Arch Scientific Journal of Rehabilitation Medicine

July, August 2022. Volume 11. Number 3

Research Paper

The Effect of Resistance Training With Electrical Muscle Stimulation on Atrogin-1 and Muscle Ring Finger-1 in Elite Male Athletes After Anterior Cruciate Ligament Surgery

Abouzar Alavi[1], ***Najmeh Rezaeian**[1], **Reza Ganji**[2], **Ali Yaghoubi**[1]

1. Department of Sport Physiology, Faculty of Humanities, Bojnourd Branch, Islamic Azad University, Bojnourd, North Khorasan Province, Iran.
2. Department of Orthopedic Surgery, Faculty of Medicine, North Khorasan University of Medical Sciences, Bojnurd, North Khorasan Province, Iran.

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Citation Alavi A, Rezaeian N, Ganji R, Yaghoubi A. [The Effect of Resistance Training With Electrical Muscle Stimulation on Atrogin-1 and Muscle Ring Finger-1 in Elite Male Athletes After Anterior Cruciate Ligament Surgery (Persian)]. Scientific Journal of Rehabilitation Medicine. 2022; 11(3) 426-437. https://dx.doi.org/10.32598/SJRM.11.3.6

https://dx.doi.org/10.32598/SJRM.11.3.6

Received: 20 Feb 2021

Accepted: 05 Mar 2021

Available Online: 23 Jul 2022

ABSTRACT

Background and Aims The cellular mechanisms preventing muscle atrophy after Anterior Cruciate Ligament (ACL) regeneration are not well understood. The aim of the present study was to evaluate the effect of resistance training combined with electrical muscle stimulation on serum levels of atrogin-1 and muscle RING finger-1 (MuRF1) in elite athletes following ACL surgery.

Methods Among the elite athletes of Razavi Khorasan Province, 20 athletes voluntarily participated in the study and were divided into two groups (ten cases each), including 1) resistance training- electrical muscle stimulation (RT-EMS), and 2) resistance training (RT). The subjects in both groups performed 2-4 sets of resistance exercises (knee extension machine, squat, and knee flexion machines) at an intensity of 30-70% of ten repetitions for 12 weeks. The subjects in the RT-EMS group performed the exercises in combination with electrical stimulation at 35-70 Hz. Blood samples were collected from all subjects before and 48 hours after the last training session to measure atrogin-1 and MuRF1 levels. Analysis of covariance (ANCOVA) and paired t-test were used to compare between- and within-group changes, respectively, and a $P<0.05$ was considered significant.

Results The results showed that 12 weeks of resistance training- electrical muscle stimulation significantly decreased serum levels of atrogin 1 ($P=0.013$) and MuRF1 ($P=0.008$) in the post-test compared to the pre-test. In addition, the between-groups comparison showed a significant difference in atrogin 1 levels between the RT-EMS and RT groups ($P=0.047$).

Conclusion It can be suggested that resistance training in combination with electrical muscle stimulation is associated with lower levels of muscular atrophy proteins, such as atrogin-1 and MuRF1, and therefore, can be more effective than resistance training alone.

Keywords Muscle atrophy, Atrogin 1, MuRF1, Anterior cruciate ligament, Electrical muscle stimulation

Corresponding Author:

Najmeh Rezaeian, PhD.

Address: Department of Sport Physiology, Faculty of Humanities, Bojnourd Branch, Islamic Azad University, Bojnourd, Iran.

Tel: +98 (915) 3887389

E-Mail: rezaeian.n@gmail.com

Citation Alavi A, Rezaeian N, Ganji R, Yaghoubi A. [The Effect of Resistance Training with Electrical Muscle Stimulation on Atrogin-1 and Muscle RING Finger-1 in Elite Male Athletes After Anterior Cruciate Ligament Surgery (Persian)]. Scientific Journal of Rehabilitation Medicine. 2022; 11(3):426-437. https://dx.doi.org/10.32598/SJRM.11.3.6

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Extended Abstract

Introduction

nterior cruciate ligament (ACL) injury is one of the most common sports injuries in athletes and is accompanied by several immediate and long-term consequences, such as weakness and atrophy of the quadriceps. Numerous factors contribute to the reduction of muscle mass during sports injuries and muscle diseases, including oxidative and inflammatory damages, as well as muscle weakness caused by increased protein catabolism. Increased protein catabolism is associated with the specific activity of ubiquitin E3 ligases, which targets proteins for proteasome-induced proteolysis. Muscle-specific ubiquitin ligases MuRF1 and atrogin-1 (MAFbx) are well-known molecular markers for muscle atrophy that increase significantly in peripheral skeletal muscles under various conditions of decreased muscle mass. To increase muscle mass and strength, high-intensity resistance training at an intensity of approximately 70-85% 1RM is usually recommended. However, there are limitations to training at this high intensity following reconstruction surgery and rehabilitation of ACL. Electrical muscle stimulation (EMS) eliminates the inhibitory effects on contraction by creating action potential in motor nerves of the muscle and helps to improve muscle weakness and function. Therefore, when exercise training is not possible due to limited mobility, EMS may be a useful method during the rehabilitation period.

