Int Cardiovasc Res J.2020;**14**(2):48-53.icrj.101280

International Cardiovascular Research Journal
www.ircrj.com

Comparison of the Effects of Continuous and Non-Continuous Aerobic Exercises on Serum Vascular Endothelial Growth Factor and Endostatin in Rats with Coronary Artery Disease

Elham Dehghan [1], Reza Nouri [2,*], Ali Asghar Ravasi [3], Maryam Nekooei [4]

[1] Department of Exercise Physiology, Kish International Campus, University of Tehran, Kish, IR Iran
[2] Department of Sport Sciences, Kish International Campus, University of Tehran, Kish, IR Iran
[3] Department of Sport Physiology, University of Tehran, Tehran, IR Iran
[4] Department of Biochemistry, Shiraz University of Medical Sciences, Shiraz, IR Iran

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 22 Jan 2020
Revised: 23 May 2020
Accepted: 31 May 2020

Keywords:
Vascular Endothelial Growth Factor
Endostatin
Myocardial Infarction

ABSTRACT

Background: Physical activities are associated with a decreased risk of Coronary Heart Disease (CHD), which is one of the widespread Cardiovascular Diseases (CVDs).
Objectives: The present study aimed to compare the effects of continuous and non-continuous aerobic exercises on serum Vascular Endothelial Growth Factor (VEGF) and Endostatin (ES) in rats with coronary artery disease.
Methods: In this study, 40 healthy male Wistar rats (age: two months, weight: 200 - 250 g) were divided into continuous and non-continuous aerobic exercises with Myocardial Infarction (MI), control, and sham groups. After treatment of the rats by Isoproterenol (ISO), the experimental groups underwent continuous and non-continuous aerobic exercises on a treadmill for eight weeks. The means of the variables were compared using ANOVA. Moreover, Scheffe's post hoc test was used to clarify the exact zones of differences in the SPSS 21 software ($P \leq 0.05$).
Results: The results demonstrated that both continuous and non-continuous aerobic exercises increased VEGF in comparison to the control group ($P = 0.001$). However, no significant difference was observed between the experimental groups and the control group with respect to ES ($P = 0.09$).
Conclusion: Continuous and non-continuous aerobic exercises could increase angiogenesis in coronary artery disease. Moreover, both methods might have the same positive effect on rehabilitation of patients with MI. Therefore, such exercises could be used as a complementary treatment alongside medications for MI patients.

1. Background

Cardiovascular Disease (CVD) is the most common heart disease, which is responsible for 30% of all deaths worldwide and its mortality was estimated to be 17.3 million in 2008 (1). Myocardial Infarction (MI) is caused by damage of the myocardium, causing 12 million deaths each year (2). Isoproterenol (ISO) causes damage to the cardiac myocytes creating MI. ISO-induced damage to cardiac myocytes occurs via several mechanisms, such as hypoxia, coronary hypotension, calcium overload, energy depletion, and excessive production of free radicals (3).

Physical inactivity has been believed to be an independent risk factor for the development of Coronary Artery Disease (CAD), stroke, and Peripheral Vascular Disease (PVD) (4, 5). However, physical activity can have positive effects on blood pressure, insulin sensitivity, decrease of blood viscosity, and promotion of endothelial nitric oxide production (6). Aerobic exercises increase aerobic enzyme activity and expand the capillary network in the heart and skeletal muscles (7). The spread of this network occurs through arteriogenesis and angiogenesis processes (8). Vascular Endothelial Growth Factor (VEGF) is one of the most important growth factors in the process of angiogenesis (9, 10). VEGF is linked to the corresponding tyrosine kinase receptors and activates cascade reactions (11), leading to survival, proliferation, migration, and permeability of cardiovascular endothelial cells (12).

