

# The Effect of Concurrent Endurance and Resistance Exercise on Plasma Levels of Vascular Endothelial Growth Factor and Endostatin in Inactive Women

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## Abstract

**Introduction:** The present study aims to investigate the effect of one concurrent endurance and resistance exercise training session on plasma levels of vascular endothelial growth factor (VEGF) and endostatin in inactive women.

**Methods:** In this quasi-experimental study, 12 inactive female university students were divided into control (average age=20.0 ±1.78 years, height = 161.3 ±50 cm, weight = 58.83±5.71 kg, BMI = 22.38±3.23 kg/m<sup>2</sup>) and exercise (average age =19.5±1.04 years, height = 163.1±3.79 cm, weight = 58.67±7.69 kg, BMI =22.35±2.22 kg/m<sup>2</sup>) groups. The endurance exercise was performed on a treadmill with 65% of MHR for 16 minutes. The resistance exercises of four movements including leg press, chest compression, armpit and leg stretch were performed at 50% 1RM in two sets with 10 repetitions and 1-2 minutes' rest intervals between sets. Data were analyzed using dependent t-test and independent t-test to determine differences within the group and between-group at p<0.05.

**Results:** The results showed that VEGF concentration in training group significantly increased in comparison with the control group (p= 0.004). But, plasma endostatin levels in training group did not change in comparison with the control group (p= 0.168).

**Conclusion:** According to the findings, it seems that one session of concurrent endurance and resistance exercise has a positive influence on VEGF and probably it can help to improve the condition of angiogenesis.

**Keywords:** Vascular Endothelial Growth Factor, Endostatin, Concurrent Endurance and Resistance Exercise

## Introduction

Studies have shown that physical activity is a strong angiogenic stimulant in skeletal and cardiac muscles (1). While endurance training exercise increases capillary density in skeletal muscle (2), resistance exercise is most likely to reduce capillary density as a result of muscle fiber hypertrophy with inadequate angiogenesis (3). There is not enough knowledge about the exact mechanism of blood vessel growth following different

exercise protocols. The capillary network expands through angiogenesis, which is regarded as the process of formation of new blood vessels from the existing vessels in skeletal muscles (4). This process is regulated by a wide range of deterrent and stimulating factors which are released into the bloodstream and affect the formation of vessels (5). One of the most important angiogenesis regulators is the vascular endothelial growth factor (VEGF), which is

regarded as a homodimeric glycoprotein with a molecular weight of 35-25 kDa and a strong mitogenic effect on endothelial cells and plays an important role in the vasculogenesis and angiogenesis through the proliferation and migration of endothelial cells (6). Endostatin as a 20-kDa C-terminal fragment released from collagen XVIII is considered as another important factor affecting the angiogenesis. Endostatin is recognized as a strong angiogenesis inhibitor (7), which decreases the VEGF signaling pathway by reducing phosphorylation of endothelial nitric oxide synthase (eNOS) (8). The inhibitory mechanism of endostatin involves suppressing the endothelial cell proliferation by binding to VEGF and inhibiting its function. In addition, endostatin prevents the capillary basement membrane from being damaged, which ultimately suppresses the endothelial cells migration and consequently the growth of capillary network (9). The equilibrium between angiogenesis and angiostatic factors (10) can be disturbed in different pathophysiological conditions like exercising. During recent years, several studies have shown VEGF responses to short-term exercise. The amount of VEGF mRNA increases during and after the short-term exercise of stretching knees (11-13). However, VEGF should increase at protein level to exert its angiogenesis effects. Hiscock et al (11) showed a progressive increase in both plasma VEGF and VEGF mRNA after a three-hour session of knee stretching in healthy young men, which reached a significant level one hour after the exercise. Further, the VEGF levels of men practiced endurance exercise increased after one-hour exercise with 50% power output (14). In another study, serum VEGF levels increased after a session of intensive exercise until exhaustion (15). However, some studies indicated that VEGF reduces in healthy men with a history of performing physical activities (16) while it remained unchanged after a short duration of exercise on ergometer in healthy but

