Original Article

Alterations of plasma nitric oxide, vascular endothelial growth factor, and soluble form of its receptor (sFlt-1) after resistance exercise: An experimental study

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Abstract

Background: This study was aimed to investigate the alterations of some plasma angiogenic factors after resistance exercise in male rats.

Materials and Methods: Twenty male rats were divided into two groups: Sedentary and trained ($n = 10$ each). The animals in the trained group undertook one training session per day, 3 days/week. After 4 weeks; plasma nitric oxide (NO), vascular endothelial growth factor (VEGF), and soluble form of VEGF receptor-1 (sFlt-1) concentrations were measured.

Results: Plasma NO concentration was not different between groups ($P > 0.05$). Plasma VEGF concentration was also not different between sedentary and trained groups (142.73 ± 3.74 and 144.5 ± 5.1 pg/mL, respectively; $P > 0.05$). Resistance training did not significantly change plasma sFlt-1 concentration ($P > 0.05$). VEGF/sFlt-1 ratio did not alter after exercise.

Conclusion: Resistance training does not alter plasma angiogenic factors (NO, VEGF, and sFlt-1), at least in normal rats. More studies are needed to show the effect of resistance training on angiogenesis process.

Key Words: Angiogenesis, angiogenic factors, resistance exercise

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INTRODUCTION

The risk of cardiovascular diseases, diabetes, high blood pressure, high plasma cholesterol and triglycerides (TGs), and a variety of other diseases is heightened by lack of physical activity. Conversely, regular exercise and active lifestyle help offset many of these risk factors. Yet, what is often neglected is the fact that resistance exercise has benefits beyond just endurance, and enhances metabolic profile in high risk individuals. It has been found that isometric contractions have insulin-like effects on glucose uptake in skeletal muscle.[1,2]

Resistance training creates some adaptation in different parts of the body. The significant consequences of resistance training are transduction

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and change in muscle fibers; particularly type I fibers, hypertrophy of skeletal muscles, and prevention of muscle fatigue involving motor units during work-out.[3] In humans, resistance training reduces serum homocysteine and increases insulin-like growth factor levels.[4] This type of training can increase skeletal muscle size and capillary density, and subsequent muscle cross-section growth without an increase in the number of fibers.[5]

Angiogenesis is the formation of new blood vessels by sprouting from preexisting small vessels in adult and embryonic tissue.[6,7] This process occurs in physiological conditions such as wound healing and menstrual cycle, and also in pathological conditions such as diabetic retinopathy and tumor growth. Angiogenesis is controlled by the activator and inhibitor molecules and autocrine and the balance between them. Difference in endothelial response depends on various stress stimuli along with activators and inhibitors as mediator in angiogenesis process.[7-9] Nitric oxide (NO) is one of the most important factors released from endothelial cells. It is a free radical made from L-arginine by NO synthase and is involved in neural transmission, vascular functions, defense, and inflammation. NO causes vasodilatation and prevents accumulation of platelets and adhesion of leukocytes.[10] NO is also an important factor in angiogenesis process.[11] Other factor that involves in angiogenesis is vascular endothelial growth factor (VEGF). VEGF is a 45 kD factor and also known as vascular permeability factor.[10,12] VEGF plays an important role in the growth, proliferation, and migration of endothelial cells[13] and involves during angiogenesis process and collateral vessels formation.[14,15] This angiogenesis effect of VEGF is also observed in *in vitro*[16,17] and *in vivo*[18,19] studies. VEGF has three types of tyrosine kinase receptors: VEGF R1 (Flt-1), VEGF R2 (KDR/Flk-1), and VEGF R3 (Flt-4). The plasma soluble form of VEGF R1 (sFlt-1) binds to VEGF in plasma and has a special importance as regulator of angiogenesis function. VEGF regulates inflammatory responses, angiogenesis, and vasculogenesis through VEGF R1 and VEGF R2 signalings.[20,21]

Studies have shown that endurance training has positive effects on angiogenesis.[22] This study aims to investigate the effects of resistance exercise on plasma level of biomarkers of angiogenesis in male rats.

