The Effect of Aerobic Training on Tumor Necrosis Factor alpha, Hypoxia-Inducible Factor-1 alpha & Vascular Endothelial Growth Factor Gene Expression in Cardiac Tissue of Diabetic Rats

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Abstract

Objective: The goal of this research was to determine the influence of 4 weeks aerobic training on gene expression of tumor necrosis factor alpha (TNF-**α**)**,** hypoxia-inducible factor-1 alpha (HIF-1**α**) and vascular endothelial growth factor (VEGF) in the cardiac tissue of diabetic rats**.**

Materials and Methods: In an experimental study, 30 male wistar rats were partitioned into three groups (n=10), diabetic aerobic training, diabetic control and healthy control (n=10). Induction of type 2diabetes (T2DM) was done by intraperitoneal infusion of streptozotocin. The progressive aerobic training protocol entailed 4 weeks, 5 sessions per week running on treadmill at velocity of 20 m/min for 60 min. The gene expression of TNF-α, VEGF and HIF-1α were measured by real time & PCR. One way analysis of variance and bonferroni test were applied to analysis the data. The significant level was set at *P*-value< 0.05.

Results: The results indicated that the aerobic training induced significant decrease in TNF-α mRNA (*P*-value< 0.001) and significant increase in HIF-1α mRNA (*P*-value< 0.001) and VEGF mRNA (*P*-value< 0.001) compared to diabetic control group.

Conclusion: It appears that aerobic training with reduction of TNF-α issues an affirmative effect on angiogenesis, as a result, it improves diabetic cardiac.

Keywords: Angiogenesis process, Aerobic training, Tumor necrosis factor alpha**,** Hypoxia-inducible factor-1 alpha, Vascular endothelial growth factor.

Introduction

Lack of physical activity is related to an increase in weight and metabolic disorders such as Diabetes mellitus (type 2 diabetes (T2DM)) (1). People with type 2 diabetes are more prone to peripheral arterial disease, heart attack and other cardiovascular diseases (2). According to previous studies, T2DM is associated with hyperglycemia and changes in reactive oxygen species (ROS), angiogenic factors and cytokines. Disruption in catabolism and anabolism of glucose and persistent

inflammation in the cardiac causes diseases such as cardiomyopathy. Cardiomyopathy is the usual result of promotion metabolic disorder (3).

Tumor necrosis factor alpha (TNF-α) activates transcriptional pathways that cause oxidative stress, then the induced oxidative stress and inflammation interact with each other to destroy the cells (4).TNF-α exerts an important role in the progress of insulin resistance and reduces the glucose transporter type 4 (GLUT4) expression, which is predominantly present in adipocytes and heart muscles (5). Also, TNF-α also play a role in inhibiting the generation of hypoxia-inducible factor-1 alpha (HIF-1α) (6).

HIF-1α is an important transcription factor that intermediates cellular adaptation to skimp of oxygen pressure. Hypoxia and hyperglycemia are of the main problem of diabetes and adjust HIF-1α expression (6). Although studies have shown that hyperglycemia and high glucose disrupt the production pathway for HIF-1α, its molecular mechanism has not yet been completely determined (6).

During angiogenesis, HIF-1α activation is the initiator of vascular endothelial growth factor (VEGF) expression (7). VEGF is a major mitogenic factor engaged in the angiogenesis process. VEGF is a 45-kilodalton glycoprotein and endothelial cells and tumor cells release it. VEGF results in the survivorship, duplication, and immigration of endothelial cells and ultimately induce construct the novel blood vessels (8). T2DM is a contradictory state of angiogenesis, because on the one hand, in organs such as the eyes and kidneys, it increases angiogenesis, and on the other hand, in tissues such as the heart and muscles, it reduces or inhibits angiogenesis (9).

According to a report, the HIF-1α signaling pathway is damaged in type 2 diabetes (6), and as a result, VEGF activation and expression are reduced and downregulated inside the ventricles of diabetic patients (10). Regular aerobic exercise is a non-pharmacologically effective method for controlling T2DM. Performing arranged exercise training decelerates the endothelial dysfunction progression, ameliorate blood stream and cardiac perfusion, and leads to diminish the hazard of coronary artery disease (11,12).

