# Effect of Blood Flow Restriction Training on Serum Levels of Some Muscle Growth Factors in Male Athletes after Anterior Cruciate Ligament Surgery

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## Abstract

**Introduction** The cellular mechanisms of muscular hypertrophy resulting from Blood Flow Restriction (BFR) training have not been well studied. The aim of the present study was to investigate the effect of BFR on serum levels of proxis-1-alpha (PGC-1α), insulin-like growth factor-1 (IGF-1) and myostatin in male athletes after anterior cruciate ligament surgery.
**Materials and Methods:** 20 people were selected from the volunteers and randomly divided into 2 groups (10 people in each group) 1. BFR exercise; 2- Control. Subjects in both groups performed resistance training movements in 2 to 4 sets with an intensity of 30 to 70% RM. The subjects in the BFR group performed resistance training movements by closing the pressure cuff in the upper thigh with a pressure of 120 to 180 mm Hg. Data were analyzed using analysis of covariance and paired t-test.
**Results:** The results showed that 12 weeks of BFR training significantly increased the serum concentration of IGF-1 and significantly decreased the serum levels of myostatin in the post-test compared to the pre-test (P = 0.009). The results also showed that BFR training did not have a significant effect on serum PGC-1α concentration.
**Conclusion:** Overall, the results showed that BFR training leads to changes in serum levels of myostatin and IGF-1, which is of particular importance compared to traditional resistance training.

**Keywords:** Blood flow restriction, Myostatin, Hypertrophy, Anterior cruciate ligament injury

## Introduction

Anterior cruciate ligament (ACL) injury is the most common knee joint injury with a prevalence of 2 to 3 per 10,000 athletes in athletes (1, 2). The quadriceps muscle becomes atrophied by 34 to 40% following ACL injury and subsequent surgery phase (3, 4) and in most studies, failure to achieve optimal muscle function has been reported even for several years after surgery (5). The postoperative rehabilitation period for athletes undergoing ACL reconstruction surgery lasts about six months to a year (6). Therefore, improving the strength and hypertrophy of the knee complex muscles is very important (7).

Blood flow restriction (BFR) training is a special type of low-pressure strength training that puts less strain on the joints and ligaments compared to traditional resistance training, but is equally effective in improving strength and hypertrophy (8). BFR training induces ischemia and hypoxia in muscle leading to two primary triggers of hypertrophy, namely metabolic and mechanical stress, and provides the basis for muscle growth and hypertrophy (9). Actually; BRF training is involved in muscle hypertrophy by increasing the production of reactive oxygen species (ROS), increasing the recall of fast-twitch fibers and the production of trophic hormones (10). Among the hormones affecting muscle growth, two myostatin hormones, as

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muscle growth inhibitors and insulin-like growth factor-1 (IGF-1), as growth-promoting hormone, have been studied. Among the hormones affecting muscle growth, two myostatin hormones, as muscle growth inhibitors and insulin-like growth factor-1 (IGF-1), as growth-promoting hormone, have been studied. Unlike myostatin, IGF-1 is one of the most important stimuli for muscle growth in response to mechanical stimuli that are either regulated as an intracellular mediator by growth hormone or act independently of growth hormone (12). IGF-1 and the mammalian signal pathway rapamycin (mTOR), which are important for muscle hypertrophy, are activated by the proxy zoom-1 receptor activator (PGC-1α). Recent studies have shown that PGC-1α is involved in muscle hypertrophy by inhibiting myostatin and activating IGF-1 (13).

There are limited studies that have measured the effect of BFR training in athletes after ACL rehabilitation. Marissa (2018) showed that the use of BFR training increases the strength and muscle mass of individuals after ACL reconstruction (14). In contrast, Iverson et al. (2016) showed that the use of blood flow restriction in the first 14 days after ACL reconstruction did not prevent quadriceps muscle atrophy (15). However, the mechanism of BRF training effect on the rehabilitation of the knee control muscles after ACL surgery has not yet been investigated. Therefore, the aim of this study was to evaluate the effect of 12 weeks of blood flow restriction training on serum levels of PGC1-α, myostatin and IGF-1 in athletes after knee cruciate ligament surgery.