Although some studies have shown the effect of EMS and resistance training on the expression of atrophy-related genes in healthy individuals, the cell pathways associated with atrophy following resistance training. EMS and resistance training in athletes with ACL reconstruction have not been studied and compared. Therefore, the aim of this study was to investigate and compare the effect of 12 weeks of resistance training and EMS- resistance training on serum levels of Atrogin-1 and MuRF1 in elite athletes following ACL reconstruction surgery.

Materials and Methods

Twenty elite athletes (35-38 years) in the fields of volleyball, football, futsal, and basketball with a history of ACL reconstruction surgery from Razavi Khorasan province were selected by targeted non-random sampling and entered the research voluntarily and with written consent. The inclusion criteria were: 1) passing three months from their surgery and undergoing similar physiotherapy treatments during this period; 2) only an ACL ligament rupture and other ligaments and parts of the knee should be intact; 3) no previous injury in the lower extremities; 4) and no history of musculoskeletal and cardiorespiratory diseases. The subjects were divided into two groups of resistance training (RT) and resistance training- EMS (RT-EMS) (ten cases each). Before the training protocol, 10RM of each subject was determined, pre-test blood sampling was taken, and pre-test anthropometric indices were measured. Post-test measurements were done 48 hours after the last training session. Subjects in both groups of RT and RT-EMS performed two to four sets of resistance exercises at an intensity of 30 to 70 percent of 10RM, including 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, and leg flexion with the repetitive device.

In the RT-EMS group, one electrode (anode) was placed 5-7 cm distal to the origin of the quadriceps muscle and the other electrode (cathode) was placed on the femoral nerve in the groin area to stimulate contraction by a 200-Hz Myodine electric stimulator (United Kingdom). The contraction time was 5 seconds and the rest time was 5 seconds for 30 minutes. Electrical excitation of 35-70 Hz was simultaneously applied with performing resistance exercise training sets. Each training session consisted of warm-up at the start (including stationary and elliptical bikes, and stretching exercises) and cool-down (including bicycles and stretching exercises) at the end. In two phases of pre-test and post-test, 5 CC of blood samples were collected from the brachial vein at rest and fasting conditions. Serum levels of atrogin-1 were assessed by human ELISA kits (Cusabio, China, Cat.No: CSB-EL-008498HU). Furthermore, serum MuRF1 levels were measured by human ELIS kits (Estabiopharm, China, CK-E91770). The Shapiro–Wilk test was used to verify the data normality, and the Leven test was used to verify the homogeneity of variance. Analysis of covariance (ANCOVA) was applied to compare the changes in the dependent variables between the two groups. A paired t-test was used to assess the significance of the changes of the dependent variables in the post-test compared to the pre-test. In all cases, the criterion for statistical significance was set at $P<0.05$.

Results

According to ANCOVA, there were significant differences between groups of RT and RT-EMS in serum levels of atrogin-1 (P=0.047, F=3.585). However, no significant differences were reported for MuRF1 levels (P≥0.05, F=1.159). Moreover, serum levels of atrogin-1 (P=0.013) and MuRF1 (P=0.008) decreased significantly in the RT-EMS group in the post-test compared to the pre-test. However, there were no significant changes in atrogin-1 and MuRF1 in the RT group (P≥0.05).

Discussion

Post-injury muscle atrophy can be due to immobility and changes in the genes related to protein breakdowns, such as atrogin-1 and MuRF1. The finding of this study showed that serum levels of atrogin-1 and MuRF1 decreased significantly in the RT-EMS group. Transcription factors appear to play an important role in this field through changes in inflammatory factors, like tumor necrosis factor-alpha (TNF-α). TNF-α can increase protein catabolism through up-regulation of atrogin-1 and MuRF1 expression. Therefore, exercise training as an anti-inflammatory modality can decrease protein catabolism by reduction of TNF-α levels and subsequent down-regulation of atrogin-1 and MuRF1 expression. Furthermore, over-regulation of SIRT-1 and deacetylation of NF-κB following training reduce inflammation and therefore, improve atrophy. Additionally, the FOXO family plays an important role to regulate muscle atrophy through transcription factors. During inactivity, FOXO is dephosphorylated and accumulated in the nucleus of muscle cells. Dephosphorylated FOXO transfer to the nucleus preferentially increases the expression of atrogin-1 and MuRF1 mRNAs. On the other hand, increasing the phosphorylation of FOXO and its transfer to the cytosol through protein kinase B, decrease the down-regulation of atrogin-1 and MuRF1. Altogether, it can be concluded that muscular resistance-stimulation training compared to resistance training alone can be a suitable training method to improve the factors associated with muscle atrophy in the rehabilitation period after anterior cruciate ligament surgery in athletes.

Ethical Considerations

Compliance with ethical guidelines

In the implementation of the research, ethical considerations were considered according to the instructions of the ethics committee of Bojnord Branch, Islamic Azad University, and the ethics code number 18248257181323416230233O was received.

Funding

This article is taken from the thesis of Abuzar Alavi under the guidance of Najma Rezaian and Reza Ganji and the advice of Ali Yaqoubi from the Department of Physical Education and Sports Sciences of Bojnord Branch, Islamic Azad University. This research has not received any financial aid from the funding organization in the public, government, commercial, non-profit sectors of the university or research center.

Authors' contributions

All authors contributed equally in preparing all parts of the research.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

We are grateful to all the people who helped us in conducting this research.