Research Article

The effect of an electrical muscle stimulation (EMS) session on changes in endostatin and vascular endothelial growth factor in inactive men

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Abstract

The effectiveness of EMS on muscle and physical performance has been demonstrated in various populations, including healthy young adults. The aim of this study was to investigate the effect of an electrical muscle stimulation (EMS) session on changes in endostatin and vascular endothelial growth factor in inactive men. In this quasi-experimental research, 16 inactive healthy men (age, 20-30 years; BMI, 18 - 24.9 kg/m2) from Tehran were selected and randomly assigned to two EMS stimulation (n=8) and control (n=8) groups. The subjects performed the exercise (squat, lunge, crunch and plank) with WB-EMS clothes for 20 minutes; frequency 80 Hz, pulse 5 seconds and pulse pause 3 seconds. The levels of VEGF and endostatin in plasma were measured using kit and ELISA method. The data were analyzed using independent and correlated t-test at the $P<0.05$. The results showed that intragroup changes of endostatin and VEGF were significant after an EMS training session ($P\leq0.05$), but not significant change was observed in the control group ($P\geq0.05$). Also, the inter-group changes of endostatin showed no significant differences between the two groups in the post-test ($P=0.1$). However, the intergroup changes of VEGF indicated significant difference between the two groups in the post-test ($P=0.001$). It seems that in inactive people, EMS stimulation increases vascular endothelial growth factor as the most important positive regulator of the angiogenesis process and decreases endostatin as one of the most important negative regulators of the angiogenesis process.

Key Words: **Angiogenesis, Inactivity, Muscle electrical stimulation, VEGF**

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Introduction

There are various mechanisms in the vascularization of tissues, one of which is angiogenesis. Angiogenesis is a complex intercellular process leading to the migration of endothelial cells into the interstitial space and their sprouting, the expansion and proliferation of endothelial cells and the formation of new capillaries (Adair & Montani, 2010). The most important angiogenic factor is vascular endothelial growth factor (VEGF). VEGF is a homodimeric glycoprotein that is necessary for the differentiation of endothelial cells and angiogenesis during the growth and development of the capillary network (Iversen et al., 2011; Schlager et al., 2011). When VEGF binds to its special receptors on endothelial cells, it activates signals that cause proliferation and migration of endothelial cells and increase vascular permeability (Wahl et al., 2010).

On the other hand, angiostatic factors are factors, each of which acts at a specific stage of the process of angiogenesis or arteriogenesis (increasing the diameter of blood vessels) and prevents the process of angiogenesis and arteriogenesis. Endostatin is one of the most important angiostatic factors; this inhibitory factor is produced by different tissues in the body (Li et al., 2008). The inhibition mechanism of endostatin is such that this factor is connected to the angiogenic factor VEGF and prevents its function, thus preventing the proliferation of endothelial cells. In fact, endostatin is an obstacle to the growth of the capillary network by preventing the proliferation and migration of endothelial cells (Egginton et al., 2009).

In a normal state, there is a balance between angiogenic and angiostatic factors (Goodwin et al., 2007). However, the balance between angiogenic and angiostatic factors is always disturbed in physiological and pathological situations, and one of these situations can be exercise training. Several studies in recent years have shown the response of VEGF to exercise. VEGF mRNA increases during and after exercise in humans (Hiscock et al., 2003; Gavin et al., 2004); though, to exert its angiogenic effects, VEGF must be increased at the protein level. Hickok et al show a progressive increase in both plasma

VEGF and VEGF mRNA after a three-hour knee extension activity session in healthy young men, which reaching significant levels one hour after exercise (Hiscock et al., 2003). Also, it has been shown that VEGF levels in endurance trained men (Kraus et al., 2004), and healthy untrained men and women (Nemet et al., 2009) increase after exercise. However, VEGF has been shown to decrease in active healthy men (Gu et al., 2004) and healthy military subjects (Gunga et al., 1999), and remained unchanged after exercise in healthy untrained men (Kraus et al., 2004).

