Research article

Exercise increases endostatin in circulation of healthy volunteers

Jian-Wei Gu*, Giovani Gadonski, Julie Wang, Ian Makey and
Thomas H Adair

Address: Department of Physiology & Biophysics, University of Mississippi Medical Center, Jackson, Mississippi 39216 USA

Email: Jian-Wei Gu* - jgu@physiology.umsmed.edu; Giovani Gadonski - ggadonski@physiology.umsmed.edu;
Julie Wang - jwang@physiology.umsmed.edu; Ian Makey - imakey@physiology.umsmed.edu;
Thomas H Adair - thadair@physiology.umsmed.edu

* Corresponding author

Abstract

Background: Physical inactivity increases the risk of atherosclerosis. However, the molecular mechanisms of this relation are poorly understood. A recent report indicates that endostatin, an endogenous angiostatic factor, inhibits the progression of atherosclerosis, and suggests that reducing intimal and atherosclerotic plaque tissue neovascularization can inhibit the progression atherosclerosis in animal models. We hypothesize that exercise can elevate the circulatory endostatin level. Hence, exercise can protect against one of the mechanisms of atherosclerosis.

Results: We examined treadmill exercise tests in healthy volunteers to determine the effect of exercise on plasma levels of endostatin and other angiogenic regulators. Oxygen consumption (VO_2) was calculated. Plasma levels of endostatin, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) were determined using ELISA. The total peak VO_2 (L) in 7 male subjects was 29.5 ± 17.8 over a 4–10 minute interval of exercise. Basal plasma levels of endostatin (immediately before exercise) were 20.3 ± 3.2 pg/ml, the plasma levels increased to 29.3 ± 4.2, 35.2 ± 1.8, and 27.1 ± 2.2 ng/ml, at 0.5, 2, and 6 h, respectively, after exercise. There was a strong linear correlation between increased plasma levels of endostatin (%) and the total peak VO_2 (L) related to exercise (R^2 = 0.9388; $P < 0.01$). Concurrently, VEGF levels decreased to 28.3 ± 6.4, 17.6 ± 2.4, and 26.5 ± 12.5 pg/ml, at 0.5, 2, and 6 h, respectively, after exercise. There were no significant changes in plasma bFGF levels in those subjects before and after exercise.

Conclusions: The results suggest that circulating endostatin can be significantly increased by exercise in proportion to the peak oxygen consumption under physiological conditions in healthy volunteers. These findings may provide new insights into the molecular links between physical inactivity and the risk of angiogenesis dependent diseases such as atherosclerosis.

Background

Epidemiological data has established that physical inactivity increases the risk of many chronic diseases including atherosclerosis [1-3]. Coronary heart disease, ischemic stroke, and peripheral vascular disease are the clinical manifestations of atherosclerosis. Physical inactivity is believed to be an independent risk factor for the development of coronary heart disease [4], stroke [5], and peripheral vascular disease [6]. However, exercise can exert a beneficial influence on the risk factors for atherosclerosis by reducing hyperlipidemia, hypertension, obesity, platelet aggregability; increasing insulin sensitivity; and

improving glucose tolerance [7]. Despite these findings, the molecular basis of interactions between body and exercise in relation to atherosclerosis are poorly understood.

Although atherosclerosis has complex and multifactorial mechanisms, recent evidence also suggests that angiogenesis plays a critical role in the progression of atherogenesis [8]. Angiogenesis, defined as the formation of new blood vessels from an existing capillary bed, plays an important role in wound healing, body development, and inflammation, which also occurs in a variety of pathological states. Progressive angiogenesis in a primary atherosclerotic lesion has been considered as a cause of plaque expansion, plaque vulnerability, and the risk of significant disease complications such as plaque rupture and vascular thrombosis [8-11]. Mounting evidence [12,13] suggests that angiogenesis is regulated by a net balance between positive (angiogenic) and negative (angiostatic) regulators of blood vessel growth. A balance shifted towards predominantly positive regulators is an angiogenic-phenotype, whereas, a shift favoring negative regulators is an angiostatic-phenotype. Therefore, the impaired regulation of angiogenesis is often associated with the development of angiogenesis-dependent diseases such as atherosclerosis. We hypothesize that exercise can increase endostatin (an angiostatic factor) in circulation, and thereby, it may be one of the mechanisms by which exercise can protect against atherosclerosis.