Endostatin (ES) is a heparin sulfate proteoglycan that inhibits the proliferation and migration of endothelial cells,

*Corresponding author: Reza Nouri, Department of Sport Sciences, Kish International Campus, University of Tehran, Kish, Iran, Tel: +98-9138122729, E-mail: nuri_r7@ut.ac.ir.

thereby preventing the growth of the capillary network (13). In normal states, there is a balance between angiogenic and angiostatic agents. However, this balance is often impaired in physiological and pathological conditions, including physical activities (14). The results of the previous studies showed a positive relationship between exercising and angiogenesis (15-17). Therefore, the present study aims to compare the effects of continuous and non-continuous aerobic exercises on VEGF and ES in rats with CAD.

2. Objectives

The current study aims to compare the effects of continuous and non-continuous aerobic exercises on VEGF and ES serum levels in rats with ISO-induced MI and the control group.

3. Patients and Methods

3.1. Animal Model

In this study, 40 healthy male Wistar rats (age: two months, weight: 200 - 250 g, temperature: 22 ± 2 °C, humidity: 50 ± 5%, dark light cycle: 12:12) were randomly assigned to four groups. All groups, except for the sham group, received ISO over the two treatment days. Group I (sham group, n = 10) continued their normal life during the study period (eight weeks). The group II rats (control group, n = 10) were injected with ISO. Finally, the exercise groups; i.e., groups III (n = 10) and IV (n = 10), were trained as mentioned in the treadmill exercise protocol. All animal experiments were approved by the Ethics Committee of Bushehr University of Medical Sciences and were conducted in accordance with the institutional guidelines for animal care and use (IR.BPUMS.REC.1398.055).

3.2. Induction of Experimental Myocardial Infarction

MI was induced by 85 $mg.kg^{-1}$ of ISO (sigma, Cat No. 15627) applied intraperitoneally. The drug was dissolved in normal saline and was injected twice with 24-hr intervals. Troponin-I was measured to confirm the occurrence of ISO-induced MI, because serum Troponin-I is a sensitive marker of MI (18). Troponin-I was assessed using VIDAS troponin-I ultra assay (BioMerieux, France) and values above 1.5 $ng.mL^{-1}$ were reported as positive.

3.3. Treadmill Exercise Protocol and Measurement of Maximal Oxygen Consumption (VO2max)

After the approval of MI by ISO, the rats in the experimental groups underwent continuous and non-continuous aerobic exercises. The rats ran on the treadmill at a speed of 10 meters per minute for one week in order to become familiar with the treadmill. Afterwards, eight weeks of exercise (five sessions per week) were administered for the experimental group rats. The exercises have been described in Tables 1 and 2.

Table 1. Non-Continuous Aerobic Exercise Program (20)

Weeks	Exercise Duration (Minutes)	Activity	Vo2max
1 (preparation)	14	Warming up: 2 minutes at 5 meters/minute Running: 10 minutes at 10 meters/minute Cooling down: 2 minutes at 5 meters/minute	50%
2 and 3	26	Warming up: 2 minutes at 10 meters/minute Running: 10 minutes at 15 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 15 meters/minute Cooling down: 2 minutes at 10 meters/minute	55%
4 and 5	38	Warming up: 2 minutes at 5 meters/minute Running: 10 minutes at 20 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 20 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 20 meters/minute Cooling down: 2 minutes at 10 meters/minute	70%
6 and 7	50	Warming up: 2 minutes at 10 meters/minute Running: 10 minutes at 23 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 23 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 23 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 23 meters/minute Cooling down: 2 minutes at 10 meters/minute	74%
8 and 9	74	Warming up: 2 minutes at 10 meters/minute Running: 10 minutes at 25 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 25 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 25 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 25 meters/minute Active rest: 2 minutes at 5 meters/minute Running: 10 minutes at 25 meters/minute Active rest: 2 minutes at 5 meters/minute Cooling down: 2 minutes at 10 meters/minute	78%

Abbreviations: Vo2max; maximal oxygen consumption

Table 2. Continuous Aerobic Exercise Program (20)