Improving glucose control, the decline of plasma lipids and increase of insulin sensitivity are the actions of exercise training in diabetic patients (11,13). In STZ-diabetic models, the disruption of the expression of the VEGF index in the skeletal muscle was shown to improve with exercise performance (14,15). Exercise can lead to various changes in vascular function, such as decreased heart rate, low blood pressure, increased maximum oxygen consumption (Vo_{2max}) of heart, and endothelium-dependent vasodilation (16,17).

Recent studies have shown that aerobic training is effective on vascular function of T2DM rats (18,19).Stimulating angiogenesis and reducing glucose indicates a significant adaptation of exercise training in improving blood flow and developing insulin delivery for glucose removal. Aerobic training can improve vascular function by reducing the inflammatory cytokines expression like as TNF-α, which are associated with endothelial dysfunction and insulin resistance in T2DM.

Regarding the role of T2DM in the process of inflammation and angiogenesis and the efficacy of aerobic training on the improvement of hyperglycemia and T2DM, in the present study, the influence of 4 weeks of aerobic training on TNF-α, HIF-1α and VEGF gene expression in the cardiac texture of diabetic rats is investigated.

Materials and Methods

The present study was an experimental research study. Thirty male wistar rats weighing between 200 to 300 g and age of 6 weeks were purchased from Iran Institute of Pasteur. All rats were kept at 21 (± 2) ºC, 55% humidity and 12-12 has of light and dark cycles in polycarbonate cages (5 rats per cage).After two weeks of familiarity with the environment, for induction of type 2 diabetes in rats, following 12 hours of fasting, a solution of nicotinamide (made by Sigma Co.,

USA) solved in 120 mg/kg normal saline, and after 15 minutes, streptozotocin (manufactured by Sigma Co., USA) soluble in 0.1 molar of buffer citrate at a dose of 65 mg / kg was injected intra peritoneally (20).

To ensure diabetes, blood glucose above 250 mg/dL was determined as a criterion for type 2 diabetes, and one week after infusion, diabetic rats were selected accordingly (21). Diabetic rats were randomly partitioned into two groups: Diabetic control (n= 10) and diabetic + aerobic training (n= 10); also a group of rats with normal blood glucose was considered as the control group (n= 10).

The aerobic training intervention entailed running on treadmill for 4 weeks and 5 sessions per week. Rats in the aerobic training group first started at 15 m/min with a gradient of 15% for 5 minutes, then the term and velocity slowly enhanced to 2-3 minutes per session and 1-2 m / min per week, in the fourth week, the velocity of rats reached to 20 m / min for 60 minutes (Table 1) (22).

To extract RNA, 50 mg of heart texture was homogenized by adding 1 ml of Ytzol reagent (Yekta Tajhiz Azma Co.). Then, various steps were performed based on the RNA extraction kit instruction to extract the final RNA and to prepare pure RNA. The RNA solution extracted was purified with the DNaseI enzyme from any contamination with DNA and RNA degrading enzymes. The absorption ratio of 260-280 nm in a spectrophotometric method for all extracted specimens was 1.8 - 2. Then, electrophoresis and 1% agarose gel were used to evaluate the quality of RNA extracted. From each of the samples, 20 micrograms of mRNA were used to synthesize the first cDNA strand from the final extracted RNA samples and Oligo-dT primers (reverse transcription enzyme) according to the Pars Tus cDNA synthesis kit.

Statistical analysis

The Kolmogorov-Smirnov test was applied to evaluate the normality of data. One-way ANOVA was applied for analyzing the difference between mean of the groups, and Bonferroni test was applied to determine the place of difference between the groups. The significance level for all calculations was considered as *P*-value< 0.05. All the analyses were done by SPSS software version 22 and the charts were drawn using Microsoft Excel software version 16.

Ethical considerations

Different stages of training were conducted observing the ethical issues of treating animals to prevent their harassment and this study was approved by Islamic Azad University Central Tehran Branch (code 10121404972018).