MATERIALS AND METHODS

This experimental study was conducted on 20 male Wistar rats (288 ± 22 g). The animals were obtained from Pasteur Institute (Tehran, Iran) and maintained in the Central Animal House, School of Medical Sciences, Tarbiat Modarres University, Tehran and kept until they were 12 weeks old. They were then randomly divided into two groups (trained and sedentary). The trained group undertook 4 weeks of resistance training program. Training was accomplished using a 1-m ladder inclined at 80°. There were 26 rungs evenly spaced on the ladder. Before inducing diabetes, the rats in the trained group were familiarized with the exercise by practicing climbing up the ladder. The rats were positioned at the bottom of the climbing apparatus and motivated to climb the ladder by touching and tapping their tails. In order to minimize stress to the rats, we did not utilize electrical stimulation, forced air, food restriction/reward, and cold water to encourage the rats to perform exercise training. When the rats reached the top of the ladder, they were allowed to rest.

The initial weight was added as 30% of rats' body weight after introducing weight fixer on their tails. Weights gradually increased over 2 weeks, so that they could perform three consecutive sets with 100% body weight (six repeats in a set) by the end of 2 weeks. This exercise continued until the end of 4 weeks and 3 days per week.[23] Blood samples were taken from the heart 48 h after the last exercise and poured in Falcon tubes. After clotting, they were centrifuged at 4,000 rpm for 15 min; thereby separating their plasma, which was then frozen at -70°C for subsequent analysis. The Griess reagent kit (Promega Corp, USA) was used to measure serum nitrite level (as a major metabolite of NO). Plasma VEGF and VEGF R1 levels were measured using ELISA (enzyme-linked immunosorbent assay) method (R and D systems, USA; Cat ≠ RRV00 and Cat ≠ MVR100, respectively). Plasma TG, glucose, cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL) concentrations were measured with enzymatic and calorimetric methods.

Data is reported as mean ± SE. One-way analysis of variance (ANOVA) was used for comparison of data between groups using Tukey's *post hoc* test. $P < 0.05$ were considered as statistically significant.

RESULTS

The effects of resistance training on physiological parameters are presented in Table 1. Changes in plasma glucose level between trained group and control group was not significant ($P > 0.05$). Plasma insulin level in the control group was higher than training group, although the difference was not significant ($P > 0.05$). Also, there was no significant difference in plasma levels of TGs, cholesterol, LDL, and HDL between two groups.

Evaluation of plasma NO level showed that there was no significant difference in plasma NO level between sedentary and trained groups ($P > 0.05$) [Figure 1b].

No significant difference was found in plasma VEGF level between sedentary and trained groups (142.73 ± 3.74 and 144.5 ± 5.1 pg/mL, respectively) ($P > 0.05$) [Figure 1a]. Also, plasma level of VEFG R1 (sFlt-1) did not indicate significant difference between two groups ($P > 0.05$) [Figure 1c]. Resistance training did not alter the VEGF: sFlt-1 ratio [Figure 1d].

DISCUSSION

In the present study, there was no significant difference in plasma glucose and lipid profile between two groups, and plasma insulin level was lower in the trained group, although the difference was not significant. In a similar study by Elliott *et al.*, it was reported that resistance training did not affect lipid profile compare to sedentary group[24] and they suggested that insufficient duration and intensity of the training may be the cause of those results. In another study, Sung *et al.*, surveyed the effects of

Table 1: Plasma glucose, insulin, and lipid profile in sedentary and trained groups

Factor/group	Sedentary	Trained
Glucose (mg/dL)	126.12±2.04	130.00±2.28
Insulin (IU/mL)	0.54±0.10	0.49±0.03
LDL (mg/dL)	33.31±2.77	40.08±3.53
HDL (mg/dL)	27.95±0.76	27.61±1.17
TG (mg/dL)	64.62±3.87	59.62±2.49
Total cholesterol (mg/dL)	75.37±3.42	79.62±3.85

LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglyceride

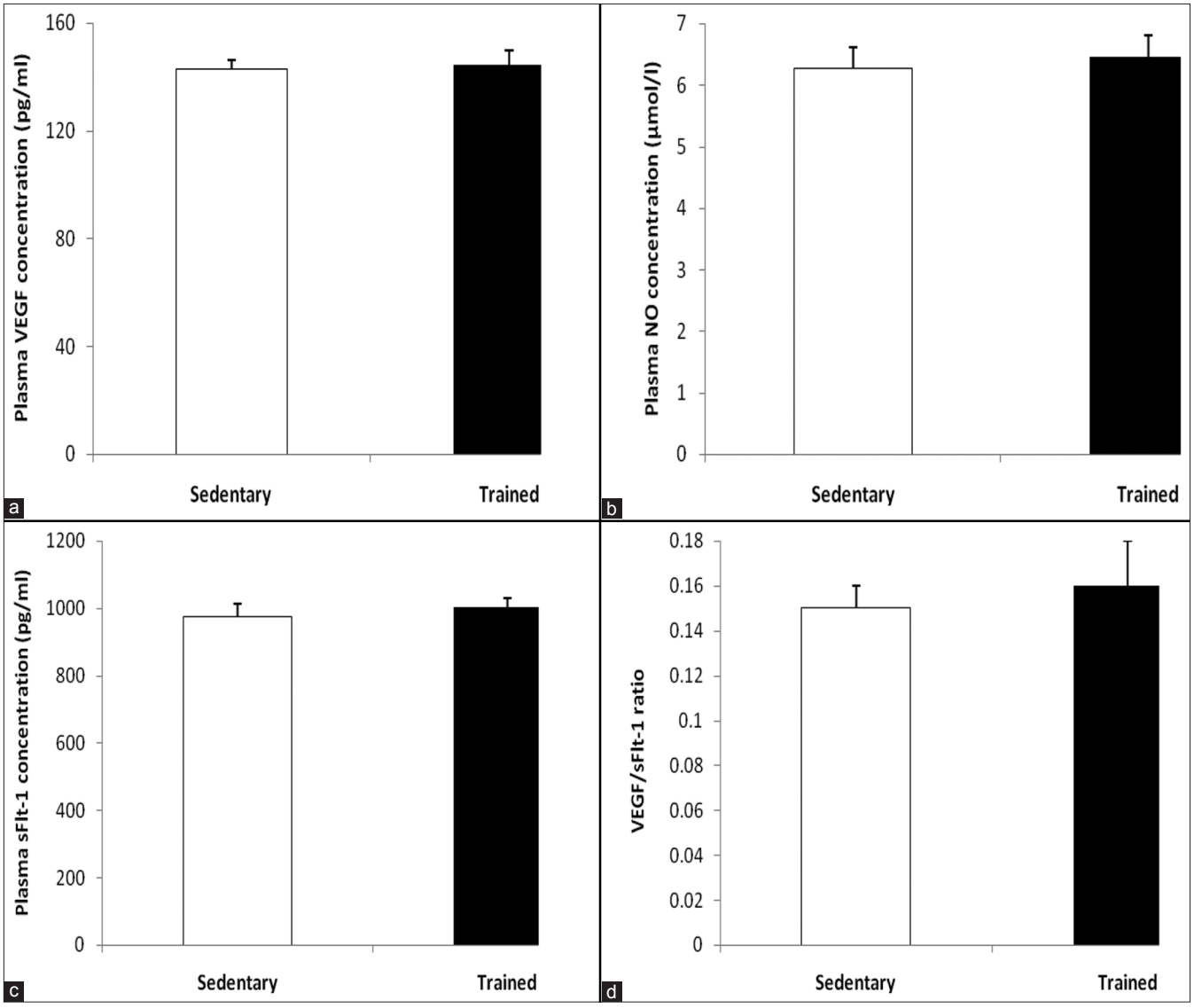

Figure 1: Plasma VEGF (a), NO (b), sFlt-1 and VEGF: sFlt-1 ratio in sedentary and trained groups. VEGF = vascular endothelial growth factor, NO = nitric oxide, sFlt-1 = soluble form of VEGF receptor-1

