ($P=0.047$).

**Conclusion:** The findings showed that BFR training reduces the concentration of major proteins associated with muscle atrophy such as atrogin-1 and MuRF1 and plays an important role in inhibiting atrophy and consequently can increase muscle mass following injury.

**Keywords:** Blood Flow Restriction, Muscle Atrophy, Anterior cruciate ligament

## Introduction

Anterior cruciate ligament (ACL) injury is one of the most common sports injuries in athletes with several immediate and long-term consequences, including pain, muscle weakness, limited mobility, and in some cases, long-term joint damage (1). Weakness of the quadriceps muscles is one of the most important factors limiting effectiveness of rehabilitation after ACL injury and its following reconstruction. This weakness can lead to a wide range of important consequences; including knee extension defects, abnormal gait, thigh atrophy, joint and muscle dysfunction, dynamic instability, persistent knee pain, and primary osteoarthritis (2).

To date, little is known about morphological and cellular changes in the quadriceps muscle following ACL injury or ACL reconstruction, and there are conflict findings

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regarding ACL injury effects on type I or type II fibers atrophy (3, 4). Numerous factors contribute to the reduction of muscle mass during injury and muscle diseases, including oxidative and inflammatory damages, as well as muscle weakness caused by increased protein catabolism (5). Normal protein reconstruction is essential to maintain muscle mass. Increased protein catabolism is associated with the specific activity of ubiquitin E3 ligases, which are target proteins for proteasome-induced proteolysis (5). Muscle-specific ubiquitin ligases; muscle RING finger protein 1 (MuRF1) and atrogin-1 (MAFbx) are valid molecular markers for muscle atrophy which increase significantly in peripheral skeletal muscle under various conditions of decreased muscle mass (6). Previous studies have shown that atrogin-1 and MuRF1 expression are increased in atrophied skeletal muscle, and Knockout mice lacking atrogin-1 or MuRF1 are resistant to muscle atrophy (7, 8). Atrogin-1 and MuRF1 expression are controlled by the nuclear factor-κB (NF-κB) and the forkhead box O (FOXO) pathways, respectively (5). Activation of NF-κB in response to the inflammatory cytokine tumor necrosis factor α (TNF-α) suppresses MyoD gene expression, while MyoD (involved in protein differentiation) has also been suggested to be a substrate for Atrogin-1 E3 ligase (5).

Therefore, prevention of muscle atrophy and early recovery of muscle strength and neuromuscular function are important for athletes. To increase muscle mass and strength, high-intensity resistance training at intensity of approximately 70-85% of one repetition maximum (1RM) is usually recommended (9). However, there are limitations to train at this high intensity in some cases of injury such as ACL injury and even its following reconstruction surgery and rehabilitation. Blood flow restriction (BFR) exercises, also known as KAATSU exercises introduced by Yoshiaki Sato of Japan in the 1970s and 1980s and achieved via the application of external pressure over the proximal portion of the upper or lower extremities. The external pressure applied is sufficient to maintain arterial inflow while occluding venous outflow of blood distal to the occlusion site (10). BFR training resulted in muscle strength and hypertrophy by initiating cellular signaling and hormonal changes. The potential mechanisms for these adaptation is an anaerobic environment due to a decrease in cellular oxygen, which stimulates protein synthesis, proliferation of satellite cells, preferential activation and recruitment of type II muscle fibers (11). It has been suggested that BFR training in combination with low-intensity resistance training can use in postoperative rehabilitation and increase muscular hypertrophy similar to traditional resistance protocols (12, 13).

In this regard, Marissa (2018) showed that BFR training increases the strength and muscle mass of individuals after ACL reconstruction (14). In contrast, Iversen et al. (2016) showed that the BFR training in the first 14 days after ACL reconstruction dis not prevent quadriceps muscle atrophy (15). Moreover, limited studies have investigated the effect of acute and chronic resistance training on muscle atrophy factors. They also showed that long-term resistance training reduced Atrogin-1 and MuRF1 levels in rats (16). In addition, decreased expression of genes associated with proteolysis of FOXO3a, Atrogin-1 and Murf-1, as well as negative regulator of muscle mass, myostatin, was observed 8 and 48 hours after an acute BFR training session (17, 18).

Although some studies have shown the effect of BFR training on expression of atrophy-related genes in healthy individuals, the cellular pathways associated with atrophy following BFR training in athletes with ACL reconstruction have not been investigated. Therefore, the aim of this study was to



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evaluate the effect of 12 weeks of BFR training on serum levels of Atrogin-1 and MuRF1 in elite athletes following ACL reconstruction surgery.