Results

Blood glucose and weight of rats before and after the end of aerobic training are given in table 2 as mean (± standard deviation). Comparisons of the pre and post data showed

Table 1. Aerobic training protocol

Weeks	Warm up (Incline=0%) (10 m/min)	Speed (m/min)	Time (Min)	Number of session in a week	Incline	Cool down (Incline=0%) (10 m/min)
First	5 min	15	5-17	5	15%	5 min
Second	5 min	17	20-32	5	15%	5 min
Third	5 min	19	35-47	5	15%	5 min
Fourth	5 min	20	60	5	15%	5 min

Table 2. fasting glucose and weight of rats before and after aerobic training in the healthy control, diabetic control and diabetic with aerobic training groups

Variable	Weight(gr)			Fasting Glucose(mg/dl)		
Group	Before	After	*P*-value	Before	After	*P*-value
Healthy Control	232.7(±22)	264.1(±37)	0.754	90.4(±16)	73.4(±8)	0.004
Diabetic Control	223.6(±36)	250.6(±48)	0.098	299.3*(±85)	378.3*(±105)	0.121
Diabetic+Aerobic Training	224.3(±30)	227.4(±38)	0.981	354.2* (±106)	150.7$ (±116)	0.001

Paired sample t-test was applied for Within group comparison and One way analysis of variance (ANOVA) was applied for comparing before and after training changes in three groups. *demonstrates significant changes comparison with the healthy control (*P*-value <05), and $demonstrates significant difference comparison with the diabetic control (*P*-value <05).

that the weight of rats in the three groups before and after aerobic training was not significantly different (*P*-value> 0.05).
However, their fasting glucose had a significant and remarkable reduction in the aerobic training group before and after training in comparison with the diabetic control group (*P*-value< 0.05).

The aerobic training effect on VEGF, HIF-1α and TNF-α gene expression

The results of statistical analyses for VEGF gene expression ($F_{2,27}$= 47.1, *P*-value= 0.008) showed that the changes in the three research groups were significantly different. By referring to Benfrroni's post hoc test, it was found that all of the studied groups had a pairwise significant difference in VEGF gene expression (*P*-value< 0.001) (Table3). In the case of HIF-1α ($F_{2,27}$=69.1, *P*-value= 0.002), a significant difference in the tree groups was found like VEGF. Besides, by referring to Bonferroni's post hoc test displayed the significant changes between the data in the healthy control group and the diabetic control group (*P*-value< 0.001), also it displayed significant changes between the data in the diabetic control group and the aerobic training group (*P*-value< 0.001) (Table3).
TNF-α showed a significant difference ($F_{2,27}$ = 36.3, *P*-value= 0.007) between three groups. Referring to Bonferroni's post hoc test displayed the significant changes between the data in the healthy control group and diabetic control group (*P*-value< 0.001), also it displayed significant changes between the data in the diabetic control and the aerobic training groups (*P*-value< 0.001) (Table3).

Discussion

The results showed that diabetes group significantly increased TNF-α and glucose concentrations, and significantly decreased HIF-1α and VEGF gene expression compared to the healthy subjects. Aerobic training also significantly decreased TNF-α gene expression and glucose concentration, and significantly increased HIF-1α and VEGF gene expression compared to the diabetic control group. These results represent cardiac protection by reducing inflammation and hyperglycemia and improving the gene expression of the angiogenic markers of the heart in T2DM.
Increased inflammation in T2DM has been identified as a contributing factor in endothelial dysfunction, catabolism and anabolism of glucose, and heart action (13-15,18). TNF-α increase in the cardiac endothelial vessels of diabetic rats and is recognized as a key mediator in the vascular dysfunction (23).
Reducing TNF-α gene expression indicates that aerobic training is defective and prevents vascular dysfunction advancement. In addition, researches have shown that TNF-α regulates catabolism and anabolism of peripheral glucose. In support of this, rats that did not have TNF-α showed better insulin sensitivity after a high-fat diet (24). However, reducing this cytokine after an aerobic training is expected to improve blood vessel function and glucose metabolism. Along with the present results, Lee et al.'s study also showed a decreasing effect of aerobic training on TNF-α levels (25).
Concerning the cause of T2DM effects on the variables of the study, hyperglycemia causes oxidative stress and high levels of ROS, which regulates HIF-1α itself (6).In addition, when producing ROS, the interaction between

Table 3. VEGF, HIF-1 and TNF-α gene expression of rats after aerobic training in the healthy control, diabetic control and diabetic with aerobic training groups

Variable Group	VEGF (mg/dl) Mean(±SD)	HIF-1α (mg/dl) Mean(±SD)	TNF-α (mg/dl) Mean(±SD)
Healthy control	1.00 (±0.08)	1.00 (±0.09)	1.00 (±0.06)
Diabetic control	0.41 (±0.05)	0.32 (±0.06)	2.54 (±0.20)
Diabetic+ aerobic training	0.72 (±0.07)	0.71 (±0.11)	1.58 (±0.24)

One way ANOVA was used for comparing VEGF, HIF-1 and TNF-alpha gene expression in three groups. * demonstrates significant changes comparison with the healthy control group (*P*-value< 05), and $ demonstrates significant changes comparison with the diabetic control (*P*-value< 05).

superoxide and NO leads to a reduction in the constant content of NO and thus reduces the NO-induced aggregation and activity of HIF-1α.