## Materials and Methods

The statistical population of the present study consisted of elite athletes with a history of ACL surgery in the age range of 35-38 years in Khorasan Razavi province. Targeted non-random sampling was used. Thus, the professionally operated athletes who played in the provincial teams in the fields of volleyball, football, futsal and basketball were informed by a specialist orthopedic surgeon of the knee or a physiotherapist and entered the research voluntarily. The inclusion criteria were passing three months since their surgery during which they had undergone similar physiotherapy treatments, having only an ACL ligament rupture and other ligaments and parts of the knee should be intact, having no previous injury in the lower extremities and being in perfect health in terms of musculoskeletal diseases and cardiorespiratory problems. The study protocol was approved by the Ethics Committee of Islamic Azad University, Bojnourd Branch. Also, in one session, all subjects signed the consent form and announced their readiness to participate in this study consciously and voluntarily. Among the volunteers, 20 people who were eligible to participate in the study were selected and randomly divided into 2 groups (10 people in each one): 1- BFR Training and 2- Control. Before starting the exercise protocol, 10RM using the Barzi formula (10 RM = displaced weight (kg) / -0.0278 (number of repetitions till fatigue × 0.0278)) was determined for the subjects in a session. 4 days after 10RM determination, and before the start of training protocols, fasting blood sampling (pre-test), measurement of anthropometric indices, were performed on all subjects (Table 1). Subjects then performed the intervention for 12 weeks. 48 hours after the last fasting training session, a second blood sample (post-test) was collected and transferred to the laboratory for analysis of serum levels of PGC-1α, IGF-1 and myostatin.

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

| Variables | EMS training group (n=10) | Control group (n=10) |
|---|---|---|
| Age (year) | 27.01 ± 3.42 | 25.62 ± 2.55 |
| Height (m) | 177.1 ± 5.59 | 178.30 ± 3.11 |
| Weight (Kg) | 71.16 ± 6.65 | 71.88 ± 5.92 |
| Body mass index (kg/m$^2$) | 22.69 ± 2.06 | 22.58 ± 1.64 |

EMS: Electrical Muscle Stimulation



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The research protocol was the same for both groups and included the following movements: 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. Prior to the protocol, subjects performed a warm-up program, including stationary and elliptical bikes, and stretching exercises. At the end of the training session, a cooling program including bicycles and stretching exercises was performed. Subjects in both groups performed training movements in 2 to 4 sets with an intensity of 30 to 70% of 10RM (training intensity gradually increased each week). The subjects in the BFR group performed resistance training of each movement by closing the pressure cuff in the upper part of the thigh with a pressure of 120 to 180 mm Hg. Also, the subjects in the control group performed resistance training in each movement similar to the BFR group. 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 at -20 ° C until analysis of the studied variables. Serum levels of PGC-1α using human kit (Manufactured by China Estabiopharm Company, Cat.No: CK-E91467), IGF-1 using commercial kit (Manufactured by China Estabiopharm Company, Cat.No: CK-E10161), and myostatin using commercial kit (Manufactured by China Estabiopharm Company, CK-E11241) were measured by ELISA method.

Descriptive statistical methods (mean, standard deviation, drawing tables, and graphs) were used to describe the data. To use the appropriate statistical test according to the sample size, first, the naturalness of distribution and homogeneity of variance of the studied variables were investigated through the Shapiro-Wilk test. Intergroup comparison was statistically analyzed using analysis of covariance (for differences between EMS and control groups). The paired samples t-test was used to compare pre-test and post-test in each group. Statistical software SPSS version 23 was used to analyze the data and EXCEL software version 2013 was used to draw the graphs. P value was set at $P < 0.05$.

## Results

Statistical analysis of covariance showed that there was no significant difference between groups in relation to serum PGC1-α levels ($P = 0.791$, $F = 0.237$). The results of the dependent t-test showed that in none of the groups the changes in PGC1-α levels in the post-test compared to the pre-test were significant ($P > 0.05$) (Figure 1 A).

Statistical analysis of covariance analysis showed that there was no significant difference between groups in relation to serum IGF-1 levels ($P = 0.101$, $F = 2.692$). The results of the dependent t-test showed that in BFR group, serum IGF-1 levels increased significantly in the post-test compared to the pre-test ($P = 0.006$). However, in the control group, changes in IGF-1 levels in the post-test were not significant compared to the pre-test($P = 0.433$) (Figure 1B).

