The Effect of Acute Sub-Maximal Endurance Exercise on Serum Angiogenic Indices in Sedentary Men

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Article information

Abstract

Background: Endurance training increases capillary density of skeletal muscle, but the molecular mechanism of this process is not yet clear. Therefore, the purpose of this study was to investigate the effect of acute sub maximal endurance exercise on serum levels of vascular endothelial growth factor (VEGF) and matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) in sedentary men.

Materials and Methods: Twelve healthy men (22.37±2.30 years, BMI=23.16 ±2.61 kg/m²) participated in this study. Subjects exercised for 1h at 70% of VO2 max, 3 days before the VO2 max determination. Antecubital vein blood was collected at rest, immediately and 2h after the exercise. Serum VEGF, MMP-2 and MMP-9 were measured by ELISA methods.

Results: Serum levels of VEGF and MMP-2 decreased immediately after the exercise. 2 hours after the exercise, serum levels of VEGF remained at a lower level but serum MMP-2 returned to its basal level. Also, serum levels of MMP-9 did not change significantly in response to exercise.

Conclusion: Acute sub-maximal endurance exercise decreased the main factors involved in development of capillary density in sedentary men. This might be due to the fact that, sub maximal exercise could not provide the two main stimulating factors of angiogenesis, i.e. Shear stress and hypoxia. It could also be explained by the fact that the mechanism of development of capillary network following regular endurance training is different from that following an acute exercise.

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Introduction

One of the most profound changes following exercise is increment of capillary density of skeletal muscle, which is dependent on angiogenesis process [1, 2]. Angiogenesis, the growth of capillaries from preexisting vessels [3], occurs in the normal physiology statues, in response to the exercise or in the process of wound healing [4].

The process of angiogenesis occurs as an orderly series of events: diseased or injured tissues produce and release angiogenic growth factors (proteins) that diffuse into the nearby tissues and then they bind to specific receptors which located on the endothelial cells (EC). Thereby the endothelial cells become activated. Signals are sent from the cell's surface to the nucleus. The endothelial cells machinery begins to produce new molecules including enzymes (e.g. MMPs), that they dissolve tiny holes in the sheath-like [5].

The endothelial cells begin to proliferate and then migrate out through the dissolved holes of the existing vessel towards the diseased tissue (tumor) [6]. After this, Additional enzymes (MMPs) are produced to dissolve the tissue, which is remolded around the vessel. Then sprouting endothelial cells roll up to form a blood vessel tube, beside individual blood vessel tubes connect to form blood vessel loops that can circulate blood. Finally, newly formed blood vessel tubes are stabilized by specialized muscle cells (smooth muscle cells, pericytes) that provide structural support. Then blood flow begins [7]. Growth factors and MMPs play a central role in mediating the process of angiogenesis [8, 9].

Vascular endothelial growth factor (VEGF) is a main angiogenic growth factor [10]. VEGF is a homodimeric glycopeptides and has been characterized as a potential growth factor in angiogenesis of endothelial cells. VEGF have six isoforms, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PIGF), which acts as ligands for receptors VEGFR-1, VEGFR-2, and VEGFR-3 [8, 10].

VEGF-A and VEGF-B are able to bind to activate VEGFR-1 and -2 receptors and they are considered the main growth factors for physiological and pathological angiogenesis, while VEGF-C and VEGF-D are primary lymphangiogenic factors [8]. VEGF increases the proliferation and migration of endothelial cells during capillary sprouting. VEGF is important for basal skeletal muscle capillarization as well as aerobic exercise-induced angiogenesis.
[11]. Various factors such as hypoxia [12, 13], shear stress [14], muscle contraction and adenosine [15] affects the VEGF-A production. The second stage of angiogenesis depends to secretion of protease enzymes. In turn, studies have been shown angiogenesis depends on the activity of matrix metalloproteinases (MMPs). MMPs are a group of calcium and zinc endoproteinases which acts as proteolytic enzymes in degradation of extracellular matrix of connective tissues. More than 25 MMPs family members were known, which can be subdivided based on their substrate specificity as either Collagenases (e.g., MMPs 1, 8, 13, MT1-MMP), Gelatinises (MMPs 2, 9), or Stromelysins (MMPs 3, 10, 11), and by cellular localization as either diffusible or membrane-type MMPs (MT-MMPs) [16-18]. They are secreted in latent form and activated in the pericellular or extracellular environment after a small peptide is cleaved from their N-terminus. Plasmin and later by other family enzymes are activated MMPs. Two MMPs that are thought to play an important role in skeletal muscle adaptation to changing contractile demands and in response to injury are MMP-2 (also known as gelatinase A) and MMP-9 (also known as gelatinase B) [5].