physically inactive men (14). On the other hand, there are limited studies on the effect of physical activity on the amount of endostatin. The increase (4, 16), and decrease (17, 18) in the serum levels of endostatin after physical activity were reported in this regard. Therefore, the effect of exercise on the serum levels of angiogenesis and angiostatic factors has contradictory results. Further, as far as our knowledge is concerned, no study has yet investigated the response of VEGF and endostatin to concurrent endurance and resistance exercise among inactive individuals. Since angiogenesis plays an important role in facilitating oxygen transfer to skeletal muscle mitochondria and may affect  $VO_2\max$  (19), the evaluation of factors involved in angiogenesis has attracted a lot of attention. Furthermore, VEGF is involved in the maintenance of muscle capillaries. The reduced amount of VEGF in the skeletal muscle leads to a decrease in capillary density and capillary to blood ratio and an increase in apoptosis (20). Therefore, recognition the factors influencing angiogenesis by coaches and doctors is important to improve exercise performance and contributes to the treatment of some diseases like coronary artery and peripheral vascular diseases. Considering the different effects of different exercise protocols on plasma levels of VEGF and endostatin, the present study aims to investigate the effect of endurance and resistance exercise on plasma VEGF and endostatin levels.

## Methods

The statistical population of the present study consisted of non-athlete female university students, among whom 12 were selected and divided into exercise (n=6) and control (n=6) groups. The subjects did not suffer from any specific disease at the beginning of the study. In addition, no regular exercise was observed during the past 3 years. Table 1 indicates the individual characteristics of the subjects. Two days before blood sampling and performing exercise protocol, the control and exercise

groups were asked not to do any exercises. The control group received only blood sampling and the training group performed exercise program after sampling.

The exercise group performed one session of concurrent endurance-resistance exercise. The intensity of the endurance and resistance exercise of the subjects was designed based on estimating their maximum heart rate (MHR) ( $MHR=220 - \text{age}$ ), and measuring one repetition of maximum during one session (1RM), respectively. In addition, the subjects were familiarized with the bodybuilding equipment used and weight training and learned how to run on the treadmill, and were presented safety tips again.

$$1RM = \frac{\text{Weight}}{[1.0278 - (\text{repetition} \times 0.0278)]}$$

At the beginning of the session, the exercise group performed the warm-up program for 10 minutes including slow running and stretching exercises and then started their exercise activities as follows: the endurance exercises were performed on the Italy made Cosmed T 150 DE MED Treadmill. Each subject stood on a treadmill with aero gradient and exercised with a work intensity of 65% for 16 minutes (21). The heart rate of the subjects was monitored during the exercise using Finland made Polar T31 Heart monitor, which was attached to their chest. The subjects performed resistance exercises with bodybuilding equipment, which included foot-press, chest-press, axillary stretching and stretching the legs. All activities (upper limbs and lower limbs) were performed during 2 periods with a rest period of 1-2 min between each, with 50% 1RM and 10 repetitions (21). At the end of the exercise, the subjects performed 10 minutes of movement to back to initial state.

In order to study biochemical variables (VEGF and endostatin values), blood sampling was done after 10-12 hours of fasting before and after the exercise session. In the first stage of blood sampling, the subjects were asked not to do any exercises for two days before blood sampling. First, 7cc of blood was taken in a