Moreover, previous in vitro and in vivo studies have shown that electrical stimulation (ES) is able to stimulate angiogenesis in types of tissues and cells (Kim et al., 2009; Sebastian et al., 2011; Ud-Din et al., 2015). The exact mechanisms have not yet been determined, although in endothelial cells, increased release of VEGF and activation of VEGF receptors, phosphatidylinositol-3-kinase (PI3K), Akt and Rho-ROCK elements play an important role (Bai et al., 2011). Electrical muscle stimulation (EMS) has long been used as an adjunctive exercise modality, applied locally or to the whole body (Matos et al., 2022; de Oliveira et al., 2021; Ludwig et al., 2019). The electrical current causes involuntary muscle contractions on the target muscles providing the same exercise benefits without as much discomfort (Seyri et al., 2011). Specifically, it is designed to facilitate the passive activation of a large number of motor units and induce simultaneous recruitment of muscle fibers, with the goal of strengthening or maintaining muscle mass. Portable and low-cost EMS devices have been developed and can be used in many settings (eg, hospitals, clinics, homes, and leisure sports) (Yoo et al., 2023).

Previous studies have shown the effectiveness of EMS on muscle and physical performance in different populations, including healthy young and elderly people, as well as the patients with muscle wasting diseases such as sarcopenia (Langeard et al., 2017; Matos et al., 2022; Tanaka et al., 2022; Wirtz et al., 2016). This method is also a practical and effective approach for people with little physical activity. Due to the busyness of life, people do not usually have enough time to do continuous activities with relatively long periods, hence, exercise with electrical muscle stimulation provides unique benefits in the clinical context and it produces positive training adaptations with lower intensity and duration tailored to daily activities. Therefore, it should be used by non-athletes who are limited in performing aerobic exercise for relatively long time or those who do not want to bear high pressure. However, the vascular benefits of the effectiveness of EMS combined with exercise in sedentary heal-thy subjects are unclear. Thus, the current research aimed to investigate the effect of an electrical muscle stimulation (EMS) session on changes in endostatin and vascular endothelial growth factor in inactive men.

Materials and Methods

Design

The current research adopted an applied quasi-experimental design conducted as pre-test-post-test with control group.

Participants

The statistical population of this research was made up of inactive healthy men (aged 20 - 30 years) in Tehran who were selected through invitations in public and administrative centers. After interviewing the volunteers and obtaining their consents, 16 eligible participants were randomly assigned to EMS stimulation (N=8) and control (N=8) groups. The sample size of the present study was determined based on the results of previous research, at significance level of 5% (type 1 error) and statistical power of 95% (type 2 error) using Medcalc 18.2.1 software (8 subjects in each group).

Procedure

Eligible participants submitted the written consent form and the related questionnaire one week before the start of the research and declared their readiness to start the training program. A briefing session was held with the presence of the researcher to familiarize the participants with the method of conducting the research, the day and time of the protocol and other explanations. The inclusion criteria for the study included: the age of 20 - 30 years, BMI 18 - 24.9 kg/m2, inactive lifestyle (activity less than 1 hour per week), absence of heart diseases and vascularity, not using sports supplements, not using medicine in the previous 6 months and consent to participate in the study. Also, in this research, a health certificate was obtained from the participants

Table 1. EMS clothing material specifications

	Main fabric	Conductive panel fabric	Conductive yarn
Material	Nylon 76.5% Polyurethane 23.5%	Nylon 100% Metal plated 99% pure silver	Nylon 59%, metal plated 99% pure silver Polyurethane 35% Polyester 6%
Density	Wale: 148.0 cm Course: 218.6 cm	Wale: 92.6 cm Course: 91.6 cm	-
Mass	217.1 g/m^2	106.4 g/m^2	-
Thickness	0.7 mm	0.4 mm	-
Elongation recovery	Wale: 97.5% Course: 96.0%	Wale: 80.0% Course: 75.0%	-
Elongation	73%	50%	22%
Tenacity	Wale: 328.6 N Course: 392.7 N	Wale: 136.2 N Course: 228.7 N	92 N (61.6%)
Resistance	-	0.7 Ω	1.8 Ω
Yarn count	-	-	Nylon: 250 d/10 Polyurethane: 560 d/3 Polyester: 150 d/2