Also, TNF-α and angiotensin II exert a role in inhibiting the generation of HIF-1α (6). Hyperglycemia causes the production of ROS and superoxide, the accumulation of which leads to the removal of superoxide dismutase (26).

Increasing the reaction of superoxide levels with NO leads to a decrease in the constant concentration of NO and therefore change in the signaling of the response due to NO (26).While increasing NO activity and accumulation increases the expression of HIF-1α (6), reducing NO inhibits and suppresses HIF-1α expression, and subsequently decreases the expression of VEGF (26).

The outcomes of the present research are in line with prior report that have shown negative regulation of VEGF in the diabetic heart and the status of insulin resistance in response to training (27). Hence, our study confirms previous reports of significant alteration in the VEGF gene expression in the diabetic cardiac compared to the healthy heart (28).

Along with our results, previous studies have reported that VEGF levels increase after training (29,30), while other studies have not observed a change in the amount of VEGF after training (31,32).Gu et al. indicated a reduction in concentration of VEGF after training (33), which is inconsistent with our results. Wagner et al. (2016) examined the influence of 12 weeks of parallel training (aerobic training with resistance, 3 sessions per week) on VEGF gene expression in T2DM rats. The aerobic training caused the augment the expression of VEGF, and one of the factors influencing this increase was the improvement of insulin sensitivity (34).

Bashiri et al. (2015) examined the 8 weeks of endurance training influence on muscular angiogenesis and serum level of VEGF in diabetic rats. The results indicated that diabetes induced the significant decline in the capillary density of heart muscle and serum level of VEGF. Also, endurance training for 8 weeks significantly increased capillary density of heart muscle and serum level of VEGF (35). Akf et al. (2011) examined the angiogenesis action as a regularizer of cardiovascular problems through stimulating aerobic training, NO and angiostatin in diabetic rats. The results showed that both of the aerobic training and the NO supplement groups had a significant augment in blood VEGF levels and gene expression, and the group taking angiostatin had a reduction in VEGF (36).

Glucose of blood changes in the diabetic rats with aerobic training showed a significant decrease after last session, which indicates the appropriateness of the intensity and duration of aerobic training. Reducing blood glucose reduces the production of ROS. Also, the aerobic training increases the NO release through various ways like as hypoxia, shear stress in the vessels (increasing the vasodilators expression, especially NO, Prostacylins and Prostanoids), and endothelial vessel stretch and pressure. Since ROS is reduced and less NO is combined with ROS indicators such as superoxide, NO accumulation increases the HIF-1α gene expression and subsequently increases the VEGF gene expression in the heart tissue (36).

Also, aerobic training increases the expression of PGC-1α, which can lead to increased VEGF gene expression in the heart tissue. HIF-1α is increased in low oxigen and ischemia state due to exercise activity (37).This factor by affecting the promoter region of the VEGF gene increases it. Also, as the intensity of exercise increases, lactate aggregation and adenosine enhances. Lactate and adenosine increase the concentration of cAMP and subsequently increase the levels of VEGF gene expression by activating the A2 receptor (37,38)

Since the most researches have evaluated the influence of aerobic training on the angiogenesis process in the muscle tissue of healthy people or healthy animals, Probably, the results of the present study can encourage physicians and physical education specialists

to further study about aerobic training influence on cardiac tissue angiogenesis in T2DM and to prevent the reduction of angiogenesis in their heart tissue.

The limitation of the present study was the temperature of the site where the training of rats was performed, so that even with all the attempts to keep the temperature steady at 21˚C, the temperature fluctuated at a range of 21 ± 2 ˚C.

Finally, considering the pattern of food in the development of type 2 diabetes, the implementation of aerobic training with a high-fat diet and its effect on ROS activity and TNF-α, NO, HIF-1α, and VEGF gene expression in the heart texture of type 2diabetic is suggested.