Why is endostatin thought to be an important molecule linked to angiogenesis-dependent diseases such as atherosclerosis? Endostatin is an endogenous angiostatic factor (inhibitor of angiogenesis) identified originally in conditioned media of Murine hemangioendothelioma cells [13,14]. In mice, recombinant endostatin decreased the size of established primary and secondary tumors, and repetitive application of the peptide prevented recurrence of the tumors by inhibiting tumor angiogenesis [12]. Recent evidence has indicated that endostatin can inhibit the progression of atherosclerosis in an ApoE-deficient mouse model by reducing intimal and plaque tissue neovascularization [15].

The present study seeks to determine: 1) whether exercise can increase plasma levels of endostatin in healthy volunteers; 2) whether increased plasma levels of endostatin are correlated with the intensity of exercise assessed by the peak oxygen consumption; and 3) whether exercise can affect plasma levels of angiogenic factors, such as VEGF and bFGF.

Results

General Characteristics and Response to Exercise

7 healthy-male-volunteers were assigned to the treadmill-exercise study (years of age, 33 ± 13; body weight [kg] 78 ± 13; mean ± SD). The peak speed and the time spent at peak speed on the treadmill were 4.85 ± 0.99 miles per hour and 6.86 ± 2.67 minutes (ranging 4 to 10 minutes), respectively. The total duration of treadmill-exercise lasted an average of 18.86 ± 6.96 minutes. At peak speed, the heart rate was significantly increased by 87% (P < 0.01) from 80 ± 8 beats/min (resting heart rate) to 167 ± 9 beats/min (peak heart rate). Neither abnormal ECG signs nor abnormal symptoms were observed during or after exercise.

Effect of Exercise on Oxygen Consumption

In this study, termination of exercise was based on the attainment of 80–93% of the maximal predicted heart rate and the tolerance of each individual. Therefore, these conditions caused differences in peak oxygen consumption for each volunteer. Using the mathematical equation [16], the estimated peak oxygen consumption (peak VO_2; L/min) was 4.06 ± 1.08 (mean ± SD; n = 7), and ranged from 2.67 to 5.88. The peak oxygen pulse (peak O_2 pulse, ml/beat), calculated by dividing peak VO_2 by heart rate, was 24.36 ± 6.77, and ranged from 16.18 to 37.45. Regression analysis shows a strong linear correlation between peak O_2 pulse and peak VO_2 in different individuals (R = 0.9571; R^2 = 0.9505; P < 0.01). The total peak VO_2 (L), considered as the product of peak VO_2 and the time spent running at that peak, was 29.5 ± 17.8, and ranged from 12.7 to 58.8.

Effect of Exercise on Plasma Levels of Endostatin

Figure 1 shows that exercise increased plasma levels of endostatin in healthy volunteers. Basal plasma levels of endostatin (immediately before exercise) were 20.3 ± 3.2 ng/ml (mean ± SE; n = 7). The plasma levels increased to 29.1 ± 4.2, 35.2 ± 1.8, and 27.1 ± 2.2 ng/ml, respectively, at 0.5, 2, and 6 h after treadmill exercise. The percent increases in plasma endostatin concentrations were 43% (P = 0.014), 73% (P = 0.004), and 33% (P = 0.034) at 0.5 h, 2 h, and 6 h after exercise, respectively, compared to the pre-exercise levels. Figure 2 shows a strong linear correlation between % increase in plasma levels of endostatin and the total peak VO_2 (R = 0.969; R^2 = 0.9388; P < 0.01).