by a specialist physician (with cardiovascular approach, high blood pressure and peripheral nerve disorders). Exclusion criteria from the research-included smoking, sleep disorder, being on a diet, diagnosis of other underlying diseases during the implementation of the protocol, such as cardio-pulmonary problems, skeletal and neurological disorders during activities that prevented the implementation of the activity, and the feeling of danger of the implementation of the exercise. After filling out the personal information questionnaire and signing the consent form, each of the participants appeared the next day to perform the tests at the test venue. At the beginning of the session, anthropometric characteristics including height, weight and body mass index were measured for all participants. The height (in centimeters) was measured using a seca measuring device made in Germany with an accuracy of 0.1 cm and their body weight (in kilograms) was measured and recorded using a digital scale made in seca, Germany with an accuracy of 0.1 kg. After measuring the height and weight of the participants, their body mass index was calculated using the formula [square of height in meters/weight (kg) = (body mass index) BMI]. After two days, the participants went to the laboratory and their blood was taken to evaluate the VEGF and endostatin of plasma levels. Then the experimental group performed the EMS training protocol. After the protocol, anthropometric characteristics and blood sampling were again obtained.

EMS training protocol

At first, the participants should know how to work with special training clothes, and then they did their sports session in the training session for 20 minutes. The participants were evaluated before and after the exercise by a physician and a physiotherapist. During the exercise session, all participants wore WB-EMS (Bodyfriend, Seoul, Korea) and performed a low-resistance exercise protocol guided by an instructor (Delly et al., 2014). This outfit was chosen due to the greater benefit in a shorter time and checking the results from the training session. The WB-EMS suit is equipped with 20 conductive plates on the shoulders (2 plates), arms (4 plates), chest (2 plates), back (4 plates), abdomen (2 plates), hips (2 plates), and legs (4 plates) used to stimulate the brachial muscle belly, triceps, pectoralis major, latissimus dorsi, rectus abdominis gluteus maximus rectus femoris, biceps femoris, semitendinosus, etc. The clothing material specifications are shown in Table 1. The protocol of the WB-EMS device is set by the controller and the program. The duration was 20 minutes, the frequency was 80 Hz, the pulse duration was 5 seconds, and the pulse pause was 3 seconds. Intensity was adjusted before each exercise.

Dynamic stretching exercises were performed before and after the main exercise, and the exercise lasted 20 minutes. Core exercises were designed with squats, lunges, crunches, and planks (Daley et al., 2014). Squat, lunge and crunch were performed 20 times each, and plank exercise was performed for 1 minute with appropriate intensity in each group. During the exercise, the electrical current for the WB-EMS suit was turned on. In addition, a physician was present during the exercise to prevent dizziness, chest discomfort, muscle pain, or other symptoms.

Statistical analysis

Blood samples of the patients before and after the protocol were collected from the pre-elbow vein of the right hand of the participants in a sitting position in the amount of 5 cc. The blood samples taken were kept at -70 degrees Celsius. It should be noted that all the stages of the test were conducted in the same and standard conditions at 8 to 10 in the morning. VEGF plasma concentration with the kit (VEGF Human, OKKB00271, Aviva System Biology California, USA) (with a range of 31.2 to 2000 pg/ml (nanogram/liter), internal variation coefficient 4.89% and external variation coefficient 8/8 3%) and endostatin (Endostatin Human, MyBioSource, MBS8243214, California, USA) (with a range of 2 to 200 μmol/liter) were measured by ELISA and Griess assay, respectively, according to the manufacturer's protocol.

The Shapiro-Wilk test was used to ensure the normality of the distribution of the variables. Independent T-test was used to compare the means between groups. Also, Correlated T-test was used to check intra-group changes. Calculations were done using SPSS version 26 statistical software and the significance level of the tests was considered as $p \leq 0.05$.