Weeks	Exercise Duration (Minutes)	Activity	Vo2max
1 (preparation)	14	Warming up: 2 minutes at 5 meters/minute Running: 10 minutes at 10 meters/minute Cooling down: 2 minutes at 5 meters/minute	50%
2 and 3	24	Warming up: 2 minutes at 10 meters/minute Running: 20 minutes at 15 meters/minute Cooling down: 2 minutes at 10 meters/minute	55%
4 and 5	34	Warming up: 2 minutes at 10 meters/minute Running: 30 minutes at 20 meters/minute Cooling down: 2 minutes at 10 meters/minute	70%
6 and 7	44	Warming up: 2 minutes at 10 meters/minute Running: 40 minutes at 23 meters/minute Cooling down: 2 minutes at 10 meters/minute	74%
8 and 9	64	Warming up: 2 minutes at 10 meters/minute Running: 60 minutes at 25 meters/minute Cooling down: 2 minutes at 10 meters/minute	78%

Abbreviations: Vo2max; maximal oxygen consumption

Table 3. The Effects of Continuous and Non-Continuous Aerobic Exercises on VEGF Level ($pg.mL^{-1}$)

Group	N	Mean (SD)	F	Significant
Continuous exercise ($pg.mL^{-1}$)	10	60.40 (5.35)	8.85	0.01 *
Non-continuous exercise ($pg.mL^{-1}$)	10	57.78 (3.95)		
Control ($pg.mL^{-1}$)	10	51.00 (3.36)		
Sham ($pg.mL^{-1}$)	10	50.84 (5.22)		

* Significant difference between groups

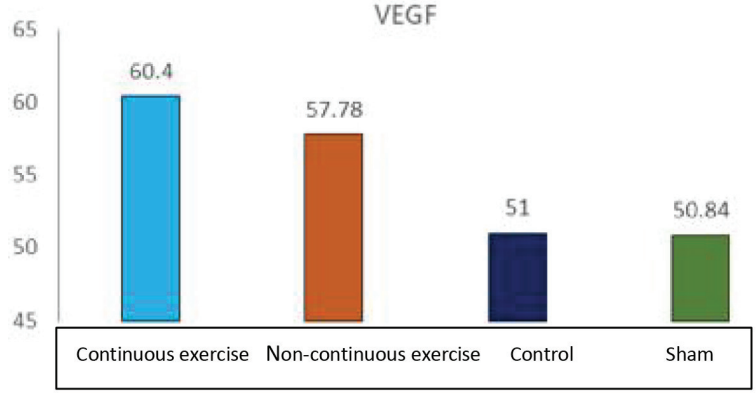

Figure 1. The Effects of Continuous and Non-Continuous Aerobic Exercises on VEGF Level ($pg.mL^{-1}$)

Maximal Oxygen Consumption (VO2max) was measured by a treadmill ramp test protocol as previously described in details (19).

3.4. Sample Collection

In order to avoid the possible acute effects, 72 hours after the last exercise session, blood samples were collected for measurement of VEGF and ES levels (21). In doing so, all rats were anaesthetized using respiratory isoflurane (4%). After confirmation of deep anesthesia, the rats' abdomens were opened up and 5 cc blood was taken from their inferior vena cava. The sera were centrifuged at 3000 rpm for ten minutes. After that, the sera were poured into test tubes and were kept at -80 °C until analysis. VEGF and ES levels were measured using Elisa kits (Cat No. CK-E91384 and Cat No. CK-E30444, respectively) from Eastbiopharm Company.

3.5. Chemical Analysis

ISO was purchased from Sigma (UK) and Troponin-I assay kit was provided by BioMerieux Compacy (France). VEGF and ES levels were measured using Elisa kits (Cat. No. CK-E91384 and Cat. No. CK-E30444, respectively) from Eastbiopharm Company (China).

3.6. Statistical Analysis

The data were entered into the SPSS 21 software and were analyzed using descriptive and inferential statistics. Kolmogorov-Smirnov test was used to check the normal distribution of the data. ANOVA was used to compare the study groups regarding the means of the study variables. Additionally, Scheffe's post hoc test was used to clarify the exact zones of differences ($P \leq 0.05$).