Effect of Exercise on Plasma Levels of VEGF and bFGF

We also investigated the effect of exercise on plasma levels of VEGF and bFGF. We found that acute treadmill-exercise causes a decrease in plasma levels of VEGF in healthy volunteers. These changes are statistically significant at 2 h after exercise compared to the basal levels immediately before exercise (P = 0.0347; n = 7). Basal plasma levels of VEGF were 37.4 ± 7.8 pg/ml (mean ± SE). Plasma VEGF
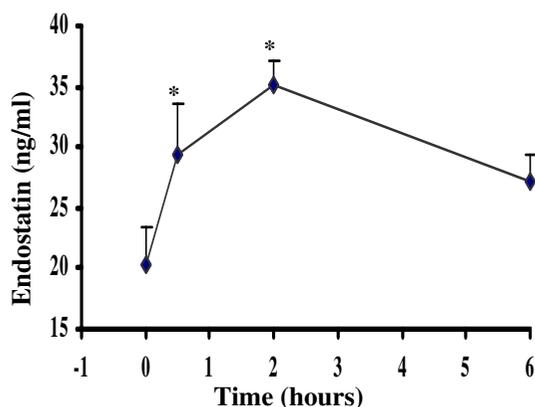
Figure 1
Changes in plasma levels of endostatin before and after exercise at different time point in 7 healthy adults. The plasma endostatin concentrations were increased by 43% (*P = 0.0136), 73% (*P = 0.004), and 33% (P = 0.034) at 0.5 h, 2 h, and 6 h after exercise, respectively, compared to the pre-exercise levels (20.3 ± 3.2 ng/ml, mean ± SE).
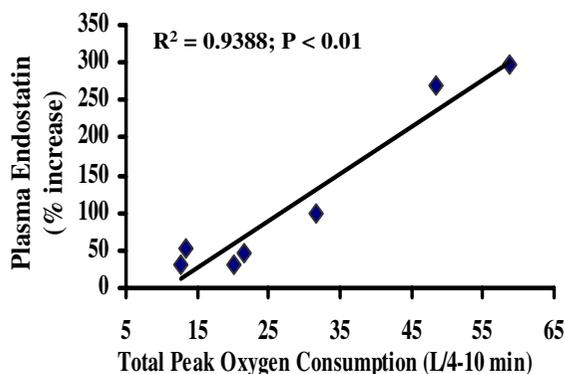

Figure 2
Oxygen consumption (VO_2) during treadmill-exercise in relation to % increased plasma endostatin. The peak speed and the time spent at peak speed on the treadmill were 4.85 ± 0.99 (mean ± SD) miles per hour and 6.86 ± 2.76 minutes (n = 7; ranging 4–10 min), respectively. The total peak VO_2 was considered as the product of peak VO_2 and the time spent running at that peak. The % increased plasma endostatin after exercise ranged from 30% to 300% for different individual. There was a strong linear correlation between % increase in plasma levels of endostatin and the total peak VO_2 (R = 0.969; R^2 = 0.9388; P < 0.01).

levels decreased to 28.3± 6.4, 17.6 ± 2.4, and 26.5 ± 12.5 pg/ml, respectively, at 0.5, 2, and 6 h after treadmill-exercise. These values reflect percent changes of 24% (P = 0.159), 53% (P = 0.035), and 29% (P = 0.182) at 0.5 h, 2 h, and 6 h after exercise, respectively, compared to the basal plasma levels. The regression analysis also indicated a good linear correlation between % decrease in plasma levels of VEGF and the total peak VO_2 (R = 0.6410). Basal levels of plasma bFGF (immediately before exercise) were detected in only 4 of 7 subjects using an assay (R&D, catalog number DFB50). The plasma levels of bFGF in those 4 subjects averaged 6.3 ± 0.5 and 6.8 ± 0.8 pg/ml, respectively, before and 2 h after exercise (P = 0.483).

Correlation between Plasma Endostatin and VEGF Levels
Interestingly, regression analysis indicated a significant negative correlation between basal plasma endostatin and VEGF levels among the 7 male volunteers (R = 0.7235; R^2 = 0.8510; P = 0.015), in which a higher endostatin level is associated with a lower VEGF level. A good linear correlation between % increase in endostatin and % decrease in VEGF levels was also found 2 h after exercise (R = 0.6541; P = 0.0811). These findings were consistent with the possibility that exercise may induce an angiostatic-phenotype (induction of endostatin and reduction of VEGF) in circulation of humans.

