Effect of Resistance Training along with Electrical Muscle Stimulation on Serum Levels of Some of the Molecular Markers of Muscle Hypertrophy in Male Athletes after Anterior Cruciate Ligament Surgery

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Abstract

Introduction: To the best of our knowledge, there is no study to examine the effect of a combination of resistance training and electrical muscle stimulation (EMS) on muscle hypertrophy factors in injured athletes. This study aimed to evaluate the effect of EMS on serum levels of some molecular markers of muscle hypertrophy in male athletes after anterior cruciate ligament surgery.
Materials and Methods: For the study, 20 volunteer men were randomly divided into 2 groups (10 people in each group), the EMS-resistance training and control groups. Subjects in both groups performed 2-4 sets of lower body resistance training movements with an intensity of 30-70% of ten repetitions maximum for 12 weeks. The subjects in the EMS-resistance group performed exercise movements combined with electrical stimulation at 35-70 Hz. Blood samples were collected from all subjects before and 48 hours after the last training session and used to measure the levels of sirtuin-1 (SIRT1), visfatin, and nitric oxide (NO). Data were analyzed using analysis of covariance and paired sample t-test.
Results: The results showed that 12 weeks of EMS training significantly increased serum levels of SIRT1 ($P < 0.001$), visfatin ($P = 0.02$), and NO ($P = 0.01$) in the post-test compared to the pre-test. Significant differences were observed between the EMS and control groups in SIRT1 ($P < 0.001$) and NO ($P = 0.021$) levels. In addition, there was no significant difference between the groups in serum visfatin level ($P = 0.098$).
Conclusion: The findings suggest that EMS in combination with resistance training could be a good alternative to traditional resistance training to stimulate factors related to muscle protein synthesis after anterior cruciate ligament reconstruction.

Keywords: Electrical muscle stimulation, Visfatin, Nitric oxide, Anterior cruciate ligament injury.

Introduction

Anterior cruciate ligament (ACL) injury is one of the most common knee injuries that occur during physical activity (1). A review study showed that only 65% of individuals return to pre-sports injury levels and 55% return to competitive exercise levels after ACL surgery (2). Decreased ability to voluntarily contract the quadriceps muscle is a common problem after knee injuries, although the muscle or nerve innervation is not damaged. This condition is often referred to as "Atherogenic Muscle Inhibition" (3). Muscle weakness after injury or surgery can be partly due to muscle atrophy as well as a decrease in the ability to activate muscle fibers (4, 5). In cases where voluntary muscle contractions are inhibited

after injury or surgery, neuromuscular electrical stimulation (NMES) is recommended to eliminate the inhibitory effects on contraction while creating action potential in the motor nerves (3, 4). Review studies have shown that NMES in combination with exercise may be more effective in improving quadriceps muscle strength than exercise alone (6-8).

Many transcription factors play a significant role in post-injury muscle atrophy. Sirtuin 1 (SIRT1) is one of the regulators of autophagy. Down-regulation of SIRT1 delays the activation of satellite cells and possibly skeletal muscle regeneration (9). SIRT1 has been shown to regulate energy metabolism during myogenesis (10). On the other hand, the inactivation of SIRT1 deacetylase activity reduces the size and regeneration of muscle fibers and reduces some important genes involve in muscle growth (11). In addition, SIRT1 can affect the signaling activity of nitric oxide (NO) and the activity of nitric oxide synthase endothelial synthase (eNOS), which is regulated by acetylation or distillation. NO is one of the most important mediators of intracellular and extracellular processes.

Because it has the function of dilating arteries, NO acts under conditions of muscle stress and stimulates changes between different types of muscle fibers (12). SIRT1-induced eNOS overexpression can stimulate mitochondrial biogenesis and increase satellite cell proliferation by increasing NO (10). NO-induced satellite cell proliferation is also essential for skeletal muscle hypertrophy (10).

