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THE SERUM LEVELS OF GROWTH FACTORS: PDGF, TGF-BETA AND VEGF ARE INCREASED AFTER STRENUOUS PHYSICAL EXERCISE

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Strenuous physical exercise induces muscle fibers damage and non-specific inflammatory response. Activated by inflammatory process cells may serve as the source of wide spectrum of inflammatory mediators and growth factors. Namely Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-ß (TGF-ß) and Vascular Endothelial Growth Factor (VEGF) could be released. The aim of present study was to assess the impact of physical exercise on growth factors generation in healthy young people. 14 young sportsmen were enrolled into the study. They performed strenuous physical exercise. Blood samples were drawn before, immediately after, and 2 hours after the exercise bout. Serum PDGF, TGF-beta and VEGF concentrations were measured using commercially available ELISA kit based on immunoenzimatic method. Serum level of PDGF increased significantly from 1.7 ng /ml before to 4.64 ng /ml (2.73-fold) immediately after, and to 3.3 ng /ml (1.94-fold) 2 hours after exertion. Serum level of TGF-beta increased significantly from 20.58 ng /ml before to 55.37 ng /ml (2.7-fold) immediately after, and to 40.03 ng /ml (1.95-fold) 2 hours after exertion. Serum level of VEGF increased significantly from 91.83 pg /ml before to 165.61 pg /ml (1.8-fold) immediately after the exercise. Two hours after the exertion serum level of VEGF was 137.22 pg /ml, what is 1.49-fold above the basal level; however not being significantly different. In summery, observed increased level of growth factors could be involved in the process of adaptation of human organism to physical training. In addition, in the context of the role of inflammation in the pathogenesis of various diseases, our results point to the potentially deleterious effect of strenuous physical exercise.

Key words: *Physical exercise, PDGF, TGF-beta, VEGF, atherosclerosis, cancer*

INTRODUCTION

It is widely established that physical exercise plays a beneficial role in the prevention of several diseases, especially coronary artery disease, hypertension, and glucose intolerance. (1-5)

Recently published data suggests that physical exercise induces muscle damage and non-specific inflammatory response, which is manifested by elevated concentrations of circulating proinflammatory cytokines, for example IL-1, TNF-α and IL-6. (6) Activated in inflammatory response cells such as macrophages, platelets, endothelial cells and connective tissue cells may serve as the source of other inflammatory mediators and growth factors, for instance Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-ß (TGF-ß) and Vascular Endothelial Growth Factor (VEGF) (7, 8).

PDGF is a member of the family of dimmers consisting of two "classic" chains: A and B. Recently C and D chains were isolated. (9) PDGF causes cell migration and proliferation. It also acts as chemoatractant for monocytes, fibroblasts, neutrophils and myocytes. PDGF increases synthesis of collagen and regulates angiogenesis. PDGF plays also significant role in embryogenesis. (8, 10)

TGF-ß belongs to the family of dimmers that includes three isoforms: TGF-ß 1, TGF-ß2 and TGF-ß3. It is a growth inhibitor to most epithelial cells in the culture. Its effect on fibroblasts and smooth muscle cells depend on its concentration: in low concentration it stimulates production of PDGF and is thus indirectly mitogenic; in high concentration it is a growth inhibitor because of its ability to lower PDGF receptor expression. TGF-ß also regulates embryonic development, collagen production and degradation and angiogenesis. (7)

VEGF belongs to the family of at least 6 isoforms. VEGF stimulates endothelial cells proliferation and differentiation, increases vascular permeability, prevents endothelial apoptosis and regulates vasodilatation. VEGF promotes angiogenesis in cancer, chronic inflammatory states and in healing wounds. It also plays role in matrix remodeling, acts as chemoatractant for monocytes and promotes expression of adhesion molecules. Hypoxia is the main stimulus for VEGF production/expression. Other growth factors, such as TGF- ß and PDGF as well as cytokines IL-1 and IL-6 also have the potential to up-regulate VEGF expression. (11-14)

Data about the regulation of TGF-beta, PDGF and VEGF by physical exercise is very limited and conflicting.