Discussion
The present study indicates that detectable amounts of endostatin are present in the plasma of healthy subjects. Basal plasma endostatin levels were 20.3 ± 3.2 ng/ml (ranging 10.3 to 29.6 ng/ml in males). These results are consistent with other reports using a similar assay in which median serum endostatin levels were 13.8 ng/ml (ranging 11.1 to 15.4 ng/ml) in healthy male subjects [17]. The circulating form of human endostatin has also been detected by other methods such as Western blot [17] and mass spectrometry [18]. These basal levels of endostatin may be generated as a by-product of physiological collagen turnover. The presence of endostatin in the circulation of healthy subjects suggests that angiostatic factors may play an important role in the homeostatic regulation of angiogenesis under a normal condition [13].

Feldman et al [17] using the same endostatin assay (Cytimmune Sciences Inc.; College Park, MD) showed that the immunoreactivity of endostatin detected in normal human serum and plasma is essentially identical to that of recombinant endostatin. However, the functional status of circulating endogenous endostatin in humans remains unclear. Further studies are needed to determine whether circulating endogenous endostatin or what levels of endostatin can have a physiological function.

To our knowledge, the present study is the first to demonstrate that circulating endostatin can be significantly increased by exercise in proportion to the peak oxygen consumption under physiological conditions. Although the mechanism of endostatin release remains unclear, many studies have shown that the proteolytic release of endostatin from collagen XVIII is mediated by proteases of many classes, such as cysteine proteases, matrix metalloproteases, and aspartic proteases [19,20]. Many of these proteases are associated with physiological collagen turnover that could be enhanced by physical exercise [21,22]. Therefore, we can speculate that exercise-induced endostatin release is associated with a higher collagen turnover rate.

Another mechanism of endostatin release may involve activation and release of extracellular matrix proteases associated with the angiogenic process. Many *in vivo* studies have demonstrated that exercise induces angiogenesis in cardiac [23] and skeletal muscles [24]. It is therefore conceivable that these proteases are also responsible for releasing endogenous angiostatic factors such as endostatin. Furthermore, exercise may alter the binding characteristics of endostatin in metabolically active tissues and may increase blood flow to these tissues. These conditions may wash endostatin into the general circulation. Our preliminary data suggest that 3 weeks of exercise-training can significantly decrease endostatin levels in rat tibialis anterior muscle and increase circulating endostatin levels (unpublished observations). This latter finding supports our hypothesis that exercise-induced circulating endostatin may be derived primarily from skeletal and cardiac muscles. However, further studies are needed to investigate mechanisms of exercise-induced endostatin release.

It is well-known that angiogenesis is often a compensatory response to a prolonged imbalance between the metabolic requirements of the tissues and the perfusion capabilities of the vasculature [25]. Alteration in the expression of angiogenic factors, such as VEGF and bFGF, in tissues or circulation of the subjects following exercise, has been observed in animals [24,26] and humans [27-31]. Several human studies [27,29] have shown that exercise causes a transient decrease in circulating VEGF levels, while exercise increases VEGF expression in human skeletal muscle [30,31]. The present study confirms these previous reports and further shows that the transient decrease in circulating VEGF levels caused by exercise in healthy volunteers is proportional to the total peak oxygen consumption. The transient decrease in circulating VEGF levels caused by exercise in healthy adults does not necessarily mean that exercise can cause down-regulation of VEGF in the local muscle [29,30].

The mechanism responsible for the transient decrease in circulating VEGF caused by exercise is poorly understood. 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 circulating VEGF binding proteins, which would protect the vascular system from a deleterious increase in VEGF-induced hyperpermeability [29,32].

In the present study, we have found that acute exercise caused a significant increase in circulating endostatin levels. Moreover, the highest induction of endostatin in circulation after treadmill exercises was observed in a subject who was a triathlon athlete and performed the greatest intensity of exercise. In addition, as mentioned above, our preliminary data suggest that 3 weeks of exercise-training can significantly decrease endostatin levels in the rat tibialis anterior muscle and increase circulation endostatin levels. Therefore, it is likely that exercise can increase endostatin in circulation acutely as well as chronically. However, further studies are needed for evaluating the long-term effect of exercise on increasing circulatory endostatin levels.