resting state from anterior vein of the forearm in the sitting position. The samples were then centrifuged at 4°C and 540×g for 10 minutes. Then, the resultant plasma was collected and stored at -80° C in order to maintain the stability of biochemical parameters (VEGF and endostatin). In the next stage, the subjects were placed in their groups and each group followed their own instructions. The second stage of blood sampling was done immediately after the exercises. The expression level of VEGF protein and the amount of endostatin were measured by ELISA method, along with corresponding kits (Vascular Endothelial Growth Factor (VEGF) ELISA Kit, antibodies, USA, Catalog number: ABIN772617 with sensitivity of <2pg/ml, endostatin ELISA Kit, Catalog number: ABIN771777, antibodies Co. USA), followed by manufacturer's instructions. The height and weight of the subjects were measured in cm and kg, respectively, using a CARMY EB9003 medical scale equipped with a height rod. Finally, the body mass index was calculated using the following formula:

$$BMI = \text{weight(Kg)} / \text{height (m)}^2$$

Kolmogorov-Smirnov and Levene's test were used to investigate the normal distribution of data between the groups and the consistency of variance between the groups, respectively. Data were analyzed using dependent t-test and independent t-test to determine differences within group and between-group at  $p < 0.05$ . All calculations were performed using SPSS 23 software.

## Results

Table 1 shows the anthropometric and physiological characteristics of the subjects.

Table 2 presents the values of VEGF and endostatin plasma as mean and standard deviation in two measurement steps. Based on the dependent t-test results, a significant difference was found between the plasma concentration of VEGF of the training group before and after the concurrent endurance and resistance exercise ( $p=0.015$ ). In other words,

the plasma concentration of VEGF increased significantly in response to concurrent endurance-resistance exercise. Also, Table 2 illustrates the plasma concentration of endostatin in both training and control group before and after a session of concurrent endurance-resistance exercise. As observed, the difference was not statistically significant although the endostatin plasma levels in the

training group decreased after a training period ( $p=0.071$ ).

Also, independent t-test results showed that VEGF concentration in training group significantly increased in comparison with the group control ( $P=0.004$ ) (Table 3). But, Plasma endostatin levels in training group did not change in comparison with the control group ( $P=0.168$ ) (Table 3).

**Table 1.** Mean and standard deviation of subject's individual characteristics in different groups

Groups Index	Control Group (CG)	Training Group (TG)
Age (years)	20.00 ± 1.78	19.50 ± 1.04
Weight (Kg)	58.83 ± 5.71	58.67 ± 7.69
Height (cm)	161.50 ± 4.23	163.00 ± 3.79
BMI (Kg/m <sup>2</sup> )	22.88 ± 3.23	22.35 ± 2.22

**Table 2.** VEGF values of the subjects during pre- and post-test by dependent t-test

Variable	Group	Pre-test	Post-test	t score	p
VEGF (pg/ml)	CG	524.80 ± 81.6	499.00 ± 52.60	-1.22	0.277
	TG	484.03 ± 72.1	608.00 ± 97.00	3.65	0.015*
Endostatin (pg/ml)	CG	5.42 ± 3.05	5.06 ± 1.71	-0.39	0.715
	TG	6.166 ± 2.63493	3.75 ± 2.95	-2.29	0.071

\* significant difference with the before the intervention

**Table 3.** VEGF and endostatin values of the subjects by independent t-test

Variable	Group	Mean difference	t score	P
VEGF (pg/ml)	CG	25.8	3.75	0.004*
	TG	-123.8		
Endostatin (Pg/ml)	CG	0.35	-1.49	0.168
	TG	-2.42		

\* significant difference with the control group after the intervention

## Discussion

The present study investigated the effect of one session of concurrent endurance-resistance exercise on the profile of angiogenesis

regulators. The results of this study showed that one session of concurrent endurance and resistance exercise increased significantly the level of VEGF in the post-test compared to the pre-test in the experimental group. Also, there