Conclusions

Aerobic training reduces TNF-α gene expression and increases HIF-1α and VEGF gene expression in the diabetic texture of heart, which shows the effectiveness of aerobic training compared to inactivity on improving angiogenesis and T2DM.

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Conflict of Interest

The authors express that they don't have any contrasts of interest.

References

1. Booth FW, Roberts CK, Thyfault JP, Ruegsegger GN, Toedebusch RG. Role of inactivity in chronic diseases: evolutionary insight and pathophysiological mechanisms. Physiological reviews. 2017;97(4):1351-402.
2. Tune JD, Goodwill AG, Sassoon DJ, Mather KJ. Cardiovascular consequences of metabolic syndrome. Translational Research. 2017;183:57-70.
3. de Laat MA, Gruntmeir KJ, Pollitt CC, McGowan CM, Sillence MN, Lacombe VA. Hyperinsulinemia down-regulates TLR4 expression in the mammalian heart. Frontiers in endocrinology. 2014;5:120.
4. Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxidative medicine and cellular longevity. 2015;2015.
5. Olson AL. Regulation of GLUT4 and insulin-dependent glucose flux. International Scholarly Research Notices. 2012;2012.
6. Xiao H, Gu Z, Wang G, Zhao T. The possible mechanisms underlying the impairment of HIF-1α pathway signaling in hyperglycemia and the beneficial effects of certain therapies. International journal of medical sciences. 2013;10(10):1412.
7. Zhao D, Tu Y, Wan L, Bu L, Huang T, Sun X, et al. In vivo monitoring of angiogenesis inhibition via down-regulation of mir-21 in a VEGFR2-luc murine breast cancer model using bioluminescent imaging. PloS one. 2013;8(8):e71472.
8. 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.
9. Werner GS, Ferrari M, Betge S, Gastmann O, Richartz BM, Figulla HR. Collateral function in chronic total coronary occlusions is related to regional myocardial function and duration of occlusion. Circulation. 2001;104(23):2784-90.
10. Chou E, Suzuma I, Way KJ, Opland D, Clermont AC, Naruse K,et al. Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic States: a possible explanation for impaired collateral formation in cardiac tissue. Circulation. 2002;105(3):373-9.