It is considered, that during physical exercise VEGF is produced locally in skeletal muscle. (15) Increased expression of VEGF mRNA was found in skeletal muscle fibers and in interstitial cells between the muscle fibers. (16, 17) It means that different cell types may contribute to increase production of VEGF in response to physical exercise. Conflicting data exist regarding to serum level of VEGF after exercise. Kraus et al. found that acute bout of physical exercise increases serum level of VEGF right after and 2 hours post exercise (15), and

Schobersberger et al. showed that serum level of VEGF was significantly higher immediately after the marathon race. (18) However, another studies revealed that both, short acute physical exercise and short-time exercise training, in contrast to increased tissue expression, decrease the serum level of VEGF (16, 19)

Hering et al. demonstrated that serum level of TGF-beta increase significantly after 4-week intense strength training. (20) However, the source of this factor is unknown. TGF-beta is one of the major stimuli maintaining bone formation, so it can't be excluded, that it is released locally in the bones due to intense mechanical stimuli. On the other hand Breen et al. reported changes in TGF-beta mRNA expression in muscle after mechanical load (21) and O'Callaghan et al. found increased TGF-beta mRNA expression in vascular smooth muscle cells. (22)

Connolly et al. investigated gene expression for PDGF in human peripheral blood mononuclear cells (PBMCs) after 30 minutes of cycling at 80% of peak O_2 uptake in healthy men. Blood samples were obtained before the exercise started, at the end of exercise and 1hour post exercise. The PDGF gene was upregulated by 1.55-fold after the exercise. (23)

Inflammation with its mediators, among them growth factors, is known to play a very important role in the pathogenesis of various diseases.

The goal of the present study was to evaluate the influence of strenuous physical exercise on growth factors serum level in young healthy well trained males.

MATERIALS AND METHODS

Subjects

Fourteen subjects (males), cyclists, were enrolled into the study. The mean age of each participant was 18 ± 0,5 years. Characteristics of subjects enrolled into the study are shown in *Table 1*.

The Ethics Committee of the Medical University of Warsaw approved the experimental protocol. All subjects were informed about the risks and purposes of the study. They all signed written consent for participating in the study.

The subjects performed graded cycling on the running-truck (Saturn, H-P Cosmos, Nussdorf, Germany) to exhaustion. Mean time of the exercise was 17 min 50 sec. (15 min 00 sec - 22 min 00 sec.) The exercise was preformed above the lactate threshold. The values of lactates and bicarbonates are shown in *Table 1*. The exercise started with short warm-up consisting of 5 minutes of cycling with the speed of 20 km/h and the grade of running-truck equal 0%. After 2 minutes of break, the proper exercise test started. The speed of cycling was 20 km/h and was constant during the whole test. The running truck was upgraded every 3 minutes of cycling: by 1,5 % for the first 3 stages, and than by 1 % up to the cessation of exercise.

The mean values of VO_{2max} for all participants was 65,71 ml/kg/min (± 4,76) and mean values of total O_2 consumption during the whole test was 49,69 (±7,82) L.

The subjects were allowed to consume fluid ad libitum.

Blood samples (n=14) were drawn from antecubital vein before, immediately after and 2 hours after the exercise bout.

2 ml of blood was drown in polypropylene tubes and was allowed to clot for 30 minutes before centrifugation. The serum was obtained by centrifugation for 10 minutes at approximately 1000 x

g. PDGF, TGF-beta, VEGF, potassium, sodium, calcium, creatinine, lactate, and bicarbonate were measured in serum. Before the measurements of PDGF, TGF-beta and VEGF, the samples were stored at - 70°C (not longer than 3 months).

Determination of serum: PDGF, TGF-beta and VEGF in serum

Serum PDGF concentrations were measured using commercially available ELISA kit: Quantikine Human human PDGF-AA Immunoassay.

Serum TGF-beta concentrations were measured using commercially available ELISA kit: Quantikine Human TGF-beta1 Immunoassay.

Table 1. Characteristics of subjects included in the study.

Parameter (norm, units)	**Preexertional**	**Postexertional**	
		Immediately after	2 hs after
Hemoglobin (g/l)	144.2 ± 6.4	150.7 ± 6.8	142 ± 6.1
Potassium (mmol/l)	4.33 ± 0.44	4.31 ± 0.37	4.27 ± 0.29
Sodium (mmol/l)	142.2 ± .,5	143.3 ± 1.44	140.0 ± 2.6
Calcium (mmol/l)	10.44 ± 0.3	10.63 ± 0.30	10.33 ± 0.30
Creatinine (mg/dl)	0.78 ± 0.12	0.89 ± 0.13	0.79 ± 0.10
Lactate (0,8-1,9 mmol/l)	1,81 ± 0,3	13.83 ± 2.8	
Bicarbonate (23-29 mEq/l)	22.07 ± 2.4	9.685 ± 1.88	20.5 ± 2.9
N^O 14; gender males; age: 18 ± 0.5 years			