Human MMP-2 and MMP-9 have been demonstrated be able to degrade several substrates, including type IV collagen, elastin and fibronectin [19-20]. MMP-2 produce by the fibroblast, chondrocyte, endothelial cells and monocytes and MMP-9 produce by neutrophils, macrophages, T lymphocytes and endothelial cells and cancer cells. These gelatinises have same decomposition but the their pattern of activation and gene expression are quite different [8]. Studies have been shown that endurance exercise causes increment of capillary density of skeletal muscle, but the molecular mechanism of capillary density increment in response to endurance exercise is not well known. Craenenbroeck et al. found that acute single bout exercise increases serum levels of VEGF immediately and 2 hours after the exercise [21] and Gianni et al decreased serum MMP concentrations reported [22]. On other hand, Suher et al did not show any change in amount of VEGF in normal condition at 0.5, 1 and 4 hours post exercise [14]. On the other hand, limited information exists regarding the effects of exercise on serum levels of MMPs. Therefore, in this study we investigated the effects of acute submaximal endurance exercise on serum levels of MMP-2, MMP-9 and VEGF in sedentary men.

**Materials and Methods**

Twelve healthy sedentary men (22.37±2.30 years, VO2 max=37.99±3.82 ml/kg/min) participated in the study. The ethics Committee of Shahid Beheshti Medical sciences University approved the experimental protocol. All participants were informed about the risks and purposes of the study. They all signed written consent for participating in the study. All participants were healthy nonsmokers, with no history of cardiopulmonary disease.

Mean and standard deviation of participant's characteristics shown in table 1.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Mean±SD</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>22.37±2.30</td>
</tr>
<tr>
<td>BMI (g/m²)</td>
<td>23.16±2.61</td>
</tr>
<tr>
<td>Body fat percent (%)</td>
<td>13.79±3.51</td>
</tr>
<tr>
<td>VO2 max (ml/kg/min)</td>
<td>37.99±3.82</td>
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**Maximal O₂ consumption (VO2 max) determination:** VO2 max was measured by ergometer (Monark-Germany) and gas analyzer (Cortex-Germany). The subjects warmed up for 5 minutes on ergometer without any resistance. After warming-up, load increased to 50 watts. After that, the work load increased 25 watts every two minutes up to exhausted. The subjects were persuaded verbally in order to do their best effort. We used the following three criteria to determine VO2 max: 1-when the heart rate reaches to 90% of the maximum heart rate that calculated from 220-age. 2-When the respiratory exchange ratio reaches to more than 1.1. 3-When oxygen uptake reaches to steady state, although the exercise intensity increases. Reaching to two of the three above mentioned criteria, was enough to stop the protocol [23].

**Main Protocol:** At least 3 days after determining of some anthropometrics factors and VO2 max, all subjects were invited to the lab while not to perform any vigorous exercise 48 hours before the test. All participants completed a 1-h cycle ergometer sub-maximal exercise bout at 70% of VO2 max. Blood samples (2 ml) were taken for the measurement of VEGF, MMP-2 and MMP-9 from antecubital vein before, immediately and 2 hours after the exercise. Blood sample before the test was taken after 30 min rest. During the trial, the gas analyzer was attached to subjects and subjects were allowed to drink water. Collected blood was drown in polypropylene tubes and was allowed to clot for 30 minutes before centrifugation. Then serum was obtained by centrifugation for 10 minutes at approximately 3000 x g. MMP-2, MMP-9 and VEGF were measured in serum. After that samples were stored at -80°C. Serum VEGF-A concentrations were measured using commercially available ELISA kit: (VEGF-A, ELISA, USCN LIFE Science Inc Wuhan, PR China) Intraassay CV%: 7.1 & Sensitivity: 19.8 pg/ml), serum MMP-2 concentrations were measured using commercially available ELISA kit: (MMP-2, ELISA, USCN LIFE Science Inc, Wuhan, P.R. China) and VEGF-A concentrations in serum and MMP-9 concentrations were measured using commercially available ELISA kit: (MMP-9, ELISA, USCN LIFE Science Inc, Wuhan, P.R. China) in serum.