Angiogenesis appears to have both beneficial and deleterious effects in atherosclerosis. Whereas increased angiogenesis in the heart tissue may be a favorable sign in the healing of the ischemic tissues [35], progressive angiogenesis in a primary atherosclerotic lesion could be a cause of plaque expansion [33,34]. Our previous studies [24] as well as those from other laboratories have shown that exercise induces a local angiogenic phenotype characterized by over-expression of VEGF in skeletal muscle [27,29-31] and heart [35]. This local induction of an angiogenic phenotype by exercise appears to stimulate angiogenesis and thereby prevent ischemia in these tissues. As mentioned before, endostatin is an endogenous angiostatic factor that can inhibit the progression of atherosclerosis in a ApoE-deficient mouse model by reducing intimal and plaque tissue neovascularization [15]. Therefore, exercise can also exert a beneficial effect against atherosclerosis by increasing circulating endostatin that may inhibit development of atherosclerotic plaque through blocking angiogenesis in the plaque tissue.

Conclusions

In conclusion, the present study shows that detectable amounts of endostatin are present in the plasma of healthy subjects and that circulating endostatin levels can be significantly increased by exercise. The magnitude of increased circulating endostatin appears to be proportional to the severity of exercise as assessed by total peak oxygen consumption. In the present study, exercise caused a transient decrease in circulating VEGF levels and did not

change plasma bFGF levels. These findings may provide new insights into the molecular links between physical-inactivity and the risk of angiogenesis-dependent diseases such as atherosclerosis.

Methods

Selection of Subjects

Seven healthy-male research personnel at University Mississippi Medical Center (UMMC) participated in the study (ages ranging from 18–49 years). The subjects were selected based on the absence of a history of malignancy, coronary heart disease, peripheral vascular disease, and hypertension. All subjects had different physical activity backgrounds including one who was a triathlon athlete and an individual who did little leisure-time physical activity. In addition, all subjects were non-smokers and abstained from alcohol consumption for 24 hours prior to the study. Informed consent was obtained from all subjects. The study protocol was approved by the Institutional Review Board of UMMC.

Exercise Study Protocol

The exercise was performed on a treadmill according to a modified Ellestad Protocol [16]. Briefly, each individual started the treadmill exercise at a speed of 2–3 mph at a fixed grade of 15%. The speed was increased in stages of 0.5–1.0 mph to a peak speed of 4.0–6.5 mile per hour (mph), each stage taking 1–3 min. Each individual ran on the treadmill at peak speed for 4–10 minutes. The subjects were monitored by ECG during the exercise. Termination of exercise was based on attainment of 80–93% of maximal predicted heart rate and the tolerance of each individual. All volunteers did not have any food consumption until 6 h after exercise.

Measurement of Plasma Levels of Endostatin, VEGF, and bFGF

Three-ml of blood were collected by venipuncture-tube with EDTA solution immediately before and 0.5 h, 2 h, and 6 h after exercise. The blood samples were immediately centrifuged at 700 × g in a micro-centrifuge for 5 minutes at 4°C; and the plasma was stored at -70°C. Plasma levels of endostatin (ng/ml) were measured using human endostatin ELISA kits (Cytimmune Sciences Inc.; College Park, MD) according to manufacturer protocol. Plasma levels of VEGF or bFGF were determined using human ELISA kits (R&D Systems, Minneapolis, MN).

Estimation of Oxygen Consumption

Oxygen consumption (VO_2) was estimated by the following equation [16]: $VO_2 = V \times W \times (0.073 + OC/100) \times 1.8$ (VO_2 = oxygen consumption in ml/min [standard temperature and pressure dry]; V = treadmill speed in m/min; W = body weight in kg; OC = treadmill angle in percent; 1.8 = factor constituting the oxygen requirement in ml/min

for 1 m/kg of work). This equation is applicable to a treadmill program, which gives a rough estimate of oxygen consumption at any speed and grade. In addition, this mathematical estimation provides values of oxygen consumption similar to those obtained from direct measurements when using treadmill exercise protocol [16]. The peak VO_2 (L/min) was estimated by calculation of VO_2 when the individual exercised at peak speed. The total peak VO_2 (L) was considered as the product of peak VO_2 and the time spent running at the peak level. The peak oxygen pulse (ml O_2/beat) was calculated as the quotient of peak VO_2 (L/min) and peak heart rate (beats/min) during exercise.