4. Results

The results of ANOVA indicated that eight weeks of continuous and non-continuous aerobic exercises caused a significant increase in VEGF concentration in male rats with MI ($P = 0.01$) (Table 3, Figure 1). Besides, the results

of Scheffe's post hoc test revealed that both methods were equally effective (Table 4). Moreover, the results revealed an insignificant difference between the experimental groups and the control and sham groups concerning ES level eight weeks after continuous and non-continuous aerobic exercises (P = 0.09) (Table 5, Figure 2).

5. Discussion

Up to now, no studies have been conducted on the impacts of continuous and non-continuous aerobic exercises on VEGF and ES levels in rats with ISO-induced MI. Exercise stimulates angiogenesis through increasing vascular shear stress. VEGF and ES play crucial roles in angiogenesis. Therefore, they were selected to be investigated (22). The results indicated that both continuous and non-continuous aerobic exercises could equally increase the serum level of VEGF in the rats with MI (P = 0.01). However, the results revealed no significant differences between the two groups regarding the ES level (P = 0.09). Some studies have demonstrated an increase in VEGF level after exercising (23, 24), while several studies have shown no changes in the VEGF serum level after exercising (11, 25). This contradiction can be attributed to the intensity, duration, and type of exercises and the studied populations.

Long-term exercise with moderate intensity has been considered as a positive regulator of VEGF mRNA gene expression and protein content. Hypoxia, shear stress, muscle contraction and strain, and impaired metabolism are the most important factors that affect angiogenesis (26-28). It seems that aerobic exercises increase the interstitial calcium ions (CaN and CaMK), Nitric Oxide (NO, eNOS and nNOS), and Hypoxia Inducible Factor-1 (HIF-1), thereby regulating VEGF (29). In this way, they increase VEGF levels, which results in the occurrence of angiogenesis. During exercise, cardiac filling volume increases and blood vessels suffer from more stretching (30). The blood vessel strain resulting from increased cyclic stretching leads to the upregulation of angiogenic factors, especially VEGF, Tie-1, Tie-2, and Flk-1 in vascular endothelial cells, thereby facilitating the process of angiogenesis (31). Cyclic stretching leads to a faster and longer increase in VEGF type II receptors (VEGFR-2), longer tubular length, and more vascular branches compared to static stretching (30). Cyclic stretching also contributes to the process of angiogenesis through a common pathway and in association with shear stress. Additionally, binding molecules such as integrin receptors (αvβ5 and αvβ3) expressed by platelets contribute to the process of stretching and ingrowing vascular tissue buds. In the next step, the Ephb/Ephrinb system regulates the process of vessel formation. Ultimately, pericytes and smooth muscle cells are added to this structure to stabilize the new blood vessel (32). Cyclic stretching of Human Umbilical Vein Endothelial Cells (HUVECs) causes the upregulation of angiopoietin-2 secretion and PDGF-β, thereby resulting in the migration of the endothelial cells and the formation of buds (33). Increasing the exercise intensity elevates the stroke volume and heart rate and increments the period of ischemia/reperfusion incurred

Table 4. Scheffe's Post Hoc Test for Pair Comparison of the Study Groups

Pair Comparison of the Study Groups		Mean Difference	Std. error	Significant
Continuous	Non-continuous	2.61	2.23	0.71
	Control	9.39	2.42	0.01*
	Sham	9.55	2.29	0.01*
Non-continuous	Control	6.77	2.23	0.01*
	Sham	6.93	2.08	0.01*
Control	Sham	0.15	2.29	0.99

* Significant difference between groups

Table 5. The Effects of Continuous and Non-Continuous Aerobic Exercises on ES

Group	N	Mean (SD)	F	Significant
Continuous exercise (ng/mL)	10	1.57 (0.27)	2.35	0.09
Non-continuous exercise (ng/mL)	10	1.95 (0.28)		
Control (ng/mL)	10	2.00 (0.46)		
Sham (ng/mL)	10	1.80 (0.30)		

