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, no study has been evaluated the effect of a combination of resistance training and electrical muscle stimulation (EMS) on muscle hypertrophy factors in injured athletes. The aim of this study was to investigate the effect of EMS on serum levels of some molecular markers of muscle hypertrophy in male athletes after anterior cruciate ligament surgery (ACL).
Materials and Methods: Twenty male athletes after ACL surgery were selected and randomly divided into two groups: EMS and control (10 subjects in each group) of. Subjects in both groups performed resistance training (at intensity of 30-70 percentage of 10-repetition maximum, 2-4 sets). Whereas, subjects in EMS group performed resistance training with EMS at frequency of 35-70 Hz. Blood samples were collected before and 48 hours after the last training session to measure serum levels of sirtoin-1 (SIRT1), visfatin and nitric oxide (NO).
Results: The finding showed that 12 weeks of EMS significantly increased serum levels of SIRT1 ($P < 0.001$), visfatin ($P = 0.02$) and NO ($P = 0.01$) in post-test compared to the pre-test. The results also revealed that the EMS training significantly increased SIRT1 ($P < 0.001$) and NO ($P = 0.021$) levels in comparison with the control group. However, there was no significant difference between two groups in visfatin levels ($P > 0.05$).
Conclusion: The results suggest that EMS training could possibly be a good alternative to the traditional resistance training to stimulate factors related to muscle protein synthesis.

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 occurs during exercise training (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 "Arthrogenic 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, Electrical Muscle Stimulation (EMS) is recommended to eliminate the inhibitory effects on contraction while creating action potential

in the motor nerves (6, 7). Review studies have shown that EMS in combination with exercise may be more effective in improving quadriceps muscle strength than exercise alone (6-8).

Many transcription factors play a role in post-injury muscle atrophy. Sirtoin 1 (SIRT1) is one of the autophagy regulators. Down-regulation of SIRT1 delays the activation of satellite cells and possibly skeletal muscle regeneration (9). In fact, SIRT1 has been shown to regulate energy metabolism during myogenesis (10). On the other hand, inactivation of SIRT1 deacetylate activity reduces the size and regeneration of muscle fibers and reduces some important genes for 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 stimulation 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 deacetylase 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). A number of 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 increase muscle volume and strength in the limb (16). However, the exact mechanism of effect of electrical stimulation training with resistance training on muscle volume and strength after ACL surgery is not known. Therefore, the aim of this study was 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

Subjects and Study Design

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. The study protocol was approved by the Ethics Committee of Islamic Azad University, Bojnourd Branch. 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. 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): EMS training and Control (Table 1).

Table 1. Physical characteristics of the subjects in EMS and control groups.

	Groups	
	Control	EMS
Age (year)	25.62 ± 2.55	27.00 ± 3.42
Height (cm)	178.30 ± 3.11	177.10 ± 5.59
Weight (kg)	71.88 ± 5.92	71.16 ± 6.65
BMI (kg/m^2)	22.58 ± 1.64	22.69 ± 2.06

Data are shown as mean ± SD. EMS: Electrical muscle stimulation, BMI: Body mass index.

Before starting the exercise protocol, 10 repetitions maximum (10-RM) using the Barzi formula [10-RM = lifted weight (kg) / -0.0278 (number of repetitions till fatigue × 0.0278)] was determined for the subjects in a session. 4 days after 10-RM determination, and before the start of training protocols, fasting blood sampling (pre-test), measurement of anthropometric indices, were performed on all subjects. 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 SIRT-1, visfatin and NO.

Training Protocol

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.

Laboratory Analysis

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 NO 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. In order 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 Shapiro-Wilk test. Intergroup comparison was statistically analyzed using analysis of covariance (for differences between EMS and control groups). Paired samples t-test was used to compare the pre-test and post-test in each group. Statistical software SPSS version 23 was used to analyze the data. P value was set at < 0.05.

Results

The results 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).

The results of dependent 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 showed that there was no significant difference between groups in relation to serum visfatin levels ($P = 0.098$, $F = 2.617$). The results of dependent t-test showed that in 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).

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 in the EMS group, serum NO levels increased significantly in the post-test compared to the pre-test ($P = 0.01$). However, in the control group, changes in NO levels in the post-test compared to the pre-test were not significant ($P = 0.280$) (Figure 1C).

The results of the present study showed for the first time that 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, research has not yet examined the effect of EMS training on changes in this factor, so a general mechanism for these changes cannot be stated. However, in the study of endurance training, consistent with the present study, Koltai et al. (2010) examined the effect of aerobic exercise on SIRT1 and visfatin levels in elderly rats. The results showed that exercise significantly increases SIRT1 activity (17). Also in agreement with the present study, Suva et al. (2008) showed an increase in SIRT1 protein in oxidative contractile fibers due to endurance training (18).

However, the underlying mechanism of exercise effects or EMS on changes in SIRT1 concentration is unclear. However, 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, inactivation of SIRT1 deacetylase activity reduces the size and regeneration of muscle fibers and reduces some genes important for muscle growth (11). Therefore, it can be stated that in this study, EMS training combined with resistance training increased the activation of satellite cells by increasing the concentration of SIRT1 and thus increased muscle protein synthesis too. The results of a recent study also showed that SIRT1 can increase cellular protein synthesis through mammalian target of rapamycin (mTOR) signals (19).