## Materials and Methods

### Research Plan and Subjects

Twenty elite athletes in the fields of volleyball, football, futsal and basketball with a history of ACL reconstruction surgery (35-38 years) from Khorasan Razavi province Selected by targeted non-random sampling and entered the research voluntarily and with written consent. The inclusion criteria were: 1) three months after surgery and during rehabilitation program which they had undergone similar physiotherapy treatments; 2) with only an ACL ligament rupture and other ligaments and parts of the knee should be intact; 3) without any previous injury in the lower extremities; 4) and without any history of musculoskeletal diseases and cardiorespiratory problems. The subjects were randomly divided into two groups of BFR Training and Control (10 people in each one. Before starting protocol, blood samples and anthropometric indices were measured. And 10RM of each subject was determined using the Berzicky formula [10RM = displaced weight (kg) / -0.0278 (number of repetitions till fatigue × 0.0278)]. Blood samples were taken 48 hours after the last training session similar to the pre-test condition. The study protocol was approved by the Ethics Committee of Islamic Azad University, Bojnourd Branch.

### Training Protocol

Subjects in the BFR training group performed 2-4 sets of resistance training at intensity of 30-70% of 10RM with the pressure cuff closed in the upper part of the thigh with pressure of 120-180 mm Hg. However, the subjects in the control group performed resistance training with the same sets and intensity without pressure cuff. The resistance exercises were included back-to-wall squats, stretching in four directions, Smith machine squat, Squat Hog machine, sitting and standing on a chair, step-up, lunge, adduction inner thigh machine, abduction inner thigh machine, Smith machine seated calf raise, leg extension, leg flexion, leg extension with repetitive device, leg flexion with repetitive device. Each training session was started by warm-up (including stationary and elliptical bikes, and stretching exercises) and ended by cool-down (including bicycles and stretching exercises).

### Laboratory Analysis Method

In two stages of pre-test and post-test, 5-CC blood samples were collected from the brachial vein at rest and fasting conditions. Blood samples were then kept at room temperature for 20 minutes until blood clotted. After blood clotting, blood samples were centrifuged at 4 ° C for 15 minutes at 3000 rpm. After serum isolation, blood samples were stored in the refrigerator temperature of -20 ° C until analysis of the studied variables. Serum levels of Atrogin-1 were assessed by human ELISA kit (Cusabio, China, Cat.No: CSB-EL008498HU). Furthermore, Serum MuRF1 levels were measured by human ELIS kit (Estabiopharm, China, CK-E91770).

## Statistical Analysis

The Shapiro–Wilk test was used to verify the data normality, and the Levene test was used to verify the homogeneity of variance. Analysis of Covariance was applied to compare the changes in the dependent variables between two groups of BFR training and control. And Paired t-test was used to assess the significance of the changes of the dependent variables in post- test compared to pre-test. In all cases, the



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criterion for statistical significance was set at P<0.05.

## Results

Values were expressed as mean and standard error. The anthropometric characteristics of subjects were shown in table 1. According to analysis of covariance finding, there were significant differences between two groups of BFR training and control in relation to serum Atrogin-1 levels (P=0.047, F=3.585). However, no significant differences existed between two groups for serum levels of MuRF1 (P=0.33492, F=1.159). Paired t-test showed significant decreases in serum levels of Atrogin-1 (P=0.01) and MuRF1 (P=0.035) following 12 weeks of BFR training in the post-test compared to the pre-test without significant changes in control group (P>0.05) (Figure 1).

**Table 1.** Descriptive characteristics of subjects under study in groups of BFR training and control.

| Variables | BFR training group (n=10) | Control group (n=10) |
|---|---|---|
| Age (year) | 24.13 ± 4.91 | 25.21 ± 2.55 |
| Height (m) | 177.70 ± 5.57 | 178.30 ± 3.11 |
| Weight (Kg) | 72.61 ± 21.98 | 71.51 ± 8.92 |
| Body mass index (kg/m2) | 22.22 ± 6.41 | 22.31 ± 8.64 |

* BFR: Blood Flow Resistance.