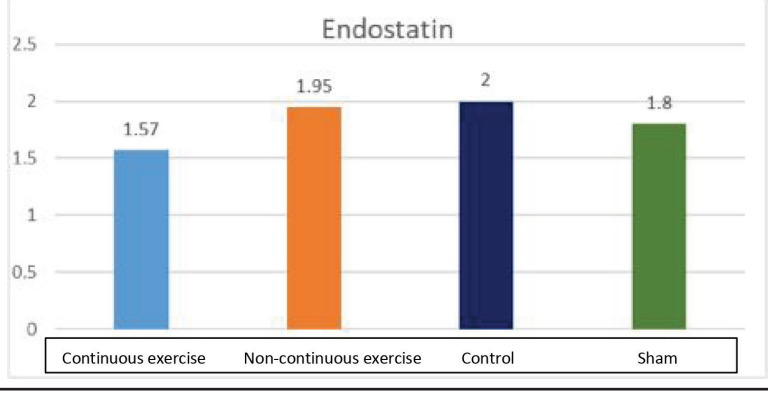

Figure 2. The Effects of Continuous and Non-Continuous Aerobic Exercises on ES Level (pg.mL^{-1})

into the coronary arteries and the cyclic stretching during systolic and diastolic periods. Therefore, angiogenesis and increased capillary density are expected in the cardiac tissue following regular exercises.

It has been shown that muscle-induced mechanical pressure induces NO release and increases eNOS. NO plays a key role in activating the VEGF signaling pathway (34). It has been found that VEGF serum levels were significantly higher in patients with MI than in those without MI (35). The results of the current study also demonstrated no changes in the rats' ES levels after continuous and non-continuous aerobic exercises. The results also revealed no significant difference between the experimental and control groups with regard to the ES level (P = 0.09). Sida and Wada conducted a study on healthy male Wistar rats and reported a non-significant lowering of the ES level after resistance training (36). Nonetheless, their results showed a significant decrease in the ES level after eight weeks of endurance training and one session of eccentric training. Brixius et al. (2008) also underlined that the ES level was reduced in response to prolonged aerobic activity in obese males (25). Overall, the level of ES has been expected to decrease following exercise. Exercising probably prevents the release of ES from collagens through changing the extracellular matrix (17). The inconsistency among the reports might be attributed to the differences in test conditions such as sampling time as well as to the intensity, duration, and type of exercises.

5.1. Conclusion

The study findings indicated that both continuous and non-continuous aerobic exercises could increase blood VEGF level (P = 0.01). However, they exerted no significant effects on the ES level (P = 0.09). Therefore, aerobic exercises might be an important therapeutic target in the prevention of heart failure in MI.

5.2. Ethical Approval

This study was approved by the Ethics committee of Bushehr University of Medical Sciences (code: IR.BPUMS.REC.1398.055).

Acknowledgements

There is no acknowledgment.

Authors' Contribution

Analysis and interpretation of data and drafting of the manuscript: E. D.; designing the work, developing the original idea and the protocol, and critical revision of the manuscript for important intellectual content: R. N.; development of the protocol and preparation of the manuscript: A. A. R.; analysis and interpretation of data: M.N.

Funding/Support

There is no funding/support.

Financial Disclosure

The authors have no financial interests related to the material in the manuscript.