Results

Table 2 shows the mean and standard deviation of the demographic characteristics of the participants in different groups.

The results of the correlated t test show that there is a significant difference between the pre-test and post-test endostatin values of the EMS group (P=0.012), but there is no significant difference between the pre-test and post-test endostatin values of the control group (P=0.206). Also, the results of the independent t-test show that there is no significant difference between the two groups of the endostatin in post-test values (Figure 1).

Table 1. Demographic characteristics of the participants

group / Variables	EMS	control
weight (kg)	75.2 ± 7.38	74.7 ± 6.21
height (cm)	175.8 ± 6.69	176.6 ± 8.64
BMI (kg/m2)	22.4 ± 1.99	22.0 ± 2.45

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Also, the results of the correlated t test show that there is significant difference between the pre-test and post-test VEGF values of the EMS group (P=0.001), but there is no significant difference between the pre-test and post-test VEGF values of the control group (P=0.714). In addition, the results of the independent t test show that there was significant difference between the two groups in the VEGF post-test values (Figure 2).

Discussion

The results of the present study showed that one session of EMS protocol significantly increased plasma levels of VEGF in inactive men. Also, plasma levels of VEGF increased significantly in the EMS training group compared to the control group. Plasma levels of endostatin significantly decreased after one session of EMS training in inactive men, but did not change significantly in the control group. In addition, inter-group changes showed no significant differences of endostatin between the two groups in post-test values. The results of this research are consistent with the findings of previous studies (Bai et al., 2011; Sheikh et al., 2005; Ud-Din et al., 2015). It seems that the mechanism of angiogenesis stimulation in tissues and endothelial cells is through increasing the release of VEGF and activation of VEGF receptors, phosphatidylinositol-3-kinase Akt (PI3K) and Rho-ROCK elements of the VEGFR signaling pathway (Bai et al., 2004; Zhao et al. al., 2004).

In our study, EMS significantly increased plasma levels of VEGF in inactive men. It is possible that exercise with EMS is effective in the production of VEGF through the application of mechanical stress (Beijer et al., 2013). It is important to mention that angiogenic responses are not only dependent on the total VEGF concentration, but also on the tissue distribution and extracellular intensity of VEGF. Also, the intensity of exercise is one of the important reasons for changes in angiogenesis factors. Yeo et al. (2012) showed that high-intensity exercise has a greater effect

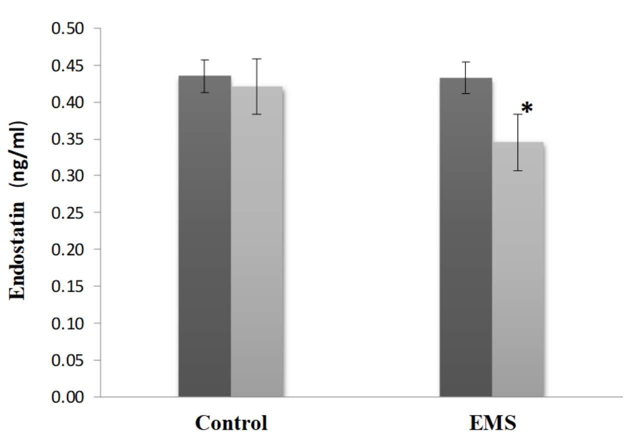

Figure 1. Endostatin values in pre-test and post-test of two groups. *The significant difference compared to the pre-test (P≤0.05).

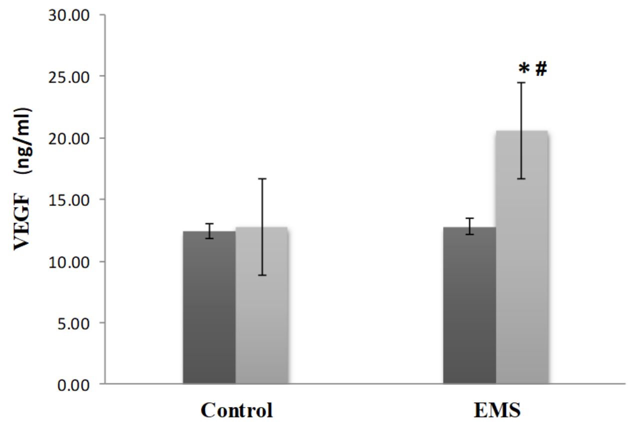

Figure 2. VEGF values in pre-test and post-test of two groups. *The significant difference compared to the control group. # Significant difference compared to the pre-test (P≤0.05)