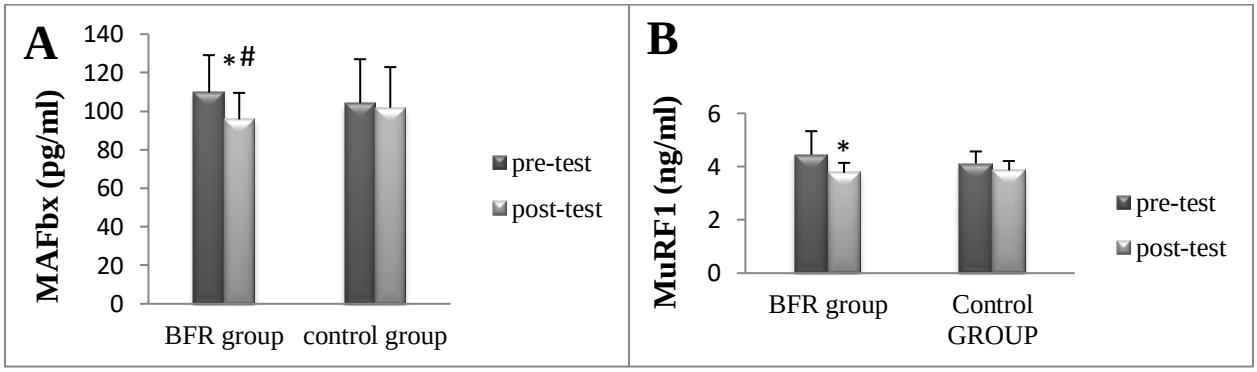



**Figure 1.** Changes in the serum levels of Atrogin-1 (panel A) and MuRF1 (panl B) following 12 weeks of BFR training in the post-test compared to the pre-test without significant changes in control group.
*Significant at P<0.05 for within-group changes.
# Significant at P<0.05 for between-group changes.

## Discussion

Decreased activity following injury can affect the genes related to proteins break down and synthesis, and therefore resulted in muscle injury- induced atrophy. In this regard, two weeks of inactivity in rodents reduced muscle protein content by 40 percentage (19) accompanied by 1.8 times significant increases in expression of atrophy related genes (20). The results of the present study showed that BFR training following ACL reconstruction surgery significantly reduces serum concentrations of two marker of atrophy means Atrogin-1 and MuRF1. Although, the effects of BFR training on levels of Atrogin-1 and MuRF1 have not investigated in subjects with ACL injury or reconstruction; Consistent with this study, Ribeiro et al. (2017) showed resistance training is an effective method to reduce the level of Atrogin-1 and MuRF1 with increasing age, and therefore, prevent muscle atrophy (21). Furthermore, Zanchi et al. (2009) concluded that resistance training reduced Atrogin-1 by 61 percentage and



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MuRF1 by 41 percentage (16). Contrary to this research, the researchers reported no change in the expression of Atrogin-1 and MuRF1 genes due to voluntary resistance training (22), which may be due to the intensity of training with limited blood flow. Although no other studies are available to elucidate the mechanism of changes in gene expression associated with muscle atrophy, it appears that transcription factors play an important role in altering inflammatory factors. One study, even reported that one day of inactivity was sufficient to increase Atrogin-1 and MuRF1 mRNA expression in plantaris muscle (23). These changes were greater in the cross-sectional area of type IIa and type IIb fibers in plantaris muscle compared to atrophied type I and type IIa fibers in horseshoe muscle after 6 and 21 days of disuse (23). It has also been reported that an increase in inflammatory factors in adult rodents is associated with an increase in mRNA content of both Atrogin-1 and MuRF1 in skeletal muscle (23). Fast twitch muscles fibers have been reported to express more Atrogin-1 and MuRF-1 mRNA than slow-twitch fibers such as horseshoe muscle after 3 days of inactivity (24). In this context, it has been shown that TNF-α stimulation increases the expression of Atrogin-1 and MuRF1 and subsequently the breakdown of muscle protein (23). Increased production of these cytokines leads to muscle atrophy resulting in a decrease in protein synthesis (25) and an increase in protein breakdown (26). In previous studies, researchers have shown that exogenous TNF-α increases the expression of ubiquity genes in skeletal muscle both in the laboratory and in the natural environment (27, 28). In this regard, Frost et al. (2007) showed that exogenous TNF-α increase is associated with an increase in Atrogin-1 and MuRF1 mRNA content (23). Another mechanism of increase in Atrogin-1 and MuRF1 due to ACL injury can be caused by a change in the expression of the FOXO gene. In this regard, Sheibani et al. (2018) consider muscle hypertrophy after resistance training as a result of decreased expression of FOXO3α and Atrogin-1 (29). The FOXO family plays an important role in regulating atrophy program through copying factors (30). FOXO (non-phosphorylated) transcription factors are mostly active in nuclear envelopes and in DNA binding (31). During inactivity, FOXO is dephosphorylated and accumulates in the nucleus of muscle cells (32). This dephosphorylated FOXO transfer to the nucleus preferentially and increase the expression of Atrogin-1 and MuRF1 mRNAs (33). On the other hand, increasing phosphorylation of FOXO leads to its transfer to the cytosol through protein kinase B and decreases the down-regulation of Atrogin-1 and MuRF1 (33). Therefore, based on the results of the present study, it seems that BFR training may have reduced the concentrations of Atrogin-1 and MuRF1 through inhibiting FOXO. However, further studies are necessary to confirm this hypothesis.

## Conclusion

The results of the present study showed that BFR training in combination with resistance training in the rehabilitation phase following ACL surgery can play an important role in inhibiting atrophy and ultimately increasing muscle mass by reducing the concentration of major proteins associated with muscle atrophy such as Atrogin-1 and MuRF1. Therefore, it can be concluded that BFR training, one of the appropriate training protocols, can be suggested to rehabilitate ACL injured athletes, as it plays an important role in the recovery of atrophied muscles by affecting the physiology of muscle.

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