References

1. Álvarez-Álvarez MM, Zanetti D, Carreras-Torres R, Moral P, Athanasiadis G. A survey of sub-Saharan gene flow into the Mediterranean at risk loci for coronary artery disease. *European Journal of Human Genetics*. 2017;**25**(4):472-6.
2. Pipaliya H, Vaghasiya J. Cardio protective effect of vitamin A against isoproterenol-induced myocardial infarction. *Journal of nutritional science and vitaminology*. 2012;**58**(6):402-7.
3. Upaganlawar A, Balaraman R. Cardioprotective effects of Lagenaria siceraria fruit juice on isoproterenol-induced myocardial infarction in wistar rats: a biochemical and histoarchitecture study. *Journal of Young Pharmacists*. 2011;**3**(4):297-303.
4. Berlin JA, Colditz GA. A meta-analysis of physical activity in the prevention of coronary heart disease. *American journal of epidemiology*. 1990;**132**(4):612-28.
5. Wannamethee SG, Shaper AG. Physical activity in the prevention of cardiovascular disease. *Sports medicine*. 2001;**31**(2):101-14.
6. Tian D, Meng J. Exercise for prevention and relief of cardiovascular disease: prognoses, mechanisms, and approaches. *Oxidative Medicine and Cellular Longevity*. 2019;**2019**.
7. Tang K, Xia FC, Wagner PD, Breen EC. Exercise-induced VEGF transcriptional activation in brain, lung and skeletal muscle. *Respiratory physiology & neurobiology*. 2010;**170**(1):16-22.
8. Suhr F, Brixius K, de Marées M, Bölck B, Kleinöder H, Achtzehn S*, et al.* Effects of short-term vibration and hypoxia during high-intensity cycling exercise on circulating levels of angiogenic regulators in humans. *Journal of applied physiology*. 2007;**103**(2):474-83.
9. Gavin T, Drew J, Kubik C, Pofahl W, Hickner R. Acute resistance exercise increases skeletal muscle angiogenic growth factor expression. *Acta physiologica*. 2007;**191**(2):139-46.
10. Kwak S-E, Lee J-H, Zhang D, Song W. Angiogenesis: focusing on the effects of exercise in aging and cancer. *Journal of exercise nutrition & biochemistry*. 2018;**22**(3):21.
11. Pandey AK, Singhi EK, Arroyo JP, Ikizler TA, Gould ER, Brown J*, et al.* Mechanisms of VEGF (vascular endothelial growth factor) inhibitor–associated hypertension and vascular disease. *Hypertension*. 2018;**71**(2):e1-e8.
12. Zachary I, Gliki G. Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family. *Cardiovascular research*. 2001;**49**(3):568-81.
13. Sponder M, Dangl D, Kampf S, Fritzer-Szekeres M, Strametz-Juranek J. Exercise increases serum endostatin levels in female and male patients with diabetes and controls. *Cardiovascular diabetology*. 2014;**13**(1):6.
14. Bloor CM. Angiogenesis during exercise and training. *Angiogenesis*. 2005;**8**(3):263-71.
15. Azimi-Nezhad M. Vascular endothelial growth factor from embryonic status to cardiovascular pathology. *Reports of biochemistry & molecular biology*. 2014;**2**(2):59.
16. Morland C, Andersson KA, Haugen ØP, Hadzic A, Kleppa L, Gille A*, et al.* Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1. *Nature communications*. 2017;**8**(1):1-9.
17. Tolouei Azar J, Ravasi A, Soori R, Akbarnejad A, Hemati Nafar M. The effect of 8 weeks aerobic training on angiogenesis (VEGF) and angiostatic (ES) factors in sedentary women. *The Journal of Urmia University of Medical Sciences*. 2017;**27**(12).
18. Joukar S, Najafipour H, Dabiri S, Sheibani M, Sharokhi N. Cardioprotective effect of mumie (shilajit) on experimentally induced myocardial injury. *Cardiovascular toxicology*. 2014;**14**(3):214-21.
19. Kemi OJ, Haram PM, Loennechen JP, Osnes J-B, Skomedal T, Wisløff U*, et al.* Moderate vs. high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function. *Cardiovascular research*. 2005;**67**(1):161-72.
20. Caponi PW, Lehnen AM, Pinto GH, Borges J, Markoski M, Machado UF*, et al.* Aerobic exercise training induces metabolic benefits in rats with metabolic syndrome independent of dietary changes. *Clinics*. 2013;**68**(7):1010-7.
21. Shamsoddini A, Sobhani V, Chehreh MEG, Alavian SM, Zaree A. Effect of aerobic and resistance exercise training on liver enzymes and hepatic fat in Iranian men with nonalcoholic fatty liver disease. *Hepatitis monthly*. 2015;**15**(10).
22. Wilson BS, Garza MA, Zhang JQ. Post-Myocardial Infarction Exercise Training Induces Angiogenesis in the heart. 2018.
23. Braga VA, Couto GK, Lazzarin MC, Rossoni LV, Medeiros A. Aerobic exercise training prevents the onset of endothelial dysfunction via increased nitric oxide bioavailability and reduced reactive oxygen species in an experimental model of menopause. *PLoS One*. 2015;**10**(4).