on increasing VEGF compared to low-to-moderate intensity exercise. Therefore, it seems that the training intensity in our EMS protocol is appropriate and has led to VEGF changes.

The amount of serum endostatin at rest indicates the amount of collagen changes. The presence of endostatin in the blood circulation of healthy people can indicate that under normal conditions, angiostatic factors play an important role in regulating the angiogenesis process. This finding is consistent with the results reported by Suhr et al. (2010) and Brixius et al. (2008) who reported significant decrease in serum endostatin following exercise. However, it is against the results of Seida et al. (2003) which is probably due to the very low baseline level of serum endostatin in the participants. The mechanism of endostatin reduction in response to exercise is still not clear. However, it has been shown that the proteolytic release of endostatin from collagen XVIII is mediated by different classes of proteases, such as cysteine proteases, matrix metalloproteases and aspartic proteases (Ferreras et al., 2000). Many of these proteases are associated with physiological collagen turnover, which can be increased by exercise. Therefore, it appears that exercise-induced endostatin levels are associated with higher collagen turnover rates.

Also, the response of endostatin to exercise depends on the anthropometric characteristics and the level of preparation of the subjects. A study has shown that the level of endostatin has an inverse relationship with capillary density and tissue metabolic characteristics (Brixius et al., 2008). It is possible that exercise reduces the amount of transformation in the extracellular matrix and this may prevent the separation of endostatin from collagen (Noris et al., 1995). Levels of Endostatin are closely related to muscle mass and aerobic capacity. Various factors such as hypo-

-xia , shear stress, muscle contraction and stretching and metabolites produced due to exercise have an effect on the expression and release of VEGF and endostatin (Taheri Chadorneshin et al., 2017; Vital et al., 2014). Therefore, in our study, it seems that EMS has led to the release of VEGF and the reduction of endostatin through the effect on the above pathways. These factors have the greatest effect on VEGF release and endostatin reduction. The induced hypoxia can increase the release of VEGF from endothelial cells and skeletal muscle (Strijdom et al., 2013).

EMS improves vascular function by increasing blood flow and shear stress in endothelial cells. It seems that EMS can have a greater effect on the expression and release of VEGF and the reduction of endostatin through the effect on the activation of the calcineurin pathway, shear stress (NO) and hypoxia (HIF-1) (Rullman et al., 2007). One of the limitations of the present study is the lack of measurement of shear stress patterns, including retrograde, anterograde shear stress patterns, and slattery index; therefore, similar studies are suggested to measure these indices in inactive people.

Conclusion

The results of the present study showed that EMS stimulation increases vascular endothelial growth factor and decreases endostatin in inactive men. These findings may provide a new insight in order to better understand capillary density changes in response to EMS protocol.

What is already known on this subject?

The effectiveness of EMS on muscle and physical performance has been demonstrated in various populations, including healthy young adults.

What this study adds?

EMS stimulation increases vascular endothelial growth factor and decreases endostatin in inactive men.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All participants were provided, prior to commen-cement of the study, with information sheets explaining intent to publish, with option to receive copies of submitted manuscripts.

Informed consent done.

Author contributions

Conceptualization: M.N, S.Sh.; Methodology: M.N, S.Sh.; Software: M.N.; Validation: S.Sh.; Formal analysis: S.Sh.; Investigation: S.Sh.; Resources: M.N.; Data curation: M.N.; Writing - original draft: M.N, S.Sh.; Writing - review & editing: M.N, S.Sh.; Visualization: M.N.; Supervision: S.Sh.; Project administration: S.Sh.; Funding acquisition: S.Sh.

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