SIRT1 is also dependent on NAD^+ levels (13). Interestingly, visfatin protein levels in the muscles of sedentary individuals increased after 3 weeks of exercise (13). Exercise increases NAD-dependent SIRT1 diestylase activity by increasing NAD (14). Visfatin suppression has been shown to reduce SIRT1 levels; As a result, the availability of visfatin is critical to maintaining NAD^+ levels and consequently SIRT1 activity (15). Several studies have examined the effect of electrical muscle stimulation (EMS) on strength and hypertrophy in patients after ACL reconstruction. For example, Hasegawa et al. (2011) showed that EMS in the initial stage of ACL rehabilitation helps maintain and increases muscle volume and strength in the limbs (16). However, the underlying mechanism of the effect of electrical stimulation training in combination with resistance training on muscle physiology after ACL surgery is not known. Therefore, this study aimed to evaluate the effect of 12 weeks of electrical stimulation training in combination with resistance training on serum levels of SIRT1, visfatin, and NO in elite male athletes after ACL 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 study protocol was approved by the Ethics Committee of Islamic Azad University, Bojnourd Branch The inclusion criteria were included 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. Then, 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 randomly divided into 2 groups (10 people in each one): EMS-resistance training and

control groups. Before starting the exercise protocol, 10 repetitions maximum (10RM) using the Barzi formula [10RM = displaced weight (kg) / -0.0278 (the number of repetitions till fatigue × 0.0278)] was determined for sessional subjects. 4 days after 10RM test, fasting blood samples were collected and anthropometric characteristics were measured (Table 1). Subjects, then, performed the intervention for 12 weeks. 48 hours after the last training session, a second blood sample (post-test) was collected and transferred to the laboratory for analysis of serum levels of SIRT1, visfatin, and NO.

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

The study 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 the repetitive device, leg flexion with the repetitive device. Subjects performed a warm-up program, including stationary and elliptical bikes, and stretching exercises. Subjects in both groups performed 2-4 sets of resistance training movements with an intensity of 30-70% of 10RM (training intensity gradually increased each week). The subjects in the EMS training group performed resistance training of each movement while wearing EMS device. Also, the subjects in the control group performed resistance training in each movement similar to the EMS group. Each training session was finished by cooling down program including bicycles and stretching exercises were performed.

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. Serum levels of SIRT1 were measured by ELISA using a human kit (Manufactured by China Estabiopharm Company, Cat.No: CB-E90449). Serum levels of visfatin were measured by ELISA using a human kit (Manufactured by China Estabiopharm Company, Cat.No: CB-E11560). Serum nitric oxide levels were measured by ELISA using a human kit (Manufactured by China Estabiopharm Company, Cat.No: CK-E11334).

Statistical Analysis

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 a significant difference

between groups in relation to serum levels of SIRT1 ($P < 0.001$, $F = 17.684$). In fact, EMS in combined with resistance training significantly increased SIRT1 levels in comparison with resistance training alone. Also, the results of paired sample t-test showed that in the EMS group, serum levels of SIRT1 increased significantly in the post-test compared to the pre-test ($P < 0.001$). However, in the control group, changes in SIRT1 levels in the post-test compared to the pre-test were not significant ($P = 0.181$) (Figure 1A). Statistical analysis of covariance analysis showed that there was no significant difference between groups in relation to serum visfatin levels ($P = 0.098$, $F = 2.617$).