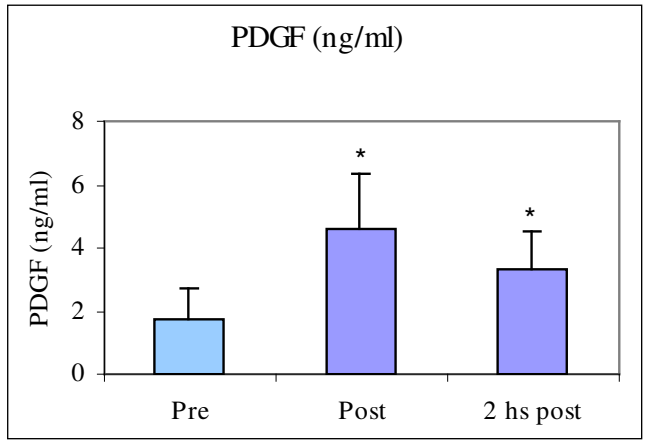

Figure 1. Serum level of PDGF preexercise (Pre), immediately post exercise (Post) and 2 hours post exercise (2h post)

Serum VEGF concentrations were measured using commercially available ELISA kit: Quantikine Human VEGF Immunoassay.

All kits used in the experiment were based on immunoenzymatic method and were produced by R&D Systems, Minneapolis, MN, USA.

Statistical analyses were performed using Statistica 6.0 softwere. Pre- and postexertional values were compared using Student's t test for independent samples. All data are expressed as mean ± SD. A p value of <0.05 was considered as statistically significant.

RESULTS

Serum level of PDGF increased significantly from 1.7 ng /ml before to 4.64 ng /ml (2.73-fold) immediately after, and to 3.3 ng /ml (1.94-fold) 2 hours after exertion. (*Figure1*).

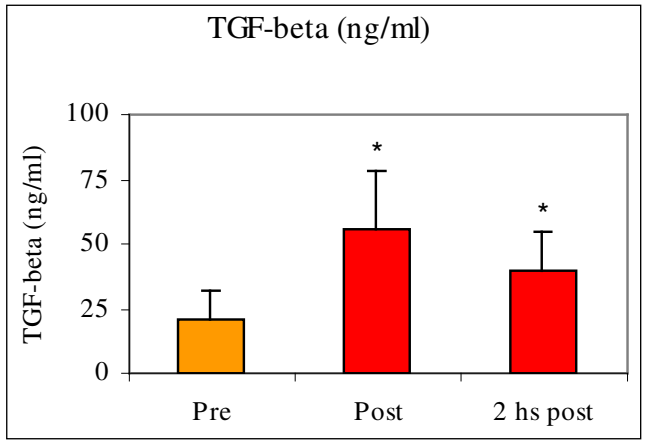

Figure 2. Serum level of TGF-beta preexercise (Pre), immediately post exercise (Post) and 2 hours post exercise (2h post)

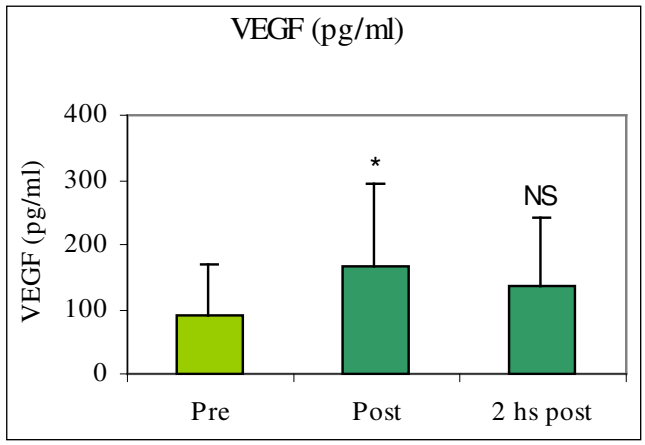

Figure 3. Serum level of VEGF preexercise (Pre), immediately post exercise (Post) and 2 hours post exercise (2h post)

Serum level of TGF-beta increased significantly from 20.58 ng /ml before to 55.37 ng /ml (2.7-fold) immediately after, and to 40.03 ng /ml (1.95-fold) 2 hours after exertion. (*Figure 2*).