11. Boule N, Haddad E, Kenny G, Wells G, Sigal R. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. Scandinavian Journal of Medicine & Science in Sports. 2002;12(1):60-1.
12. Després JP. Physical activity, sedentary behaviours, and cardiovascular health: when will cardiorespiratory fitness become a vital sign?. Canadian Journal of Cardiology. 2016;32(4):505-13.
13. Ghosh S, Khazaei M, Moien-Afshari F, Ang LS, Granville DJ, Verchere CB, et al. Moderate exercise attenuates caspase-3 activity, oxidative stress, and inhibits progression of diabetic renal disease in db/db mice. American Journal of Physiology-Renal Physiology. 2009;296(4):F700-8.
14. Erekat NS, Al-Jarrah MD, Al Khatib AJ. Treadmill exercise training improves vascular endothelial growth factor expression in the cardiac muscle of type I diabetic rats. Cardiology research. 2014;5(1):23.
15. Ghorbanzadeh V, Mohammadi M, Dariushnejad H, Chodari L, Mohaddes G. Effects of crocin and voluntary exercise, alone or combined, on heart VEGF-A and HOMA-IR of HFD/STZ induced type 2 diabetic rats. Journal of endocrinological investigation. 2016;39(10):1179-86.
16. Green DJ, Maiorana A, O'Driscoll G, Taylor R. Effect of exercise training on endothelium-derived nitric oxide function in humans. The Journal of physiology. 2004;561(1):1-25.
17. Kojda G, Hambrecht R. Molecular mechanisms of vascular adaptations to exercise. Physical activity as an effective antioxidant therapy?. Cardiovascular research. 2005;67(2):187-97.
18. Moien-Afshari F, Ghosh S, Khazaei M, Kieffer TJ, Brownsey RW, Laher I. Exercise restores endothelial function independently of weight loss or hyperglycaemic status in db/db mice. Diabetologia. 2008;51(7):1327-37.
19. Sallam N, Khazaei M, Laher I. Effect of moderate-intensity exercise on plasma C-reactive protein and aortic endothelial function in type 2 diabetic mice. Mediators of inflammation. 2010;2(1):7.
20. 20. Sanchez OA, Snow LM, Lowe DA, Serfass RC, Thompson LV. Effects of endurance exercise-training on single-fiber contractile properties of insulin-treated streptozotocin-induced diabetic rats. Journal of Applied Physiology. 2005;99(2):472-8.
21. Shahabinezhad M, MR R, KhaksariHadad M, Sepehri G, Mahmoodi M, Karimghasemi E. The effect of licorice root extract on blood sugar level in streptozotocin induced diabetic rats. Journal of Rafsanjan University of Medical Sciences. 2007;6(4):237-44.(In persian)
22. Linden MA, Fletcher JA, Morris EM, Meers GM, Kearney ML, Crissey JM, et al. Combining metformin and aerobic exercise training in the treatment of type 2 diabetes and NAFLD in OLETF rats. American Journal of Physiology-Endocrinology and Metabolism. 2014;306(3):E300-10.
23. Byrkjeland R, Njerve IU, Arnesen H, Seljeflot I, Solheim S. Reduced endothelial activation after exercise is associated with improved HbA1c in patients with type 2 diabetes and coronary artery disease. Diabetes and Vascular Disease Research. 2017;14(2):94-103.
24. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature. 1997;389(6651):610-4.
25. Lee S, Park Y, Zhang C. Exercise training prevents coronary endothelial dysfunction in type 2 diabetic mice. American journal of biomedical sciences. 2011;3(4):241.
26. Thomas DD, Ridnour LA, Espey MG, Donzelli S, Ambs S, Hussain SP, et al. Superoxide fluxes limit nitric oxide-induced signaling. Journal of Biological Chemistry. 2006;281(36):25984-93.
27. Han B, Baliga R, Huang H, Giannone PJ, Bauer JA. Decreased cardiac expression of vascular endothelial growth factor and redox imbalance in murine diabetic cardiomyopathy. American Journal of Physiology-Heart and Circulatory Physiology. 2009;297(2):H829-35.
28. Li S, Culver B, Ren J. Benefit and risk of exercise on myocardial function in diabetes. Pharmacological research. 2003;48(2):127-32.
29. Czarkowska-Paczek B, Bartlomiejczyk I, Przybylski J. The serum levels of growth factors: PDGF, TGF-BETA. Journal of Physiology and Pharmacology. 2006;57(2):189-97.
30. Van Craenenbroeck EM, Vrints CJ, Haine SE, Vermeulen K, Goovaerts I, Van Tendeloo VF, et al. A maximal exercise bout increases the

number of circulating CD34+/KDR+ endothelial progenitor cells in healthy subjects. Relation with lipid profile. Journal of applied physiology. 2008;104(4):1006-13.
31. Jensen L, Pilegaard H, Neufer PD, Hellsten Y. Effect of acute exercise and exercise training on VEGF splice variants in human skeletal muscle. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2004;287(2):R397-402.
32. 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.
33. Gu JW, Gadonski G, Wang J, Makey I, Adair TH. Exercise increases endostatin in circulation of healthy volunteers. BMC physiology. 2004;4(1):2.
34. Wagner H, Fischer H, Degerblad M, Alvarsson M, Gustafsson T. Improvement of insulin sensitivity in response to exercise training in type 2 diabetes mellitus is associated with vascular endothelial growth factor A expression. Diabetes and Vascular Disease Research. 2016;13(5):361-6.
35. Bashiri J, Gaeini A, Hadi H. Endurance training affects muscular angiogenesis and serum VEGF concentration in diabetic rats. Koomesh journal. 2015;17(1):123-32.(In persian).
36. Khoweiled A, El-Sebaee H, El-Attar S, Mansour M. Role of Angiogenesis as a Factor Modulating the Course of Cardiovascular Complications in Diabetic Rats. The Medical Journal of Cairo University. 2011;79(2).
37. Trenerry MK, Carey KA, Ward AC, Cameron-Smith D. STAT3 signaling is activated in human skeletal muscle following acute resistance exercise. Journal of applied physiology. 2007;102(4):1483-9.
38. Taheri Chadorneshin H, Nourshahi M, Ranjbar K. The Response of Vascular Endothelial Growth Factor to Exhausted Submaximal Exercise and Its Relationship with VO2max. Sport Biosciences (HARAKAT). 2011;1(7):59-75.(In persian).