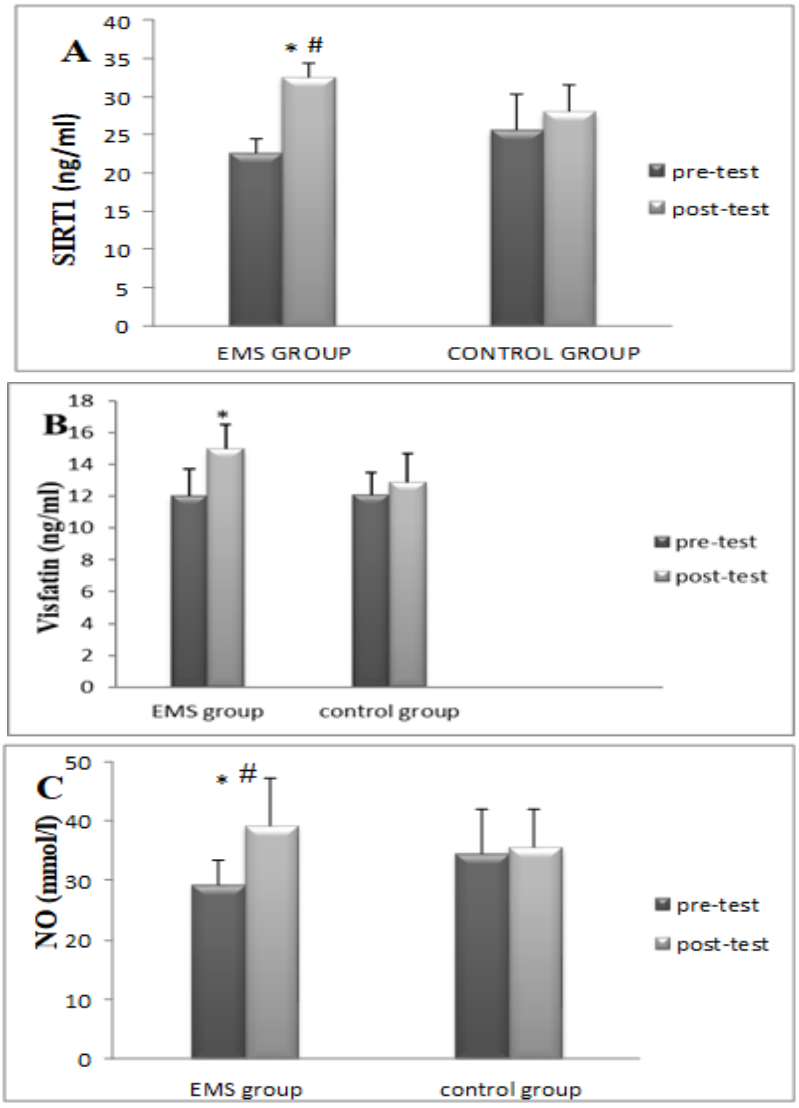

Figure 1. The effects of 12 weeks electrical muscle stimulation (EMS) and resistance training on serum levels of Sirtuin-1 (SIRT1) (Panel A), visfatin (Panel B) and nitric oxide (NO) (Panel C). *Significant difference compared to the pre-test. #Significant difference compared to the control group. Data are shown as mean ± SD.

The results of paired sample t-test showed that in the EMS group, serum visfatin levels increased significantly in the post-test compared to the pre-test ($P = 0.02$). However, in the control group, changes in visfatin levels in the post-test compared to the pre-test were not significant ($P = 0.340$) (Figure 1B).

Statistical analysis of covariance revealed that EMS combined with resistance training significantly increased serum NO levels compared to the control group ($P = 0.021$,

F = 4.744). Furthermore, the paired sample t-test showed that serum nitric oxide levels increased significantly in the post-test compared to the pre-test in the EMS group (P = 0.01). However, in the control group, changes in nitric oxide levels in the post-test compared to the pre-test were not significant (P = 0.280) (Figure 1C).

Discussion

The results of the present study showed that, for the first time, EMS training combined with resistance training as a major rehabilitation method increases the serum concentration of SIRT1 after ACL surgery in young athletes. To the best of our knowledge, so far, there is no study to examine the effect of EMS training on changes in this factor, so a general mechanism for these changes cannot be stated. However, in line with the present study, Koltai et al. (2010) examined the effect of aerobic exercise on SIRT1 and visfatin levels in elderly rats. The results demonstrated that exercise significantly increases SIRT1 activity (17). Also in agreement with the present study, Suva et al. (2008) found an increase in SIRT1 protein in oxidative contractile fibers in response to endurance training (18).