24. Mota MM, Mesquita TRR, da Silva TLTB, Fontes MT, Santos SL, Capettini LdSA*, et al.* Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals. *Life sciences*. 2015;**142**:86-91.
25. Brixius K, Schoenberger S, Ladage D, Knigge H, Falkowski G, Hellmich M*, et al.* Long-term endurance exercise decreases antiangiogenic endostatin signalling in overweight men aged 50–60 years. *British journal of sports medicine*. 2008;**42**(2):126-9.
26. Gustafsson T, Rundqvist H, Norrbom J, Rullman E, Jansson E, Sundberg CJ. The influence of physical training on the angiopoietin and VEGF-A systems in human skeletal muscle. *Journal of Applied Physiology*. 2007;**103**(3):1012-20.
27. Hudlicka O, Brown M, Egginton S. Angiogenesis in skeletal and cardiac muscle. *Physiological reviews*. 1992;**72**(2):369-417.
28. Hudlicka O, Brown MD. Adaptation of skeletal muscle microvasculature to increased or decreased blood flow: role of shear stress, nitric oxide and vascular endothelial growth factor. *Journal of vascular research*. 2009;**46**(5):504-12.
29. Ohno H, Shirato K, Sakurai T, Ogasawara J, Sumitani Y, Sato S*, et al.* Effect of exercise on HIF-1 and VEGF signaling. *The Journal of Physical Fitness and Sports Medicine*. 2012;**1**(1):5-16.
30. Zheng W, Christensen LP, Tomanek RJ. Differential effects of cyclic and static stretch on coronary microvascular endothelial cell receptors and vasculogenic/angiogenic responses. *American Journal of Physiology-Heart and Circulatory Physiology*. 2008;**295**(2):H794-H800.
31. Milkiewicz M, Ispanovic E, Doyle JL, Haas TL. Regulators of angiogenesis and strategies for their therapeutic manipulation. *The international journal of biochemistry & cell biology*. 2006;**38**(3):333-57.
32. Martínez A. A new family of angiogenic factors. *Cancer letters*. 2006;**236**(2):157-63.
33. Yung YC, Chae J, Buehler MJ, Hunter CP, Mooney DJ. Cyclic tensile strain triggers a sequence of autocrine and paracrine signaling to regulate angiogenic sprouting in human vascular cells. *Proceedings of the National Academy of Sciences*. 2009;**106**(36):15279-84.
34. Loufrani L, Henrion D. Role of the cytoskeleton in flow (shear stress)-induced dilation and remodeling in resistance arteries. *Medical & biological engineering & computing*. 2008;**46**(5):451-60.
35. Liou J-Y, Kuan P-L, Shyu K-G, Lu M-J, Chao H-H, Wang B-W. Pericardial fluid and serum levels of vascular endothelial growth factor and endostatin in patients with or without coronary artery disease. *Journal of the Formosan Medical Association*. 2006;**105**(5):377-83.
36. Seida A, Wada J, Kunitomi M, Tsuchiyama Y, Miyatake N, Fujii M*, et al.* Serum bFGF levels are reduced in Japanese overweight men and restored by a 6-month exercise education. *International journal of obesity*. 2003;**27**(11):1325-31.