**Statistical Analyses:** In order to analyze data, we used the SPSS-16. For normality of data Kolmogrov-Smirnoff was used. Repeated-measures ANOVA were used to test for differences in serum angiogenic factors within individuals in different time course. Significant level was set at p≤0.05.
Results

Result of this study showed that the serum VEGF changed significantly followed by acute sub-maximal exercise \( (p=0.0001) \). Immediately after the exercise serum VEGF decreased by 49\% \( (p=0.0001) \), while two hours after the exercise, increased significantly 124.82±39.29 pg/ml, but it was still under basal level \( (p=0.015) \) (Fig. 1). Serum MMP-2 also was affected by acute exercise \( (p=0.028) \). Amount of serum MMP-2 immediately after the exercise, decreased 41\% \( (p=0.030) \) but returned to basal level, 2 hours after the exercise (Fig. 2). The results also showed that the amount of MMP-9 was not affected by acute the exercise. This enzyme levels immediately and two hours after the exercise did not change significantly (Fig. 3).

Discussion

The main finding of the study was that acute bout of sub maximal exercise caused 40-50\% decrease in serum levels of angiogenic factors of VEGF and MMP-2 in healthy young sedentary men. These change was contrast with findings of Danzig and colleagues and Emilin et al, while was in line with the findings of Gianni and colleagues. Considering the available information, this study is the first document which reported a decrease in serum MMP-2 following acute exercise. Danzig et al. and Emilin et al. showed that VEGF levels do not change immediately after and two hours after exercise. However, according to Danzig immediately and after the exercise, MMP-9 increased while MMP-2 did not have any changes [24]. The reason for the differences between the results of this investigation and the findings by Emilin and Danzig could probably relate to the intensity of exercise. Both researches had used exhausted, progressive and short protocol while in this study the intensity of exercise was moderate, constant and prolonged (\( \text{VO}_2 \text{max} 70\% \)). The result of this study was in contrast with the findings of Koskinen et al. in which no changes of serum MMP-2 following the acute exercise was reported [25]. The reason may be related to the type of contraction, in that research the downhill running, an eccentric exercise, was used. Intensity [26] and type of exercise [27] are the main factors affecting the serum concentrations of MMPs. The mechanism responsible for the transient decrease in VEGF circulating caused by exercise is poorly understood. Gianni et al. showed that decreased amount of serum VEGF after acute exercise does not mean the decrease in the amount of VEGF production in muscle. However, possible explanations might be as follows: 1) increased VEGF binding-affinity to its receptors at the endothelium, which would stimulate angiogenesis in the local tissues such as heart and skeletal muscles, and 2) a substantial increase in VEGF circulating binding proteins such as heparin sulfate [14] and Endothelial Progenitor Cell (EPC) [28], which would protect the vascular system from a deleterious increase in VEGF-induced hyper permeability [22]. Suhr et al. showed that VEGF plays an important role in release of the EPC from the bone marrow [14]. It should be noted that EPC levels are increased following acute activity. On the other hand endostatin as angiostatic factor that prevented the activation of VEGF increases following acute exercise [29]. It is therefore possible that this decrease be the result of the increase in endostatin [30]. Hypoxia is the most stimulate for angiogenesis. Probably in this study exercise with 70\% \( \text{VO}_2 \text{max} \), did not induce decrease of level \( \text{O}_2 \) in endothelial cell. In the
presence of oxygen, the product of von Hippel-Lindau tumor-suppressor gene, pVHL, which is the main regulator of Hypoxia Inducible Factor-1 (HIF-1) attaches to hydroxylated proline residues within HIF-1. Once bound, pVHL attaches a protein ubiquitin to HIF-1, which designate HIF-1 for destruction by the proteasome. In the absence of oxygen, HIF-1 accumulates and activates the transcription of hypoxia-inducible genes. The products of genes targeted by HIF-1 are PDGF, TGF-beta, VEGF and erythropoietin [31]. Furthermore VEGF with MMP-2 has an interrelation. MMP-2 causes separation of VEGF from proteoglycans. Results of this study showed that the amount of serum levels MMP-2 decreased immediately after exercise, the possibility also exists that the reason for the 49% reduction in serum VEGF be the 41% reduction in serum MMP-2. Two hours after exercise MMP-2, increased by 44% while VEGF by 39%. Research has shown that the most important factor to regulate serum VEGF levels two hours after exercise, is transcription of VEGF in skeletal muscle [24]. Rulman et al. showed that two hours after the exercise, mRNA and protein of VEGF level in skeletal muscle, increased respectively, six and 1.6 folds [29]. This further supports that VEGF-A is mainly regulated pretranslationally in exercising human muscle. On the other hand Hoffner et al. showed that VEGF levels interstitial of the tissue after exercise increased. So we can conclude that increased serum VEGF in two hours after the exercise induced transfer of VEGF- skeletal muscle into blood flow [32]. The increase in skeletal muscle VEGF because of the transfer serum VEGF into skeletal muscle was rejected by Rulman. He also showed that decreased serum VEGF is not related to peripheral uptake of VEGF-A to the exercising leg, it must reflect uptake by tissues other than those in the exercising leg.