Statistical Analysis

Where indicated, data is presented as mean ± SE. Statistical significance was defined as a two-tail Student's t-test value of $P < 0.05$. Paired Student's t-test was used to compare variables between two groups (before and during or after exercise). Linear-regression was performed to assess the relationships between two continuous variables. All statistical calculations were performed with SigmaStat software (Jandel Corporation, San Rafael, CA).

Authors' contributions

JWG carried out the design of the study, drafted and edited the manuscript. GG reviewed and edited the manuscript. JW and JWG carried out the immunoassays. JWG, IM, and THA participated in the exercise study. JWG, JW, and IM performed the statistical analysis, and data interpretation. JW, IM, and THA conceived of the study and the manuscript. All authors read and approved the final manuscript.

Acknowledgements

References

1. Booth FW, Gordon SE, Carlson CJ, Hamilton MT: **Waging war on modern chronic disease: primary prevention through exercise.** *J Appl Physiol* 2000, **88:**774-787.
2. Francis K: **The burden of physical inactivity and cardiovascular heart disease.** *Compr Ther* 1998, **24:**87-92.
3. Manson JE, Hu FB, Rich-Edwards JW, Colditz GA, Stampper MJ, Willett WC, Speizer FE, Hennekens CH: **A prospective study of walking compared with vigorous exercise in the prevention of coronary heart disease in women.** *N Engl J Med* 1999, **341:**650-568.
4. Berlin JA, Colditz GA: **A meta-analysis of physical activity in the prevention of coronary heart disease.** *Am J Epidemiol* 1990, **132:**612-628.
5. Wannamethee SG, Shaper AG: **Physical activity in the prevention of cardiovascular disease: an epidemiological perspective.** *Sports Med* 2001, **31:**101-114.
6. Tan KH, de Cossart L, Edwards PR: **Exercise training and peripheral vascular disease.** *Br J Surgery* 2000, **87:**553-562.
7. Sacco RL, Benjamin EG, Broderick JP: **Risk factors (AHA conference proceedings).** *Stroke* 1997, **28:**1507-1517.