dietary intervention and strength training on blood lipid level in obese children. Their results showed that serum total cholesterol was significantly reduced in both groups and LDL: HDL ratio was significantly decreased in the training group. Possibly, the low calorie regimen is the main reason for improvement in lipid profile,[25] whereas in present study the animals used their normal, usual regimens.

Some important points should be considered on the effect of resistance training on lipid profile plasma. Firstly, longer duration of exercise (over 8 weeks according to some studies) appears to be more effective.[26,27] Secondly, some researchers believe that exercise rarely has little effect on total cholesterol and LDL levels, unless it is combined with calorie regimen or weight loss.[28,29] Thirdly, aerobic and resistance training does not have many effects neither on lipid profile nor particularly on HDL when TG levels is normal level.[30] In the present study, no significant change was found in the lipid profile after 4 weeks of resistance exercise, and it is probable that the desired effect on lipid profile could have been gained with longer exercise duration or with simultaneous use of specific nutrition regimen.

We also showed that resistance training enhanced plasma NO level, although it was not significant. This result concurs with that obtained by Jungersten *et al*.[31] Other studies have also shown that during exercise, many of vascular limitations could improve and influence metabolic factors by NO.[32] Also, many studies have admitted positive effects of NO, but some have expressed that increased production of NO during exercise may reduce the iron status.[33]

The effect of physical activity on plasma VEGF level also shows conflicting results. Some studies have shown that acute exercise increases serum VEGF level,[34] while others have reported no change or even reduction in VEGF concentration.[35] It does not mean that exercise causes reduction in serum VEGF, but this transient reduction may be due to VEGF connection to endothelial cell receptors. This connection stimulates angiogenesis process in heart and skeletal muscles.[35] It has been shown that VEGF transcription level in skeletal muscle is the most important factor in serum VEGF level 2 h after exercise[36] and an increase in serum VEGF could be due to transfer of skeletal muscle VEGF into the blood circulation.[37] In a study by Kraus *et al*., VEGF level increased after 2 and 4 h of aerobic exercise in active and passive people.[36] In our study, changes in plasma VEGF level 48 h after exercise were not significant, which may be due to the time of measurement. On the other hand, exercise without subsequent changes in serum VEGF level could increase angiogenesis by increasing VEGF bonding to its receptors which needs further studies.

We also found that plasma VEGF R1 level did not change after resistance training. VEGF R1 is the first of VEGF receptors, but its function is not fully clear. It causes negative regulation of VEGF action and division on endothelial cells.[38] VEGF R1 has a soluble form called soluble VEGF R1 (sVEGFR1) or soluble fms-like tyrosine kinase-1 (sFlt-1), which was measured in this study. As this soluble receptor lacks the transmembrane and intracellular signaling domains tyrosine kinase, it cannot create a message[39] and remains as an inactive receptor. By bonding to VEGF, this receptor prevents ligand to affect main VEGF receptor. Therefore, an increase in VEGF R1 (soluble form) reduces the function of VEGF. As it has been suggested that ratio of VEGF/sFlt-1 may be a better index of angiogenesis,[40] this ratio was also measured and although in the training group, it was slightly higher than in the control group, however, the difference between the two groups was not significant.

CONCLUSION

Resistance training did not alter plasma angiogenic factors (NO, VEGF, and sFlt-1 concentrations) in normal rats. However, the exact effect of resistance training on angiogenesis needs further studies.

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