was a significant difference in the level of VEGF in the experimental group compared to the control group. The results of the present study are in line with those of other studies which showed that acute exercise leads to an increase in VEGF levels (11-15, 22). In the same vein, Van Pelt *et al.* observed that after an hour of aerobic activity in 60%  $\dot{V}O_{2peak}$  VEGF levels increased among inactive individuals. In addition, Rullman *et al.* (23) reported that VEGF plasma level increases in healthy men after 65 min of cycling. The increase in vascular endothelial growth factor two hours after exercise can be related to skeletal muscle VEGF release into the bloodstream (14). Further, mechanical factors stimulate VEGF expression during exercise such as shear force and muscle stretching (24). HIF-1 exercises is considered as one of the important regulators of VEGF. Aerobic exercises can activate the muscle HIF-1 in human leading to an increase in VEGF level (25). The above-mentioned studies made use of aerobic exercises although the possible mechanism for increasing VEGF is related to the activation of HIF-1 due to these types of activities. The present study used both endurance and resistance exercises in the training session. Based on some studies, other VEGF regulators are Cascading routes including PI3K, mTOR and STAT3 (13). During performing endurance-resistance exercises, resistance exercise can cause muscle hypertrophy, which activates a growth-dependent signal network. Experiments on humans and animals showed that resistance activities lead to an increase in PI3K and mTOR (26). mTOR is the key regulator of the muscle protein synthesis which results in increasing VEGF transcription independent of HIF-1 (27). In response to resistance exercise, the VEGF level increases in a STAT3 activation behavior (28). Therefore, various paths regulate the VEGF level after the resistance exercise. Unfortunately, in the present study, the VEGF level in each exercise forms were not considered to see whether the

increase in VEGF is due to higher endurance type of activity or the resistance one.

In addition, some studies emphasized that the concentration of the vascular endothelial growth factor did not change or even decreased after acute exercise (16). The reduced level of VEGF serum after acute exercise does not mean that the exercise reduces the amount of VEGF production although the temporary reduction of this factor may be in response to the exercise due to VEGF binding to receptors on endothelial cells, which is a stimulus for the occurrence of angiogenesis in the heart and skeletal muscle (29).

Also, the results of this study showed that one session of concurrent endurance and resistance exercise did not have a significant effect on the level of endostatin in the post-test compared to the pre-test in the experimental group. Also, there was no significant difference in the level of endostatin in the experimental group as compared to the control group. Endostatin is an angiogenesis inhibitor factor. Research showed that the endostatin decreases the VEGF cascade route and there is a negative relationship between the endostatin and VEGF serum (16). It seems logical that endurance-resistance exercise that increased plasma VEGF control the release rate of endostatin so that angiogenesis can be performed in the active muscle. Lack of significant difference in endostatin before and after the concurrent endurance-resistance exercise in the present study is consistent with the findings of Rullman *et al.* (23) and Seida *et al.* (30) which observed that exercise does not change endostatin level in inactive men. However, the lack change in endostatin in the present study is inconsistent with the results of some studies (16-18). Brixius *et al.* (31) demonstrated that the endostatin level in obese men decreases in response to prolonged aerobic activity. Further, Suhr *et al.* (32) found that six months of endurance activity significantly reduces the endostatin level in runner man. The results indicate that

endostatin response to exercise depends on the anthropometric characteristics and the physical fitness of the subjects. Recent studies showed that there is reverse relationship between the endostatin level and capillary density and metabolic properties of the tissue (31). The endostatin reduction mechanism in response to exercise is not yet known, but it is probable that exercise reduces the amount of metamorphosis in the extracellular matrix, and this may prevent endostatin separation from the collagen (33). The difference can also be due to the research methodology. In the present study, a session of exercise was performed followed by an immediate blood sampling, but other studies conducted a period of exercises and blood sampling was done 24 hours after the last training session. It is suggested that other factors involved in the angiogenesis process such as angiopoietin, FGF, TGF and other angiostatic factors such as angiostatin should be evaluated for future research.

### Conclusion

According to the findings, it seems that one session of concurrent endurance and resistance exercise has a positive influence on VEGF and probably it can help to improve the condition of angiogenesis.

### Ethical issues

No applicable.

### Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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