Serum level of VEGF increased significantly from 91.83 pg /ml before to 165.61 pg /ml (1.8-fold) immediately after the exercise. Two hours after the exertion serum level of VEGF was 137.22 pg /ml, what is 1.49-fold above the basal level; however the difference was not significant. (*Figure 3*).

DISCUSSION

The main finding of our study was that acute bout of strenuous exercise caused 2-3 fold increase in serum levels of growth factors: PDGF, TGF-beta and VEGF in healthy young sportsmen. This effect was observed immediately and 2 hours after the exercise.

The sportsmen exercised mostly above the lactate threshold, what means that there was hypoxia in contracting muscles.

Growth factors are involved in the pathogenesis of atherosclerosis, which is currently known to be an inflammatory disease initiated by the endothelial cells dysfunction (24-26), and in the pathogenesis of the cancer as well. (27-31)

The group investigated in our experiment consisted of young healthy sportsmen. We would like to check whether previous training influence the serum level of growth factors, and how acute bout of strenuous physical exercise, characteristic for physical training, influence serum levels of growth factors. Such exercise as in our experiment is unusual for sedentary people during leisure physical activity, and because of its intensity, it is much more probable to induce expected changes. Resting levels of growth factors in investigated group were in normal ranges. Because of the role of growth factors in the pathogenesis of various potentially lethal diseases and because of that many young people exercise very hard to achieve the best sport results, it seems to us very important to clarify the reason and the pattern of growth factors production after physical exercise, especially in the group of young sportsmen.

Our findings regarding serum level of VEGF are in line with the results of Kraus et al., who found the increase of VEGF after physical exercise in well trained athletes, but not in sedentary individuals and with the results of Schobersberger et al, who investigated the serum level of VEGF in well trained runners after marathon race on moderate altitude. (15, 18) On contrary, our findings were different from data presented by Gustafsson et al. and Gu et al. (16, 19) However they revealed the decreased level of VEGF after physical exercise, it does not exclude the upregulation of protein level of VEGF as an early event in muscle adaptation to training. The possible reason of decreased serum level of VEGF could be increased VEGF binding to its receptors at the enodothelium, which may stimulate angiogenesis in the muscles or increase in circulating VEGF

binding proteins. (17, 32) The differences between our study and those citied above, especially the one in which serum level of VEGF decreased after acute bout of exercise, could be caused by different exercise protocol. In our study the bout of physical exercise lasted longer and was much more intense.

The angiogenic growth factor VEGF is an important element in angiogenesis, and it is likely that it is involved in the vascular remodeling that occur in response to exercise and muscle contraction. (16, 33)

Data referring to influence of physical exercise on PDGF and TGF-beta serum level is very limited. Most experiments referred to the increased mRNA for TGF-beta and PDGF gene expression. (21, 22, 23). Our findings were in line with the results of Hering et al, however the exercise protocol was different. (20)

Concomitant increase of all three growth factors after physical exercise could suggest the common stimuli for increase of their production. We hypothesize, that it could be tissue hypoxia. In the presence of oxygen, the product of von Hippel-Lindau tumor-supressor 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 proteasom. 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. (34)

However, data referring to HIF-1 is limited and conflicting. Conolly et al. found 2.40 -fold upregulation in HIF-1 gene in PBMCs after acute bout of physical exercise. (23) HIF-1alfa significantly increased in the ischemic muscle in the study performed on mice, although in the concomitant treatment with dimethyloxalylglycine (DMOG), which is activator of HIF. This effect was not so apparent in mice treated with DMOG or ischaemia of the muscle alone, however ischaemia caused 2.5 fold increases in VEGF production, irrespective of DMOG treatment. (35) On the contrary, Gustafsson et al. didn't find any changes of basal levels of mRNA of HIF-1 in the muscle biopsy post exercise training consisting of seven 45-minutes constant-load one-leg knee-extension performed during 10 days. However, they did not exclude the possible impact of HIF-1 on mRNA of VEGF tissue expression, because the main regulation of HIF-1 activity occurs at the protein level of HIF-1-alfa subunits. (16, 36)

The increased level of TGF-beta and PDGF could be involved, along with the VEGF in the process of adaptation of human organism to physical training. But taking into consideration the role of growth factors in the pathogenesis of various diseases, it is very important to recognize in details the mechanism of impact of physical exercise, especially in the context of the "dose" of exercise, on the production and release of growth factors into the circulation. Our results point to the potentially deleterious effect of particular protocols of strenuous physical exercise. Clearly, epidemiologic studies are needed to confirm or refuse above possibility.

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