8.   O'Brien ER, Garvin MR, Dev R, Stewart DK, Hinohara T, Simpson JB, Folkman J: **Angiogenesis in human coronary atherosclerotic plaques.** *Am J Pathol* 1994, **145:**883-94.
9.   Isner JM: **Cancer and atherosclerosis: the broad mandate of angiogenesis.** *Circulation* 1999, **99:**1653-1655.
10. Kahlon R, Shapero J, Gotlieb AI: **Angiogenesis in atherosclerosis.** *Can J Cardiol* 1992, **8:**60-64.
11. Walsh K, Isner JM: **Apoptosis in inflammatory-fibroproliferative disorders of the vessel wall.** *Cardiovasc Res* 2000, **45:**756-765.
12. Boehm T, Folkman J, Browder T, O'Reilly MS: **Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance.** *Nature* 1997, **390:**404-7.
13. O'Reilly MS, Boehm T, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J: **Endostatin: an endogenous inhibitor of angiogenesis and tumor growth.** *Cell* 1997, **88:**277-285.
14. Obeso J, Weber J, Auerbach R: **A hemangioendothelioma-derived cell line: its use as a model for the study of endothelial cell biology.** *Lab Invest* 1990, **63:**359-69.
15. Moulton KS, Heller E, Folkman J: **Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice.** *Circulation* 1999, **99:**1726-32.
16. Elastad MH: **Stress testing protocol.** In: *Stress Testing: Principles and Practice* Edited by: *Elastad MH. Philadelphia, PA: F. A Davis Company*; 1996.
17. Feldman AL, Tamarkin L, Paciotti GF *et al.*: **Serum endostatin levels are elevated and correlate with serum vascular endothelial growth factor levels in patients with stage IV clear cell renal cancer.** *Clin Cancer Res* 2000, **6:**4628-4634.
18. Standker L, Schrader M, Kanse SM, Jurgens M, Forssmann WG, Preissner KT: **Isolation and characterization of the circulating form of human endostatin.** *FEBS Lett* 1997, **420:**129-133.
19. Ferreras M, Felbor U, Lenhard T, Olsen BR, Delaisse J-M: **Generation and degradation of human endostatin proteins by various proteinases.** *FEBS Lett* 2000, **486:**247-251.
20. Saarela J, Rehn M, Oikarinen A, Autio-Harmainen H, Pihlajaniemi T: **The short and long forms of type XVIII collagen show clear tissue specificities in their expression and location in basement membrane zones in human.** *Am J Pathol* 1998, **153:**611-26.
21. Kovanen V, Suominen H: **Age- and training-related changes in the collagen metabolism of rat skeletal muscle.** *Eur J Appl Physiol* 1989, **58:**765-771.
22. Thomas DP, McCormick RJ, Zimmerman SD, Vadlamudi RK, Gosselin LE: **Aging- and training-induced alterations in collagen characteristics of rat left ventricle and papillary muscle.** *Am J Physiol Heart Circ Physiol* 1992, **263:**H778-H783.
23. White FC, Bloor CM, McKirnan MD, Carroll SM: **Exercise training in swine promotes growth of arteriolar bed and capillary angiogenesis in heart.** *J Appl Physiol* 1998, **85:**1160-1168.
24. Hang J, Kong L, Gu J-W, Adair TH: **VEGF gene expression is upregulated in electrically stimulated rat skeletal muscle.** *Am J Physiol Heart Circ Physiol* 1995, **269:**H1827-31.
25. Adair TH, Gay WJ, Montani J-P: **Growth regulation of the vascular system: evidence for a metabolic hypothesis.** *Am J Physiol Regul Integr Comp Physiol* 1990, **259:**R393-R404.
26. Breen EC, Johnson EC, Wagner H, Tseng HM, Sung SA, Wagner PT: **Angiogenic growth factor mRNA responses in muscle to a single bout of exercise.** *J Appl Physiol* 1996, **81:**355-361.
27. Asano M, Kaneoka K, Nomura T, Asano K, Sone H, Tsurumaru K, Yamashita K, Matsuo K, Suzuki H, Okuda Y: **Increase in serum vascular endothelial growth factor levels during altitude training.** *Acta Physiol Scand* 1998, **162:**455-459.
28. Gu J-W, Santiago D, Olowe Y, Weinberger J: **Basic fibroblast growth factor as a biochemical marker of exercise-induced ischemia.** *Circulation* 1997, **95:**1156-68.
29. Gunga H-C, Kirsch K, Rocker L, Behn C, Koralewski E, Davila EH, Estrada MI, Johannes B, Wittels P, Jelkmann W: **Vascular endothelial growth factor in exercising humans under different environmental conditions.** *Eur J Appl Physiol* 1999, **79:**484-90.
30. Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ: **Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle.** *Am J Physiol Heart Circ Physiol* 1999, **276:**H679-H685.
31. Richardson RS, Wagner H, Mudaliar RSD, Saucedo E, Henry R, Wagner PD: **Exercise adaptation attenuates VEGF gene expression in human skeletal muscle.** *Am J Physiol Heart Circ Physiol* 2000, **279:**H772-H778.
32. Anthony FW, Evans PW, Wheeler T, Wood PJ: **Variation in detection of VEGF in maternal serum by immunoassay and the possible influence of binding proteins.** *Ann Clin Biochem* 1997, **34:**276-280.
33. Celletti FL, Hilfiker PR, Ghafouri P, Dake MD: **Effect of human recombinant vascular endothelial growth factor 165 on progression of atherosclerotic plaque.** *J Am Coll Cardiol* 2001, **37:**2126-2130.
34. Lemstrom KB, Krebs R, Nykanen AI, Tikkanen JM, Sihvola RK, Aaltola EM, Hayry PJ, Wood J, Alitalo K, Vla-Herttuata S, Koskinen PK: **Vascular endothelial growth factor enhances cardiac allograft arteriosclerosis.** *Circulation* 2002, **105:**2524-2530.
35. Isner JM, Losordo DW: **Therapeutic angiogenesis for heart failure.** *Nat Med* 1999, **5:**491-492.