Statistical analysis of covariance showed that there was no significant difference between groups in relation to serum myostatin levels ($P = 0.392$, $F = 0.981$). The results of the dependent t-test showed that in BFR group, serum myostatin levels decreased significantly in the post-test compared to the pre-test ($P = 0.009$). However, in the control group, changes in myostatin levels in the post-test compared to the pre-test were not significant ($P = 0.171$)



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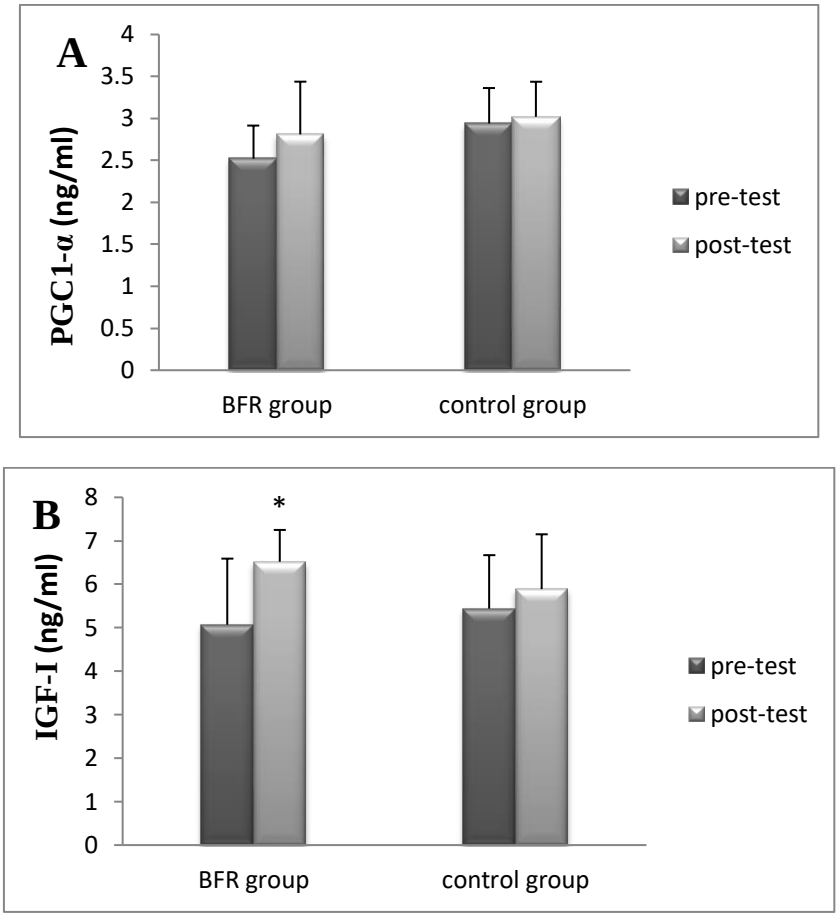



**Figure 1.** The effects of 12 weeks Blood Flow Restriction Training and resistance training on serum levels of PGC1-α (Panel A) and IGF-1 (Panel B). *Significant difference compared to the pre-test.

## Discussion

Changes in atrophy and muscle strength are associated with changes in hormone levels associated with hypertrophy and muscle nerve activation. PGC-1α has been shown to reduce the stimulation of atrophy-related genes by suppressing the transcription factor of FoxO3 (16). In the study of changes in this transcription factor in athletes after ACL surgery, the results of the present study showed for the first time that resistance training has no significant effect on serum PGC-1α concentration with and without BFR. Although there is no research in this area in in individuals with ACL rupture; its changes are likely to be small at the serum level due to the role of this transcription factor at the cellular level and the cell nucleus. In this context, it has been shown that the basal level of PGC-1α - which is present in both types of fibers I and II - increases with BFR training in both types of fibers (17). However, contrary to this research, it has been shown that PGC-1α is preferentially higher in muscle rich in type I fibers in rodents (18).