The results also showed that the amount of MMP-2 serum decreased immediately after exercise, and 2 hours after the activity returned to initial level. The changes in serum levels of MMPs are due to the changes in the tissues. Alpha two macroglobulin, TIMPs and TSP-1 are important MMPs inhibitors [33]. Previous study pointed that in normal conditions there’s a balance between the angiogenic and angiostatic factors but during exercise this balance altered to the angiostatic factors. TIMPs mRNA immediately after and 2h after exercise increased respectively 1.5 and 2.6 folds. These increases of TIMPs may decrease the serum MMP-2 in response to acute exercise [29]. Furthermore koskinen indicated an increase of complex MMP-2/TIMP-2 in response of acute exercise [26]. Alpha two macroglobulin, the largest serum antiprotease, is produced by macrophages and liver’s fibroblast. This factor affects all proteins, regardless of their classification. Alpha two macroglobulin is connected only to active MMPs [34], and shows increase of this angiostatic factor following acute exercise [35]. TSP-1 is another angiostatic factor connecting to pro-MMP-2 and pro-MMP-9 and prevents the activation of MMP-2 and MMP-9 [36, 37]. Researcher has shown the increase of the amount of TSP-1 in response to acute exercise [38]. Therefore, the increase of TSP-1 may reduce the level of serum MMP-2. Research has shown that MMP-2 has ability to connect to integrin receptors. This connection of MMP-2 to integrin receptors may also cause a decrease in serum MMP-2 [39].

This study did not measure amount of angiostatic factor, and these reasons are all just probabilities. MMP-2 levels returned to the initial level two hours after exercise, showing that the reduction of MMP-2 following acute exercise is temporary and transitory, and this change can be due to transcription, transport and secretion of MMP-2 in the skeletal muscle and endothelial cell into blood flow at 2 h after exercise [29, 40].

The serum angiogenic factors are activated by a single bout of exercise and seem to be due to a combination of pre- and posttranslational mechanism. The amount of MMP-9 immediately and two hours after the activity did not change significantly. Although some researchers reported the increase of MMP-9 following acute exercise, this difference can be due to the macrophages and inflammatory responses [14]. The rate of angiogenic factors two hours after exercise than immediately after the activity was significantly increased. These changes indicated that the reduction that was observed immediately after the activity is temporary and transitory. Furthermore, in order to see real changes of angiogenic factors after activity, it is recommended that the future researches use more than two hours duration for measuring VEGF and MMP-2 factors to consider. In summary, this study showed that the rate of angiogenic factors VEGF and MMP-2 that play a crucial role in the development of capillary network, after acute endurance exercise in inactive young men, decreased respectively, 49 and 41% percent.

This reduction was temporary and transitory. In the present study moderate exercise intensity was unable to troop Hypoxia conditions which are prerequisite of stimulating the angiogenesis. And for increasing the capillary density and therefore the aerobic capacity development the study recommends higher intensity and exhausted exercise. How the regular exercise training can develop the capillary network may have a different mechanism than a single practice session. So Molecular angiogenesis process requires further investigations in the future.

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References