Discussion

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. Furthermore, SIRT1 through other molecules is involved in increasing muscle hypertrophy. It has been shown that SIRT1 can affect the signaling activity of NO and NO endothelial synthase (12). In addition, NO can inhibit the release of Ca^{2+} from the sarcoplasmic reticulum; thereby reducing energy production (21).

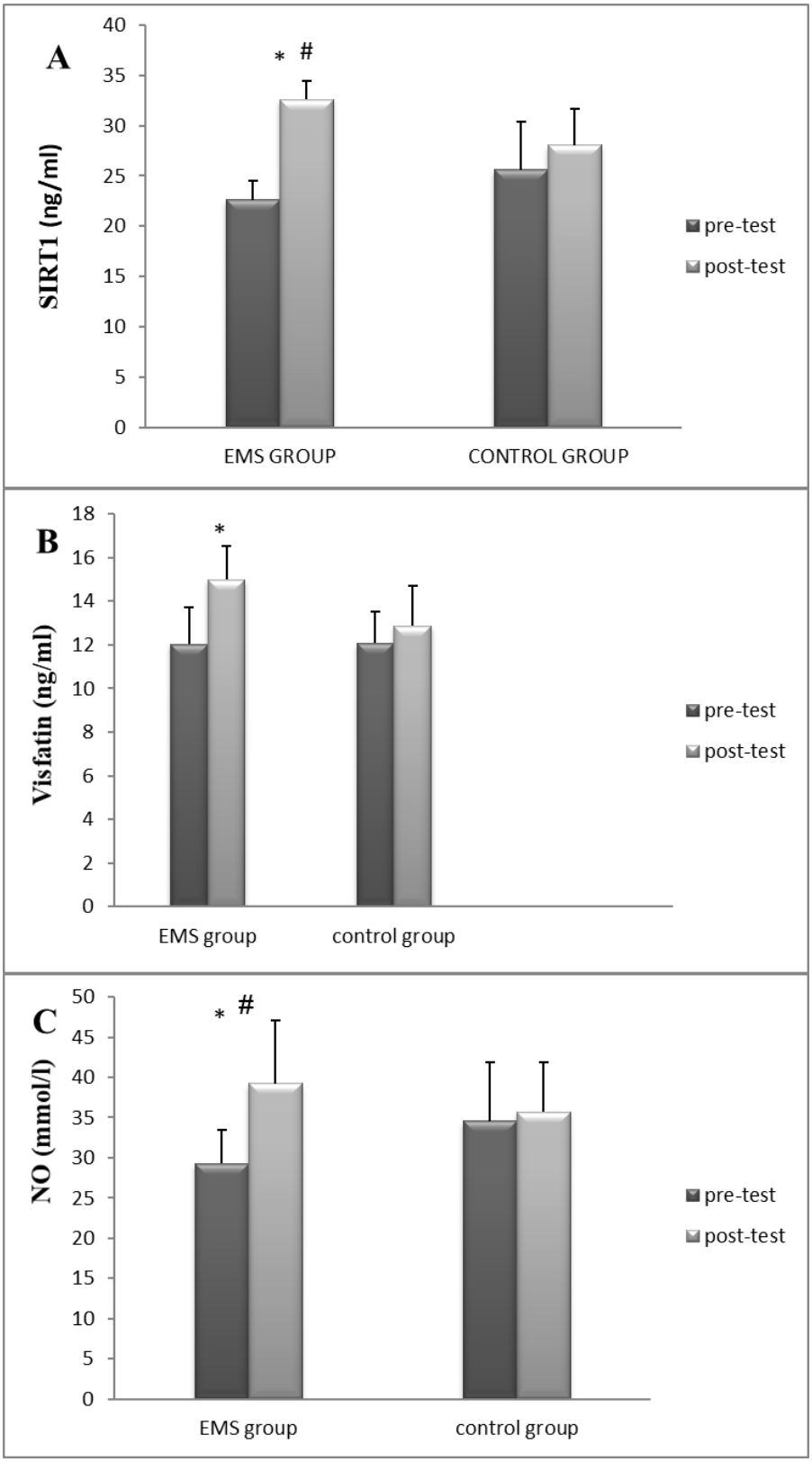

Figure 1. Changes in SIRT1 (A), visfatin (B), and NO (C) levels in the two groups under study in the pre- and post-test. *Significant difference compared to the pre-test, # significant difference compared to the control group.

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 activities and as a result lead to the gradual increase of muscle strengthening after ACL injury, which affects the 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 atherogenics (23). The results of the present study also showed that although the serum level of visfatin increased due to EMS training along with resistance training, it was not significantly different compared to resistance training alone. In line with this study, Koltai et al. (2010) showed that 6 weeks of endurance training significantly increases the concentration of visfatin which occurs through the SIRT1-dependent pathway (17). It has been showed that that a 2-week increases 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 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 (24). Therefore, it can be stated that EMS training probably plays an important role in metabolic rehabilitation and muscle hypertrophy after ACL injury through SIRT1, NO and visfatin -dependent pathways. Regarding the mechanism of communication 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 increase, 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 improvement of metabolic factors involved in muscle hypertrophy. Based on this, the results of the present study showed for the first time that 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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