Schwarz et al. (2016) also showed that resistance activity with different intensities leads to increased PCG1-a expression. In addition, Norrbom et al. (2004) in their study of the effect of resistance activity with and without BFR on PGC-1α showed that resistance exercise with BFR further increases PGC-1α mRNA in skeletal muscle (17). Therefore, due to the limitations of this study in measuring the concentration of PGC-1α at the level of muscle cells, it can be stated that this factor may have changed at the cellular level, which requires future studies.



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It has been shown that PGC-1α may alter control of histone alterations near the IGF-1 and myostatin genes and alter their transcription (19). In the present study, despite no change in serum PGC-1α level, IGF-1 concentration increased significantly after BFR training, although resistance training alone had no effect on serum levels. In line with the present study, Walker et al. (2004) showed that 10 weeks of resistance training had no effect on circulating IGF-1 concentration in healthy individuals (20). In this regard, Abe et al. (2005) showed that two weeks of resistance training with BFR increases the circulating level of IGF-1. This type of exercise also increases the cross-sectional area of the muscle and strength (21). Mechanism of action of IGF-1 on muscle hypertrophy occurs mainly through regulation and proliferation and function of satellite cells (22). IGF-1 also inhibits apoptosis (23). In general, several studies have shown an increase in IGF-1 after resistance exercise (24, 25). Several studies have also shown no effect or reduction (26, 27). These differences can be due to the type of subject (healthy versus patient) or the type of measurement (serum versus cellular). In fact, BFR induces adaptation through an increase in stress metabolites (resulting in a hypoxic / ischemic environment) (28), which leads to muscle growth by influencing other factors such as the use of rapidly contracting muscle fibers (29), an increase in systemic hormones (11), cell swelling (12) and increase in the production of reactive oxygen species (30).

Another finding of the study is that BFR exercise significantly reduces serum myostatin concentrations, which can prevent atrophy due to ACL rupture. But resistance training alone has no significant effect on serum myostatin concentration. In one study, myostatin expression was shown to inhibit the proliferation of satellite cells because the study showed that significant muscle hypertrophy occurred in mice lacking myostatin (31). Decreased regulation of myostatin gene expression has also been reported following mechanical loading due to resistance training and BFR (31). Therefore, it is hypothesized that exercise with BFR may stimulate muscle hypertrophy due to suppression of myostatin activity due to hypoxia or accumulation of exercise-induced metabolites (31). Decreased regulation of myostatin gene expression has also been reported following mechanical loading due to resistance training and BFR (31). Therefore, it is hypothesized that exercise with BFR may stimulate muscle hypertrophy due to suppression of myostatin activity due to hypoxia or accumulation of exercise-induced metabolites (31). Therefore, it is hypothesized that exercise with BFR may stimulate muscle hypertrophy due to suppression of myostatin activity due to hypoxia or accumulation of exercise-induced metabolites (31). In this regard, Walker et al. (2004) showed that 10 weeks of resistance training significantly reduced myostatin, although there was no significant difference with the control group (20). In another study, 8 weeks of resistance training with BFR resulted in increased muscle mass due to decreased myostatin expression in human skeletal muscle (1). It was also shown that BFR reduces the expression of myostatin mRNA in skeletal muscle of rodents (30). The mechanism of action of myostatin on muscle atrophy is that myostatin leads to phosphorylation and activation of Samd2 / 3 (8). The Samd2 / 4/4 complex is transported into the nucleus and inhibits genes involved in muscle cell proliferation and differentiation (32). Samd2 / Samd3 phosphorylation also disrupts protein kinase B activity and inhibits the mTOR pathway. Therefore, any decrease in myostin can be a signal for muscle hypertrophy (32). According to the results of this study, changes in myostatin concentration due to BFR training and on the other hand an increase in IGF-1 concentration can be a mechanism to reduce atrophy after ACL surgery.



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## Conclusion

The results of the present study showed that BFR is associated with an increase in serum IGF-1 growth factor and a decrease in myostatin growth inhibitor but has no effect on serum PGC-1α level. Based on previous research and the results of the present study, it seems that this type of exercise may have led to more changes in these factors at the cellular level, which requires further research, but overall the results showed that exercise with BFR leads to changes in serum levels of myostatin and IGF-1, which are of particular importance in rehabilitation after ACL surgery compared to traditional resistance training.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

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