However, the underlying mechanism of the effect of exercise or EMS on changes in SIRT1 concentration is unclear. In this regards, it has been shown that during the activation of satellite cells, the need for energy to synthesize macromolecules and maintain an acceptable level of self-efficacy or autophagy increases (9). On the other hand, the inactivation of SIRT1 deacetylase activity reduces the size and regeneration of muscle fibers and reduces some important genes related to muscle growth (11). Therefore, it can be assumed that in this study, EMS training combined with resistance training increased the activation of satellite cells by increasing the concentration of SIRT1 and eventually increases muscle protein synthesis. The results of a recent study also showed that SIRT1 can increase cellular protein synthesis through mammalian target of rapamycin (mTOR) signals (19). In addition, SIRT1 can interact with the peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) receptor, which is the major regulator of mitochondrial biogenesis. Since mitochondrial biogenesis and hypertrophy can occur simultaneously (20), this interaction can play an important role in ACL rehabilitation after surgery.

In addition, SIRT1 through other molecules contributes to increasing muscle hypertrophy. It has been shown that SIRT1 can affect the signaling activity of NO and NO endothelial synthase (12). The results of the present study showed that EMS training combined with resistance training significantly increased the serum NO concentration in young men in the rehabilitation period after ACL injury surgery. In addition, NO can inhibit the release of Ca^{2+} from the sarcoplasmic reticulum, thereby reducing energy production (21). A significant increase in NO content has been reported with muscle contusion, which is significantly associated with a decrease in maximal production force (22). It is hypothesized that an increase in NO levels may be a protective mechanism that does not allow excessive force generation due to high muscle tension and can prevent minor injuries (21). It is also well known that muscle contusion causes damage to sarcomeres due to unusual stress. This damage may be repaired and NO appears to be involved in this process by activating satellite cells (22). NO causes the proliferation of satellite cells, which is a vital process in muscle regeneration (21). Therefore, it can be concluded that EMS training combined with resistance training reduces energy production by increasing NO in response to repeated muscle contraction; leading proliferation of satellite cells during the training period causing muscular hypertrophy. This argument needs to be confirmed by future studies. Therefore, it is possible that EMS training combined with resistance training may play a role in

muscle hypertrophy after ACL surgery via the SIRT1-NO satellite cell pathway. In another study, Durigan et al. (2014) investigated the effect of electrical nerve stimulation on gene expression and muscle atrophy after ACL injury in rodents (rats). The results showed that electrical stimulation reduces the accumulation of atherogenic (23). The results of the present study also showed that although the serum level of visfatin increased in response to EMS training along with resistance training even though it did not reach a significant level compared to the resistance training program alone. In line with this study, Koltai et al. (2010) showed that 6 weeks of endurance training significantly increased the concentration of visfatin, which occurs through the SIRT1-dependent pathway (17). In another study, Kotali et al. (2017) showed that a 2-week increase in load caused a 40% increase in muscle mass with an increase in SIRT1 content and activity. Increases in levels of visfatin, endothelial NO synthetase, and SIRT1-regulated protein kinase B (AKT) were also observed in hypertrophied muscles which has a significant negative correlation with SIRT1 and visfatin. Overall, this study suggests that in addition to the role of SIRT1 in modulating catabolic and anabolic pathways, SIRT1 may play an important role in skeletal muscle hypertrophy, which requires further research (10). Therefore, it can be speculated that EMS training probably plays an important role in metabolic rehabilitation and muscle hypertrophy after ACL injury via SIRT1, NO, and visfatin-dependent pathways. Regarding the link between SIRT1, NO, and visfatin, it has been shown that increasing visfatin increases SIRT1 and thus stimulates various cellular cascades such as increasing NO, increasing glucose transporters, decreasing myostatin and protein kinase B causing protein synthesis elevation, cellular apoptosis decrease and mitochondrial function decrease (21).

Conclusion

The results of the present study show that EMS training combined with resistance training in the rehabilitation period after ACL surgery is associated with the improvement of metabolic factors involved in muscle hypertrophy. Based on this, the results of the present study showed for the first time that EMS training increases a cascade signal of NO→SIRT1→visfatin, which can play an important role in inhibiting atrophy and ultimately increasing muscle volume after injury. Therefore, based on the results of this study, EMS training in combination with resistance training can be suitable options compared to resistance training to stimulate factors related to muscle protein synthesis after ACL reconstruction.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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