

# The Effect of Interval Training on Cardiac Angiogenesis Capacity in Rats with Myocardial Infarction

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## Abstract

**Introduction:** Myocardial infarction (MI) is the destruction and permanent and irreversible cell death of part of the cardiac muscle (myocardium) which occurs due to loss of blood flow to the heart. The condition disrupts individuals' daily life and limits their performance. Evidence indicates the likely effect of exercise on increasing the capillary density of skeletal muscle and myocardium. Vascular endothelial growth factor (VEGF) and endostatin as well as their common receptors (Flt-1) are the most important factors involved in angiogenesis. Therefore, this research aimed to evaluate the effect of 6 weeks of interval training on the VEGF, Flt-1 and collagen 18 in rats with MI.

**Methods:** 12 male Wistar rats with mean age of 10 weeks and average weight of 250- 300 gr were infected with myocardial infarction and were assigned into two groups of (1) experimental (60 minutes of interval running on treadmill, each interval 4 minutes with the 60- 75 percent of  $Vo_{2max}$  and 2 minutes of active rest at 50- 60 percent of  $Vo_{2max}$  for four days in a week for a period of 6 weeks) and (2) control group (without any training). Gene expression was investigated by the PCR technique and Ejection fraction and Shortening fraction were investigated by echocardiograph. Data were analyzed by SPSS 18 using independent samples t-test ( $p \leq 0.05$ ).

**Results:** Results showed that low intensity interval training had a significant effect on increasing the expression level of Flt-1 ( $P=0.02$ ) and VEGF ( $P=0.01$ ) genes and no significant change in collagen 18 ( $P=0.34$ ).

**Conclusion:** The overall results of the study supported the role of low-intensity interval training in increasing the basic factors affecting the process of angiogenesis after MI.

**Keywords:** Training, Myocardial Infarction, VEGF, collagen 18, Flt-1

## Introduction

Myocardial infarction (MI) is one of the most important causes of morbidity and mortality in the world and the number of people with the disease is increasing. It occurs when the blood supply to the heart cells is disturbed in short term and 40 to 50 percent of the vessels are closed (1). The extent and severity of the disease varies from person to person and depends on the veins and arteries conflicts. In general, vessel blockage and blood and oxygen carrying dysfunction cause MI and limits individuals' performance. According to recent research, results in relation to the possibility of creating new blood vessels in a process called angiogenesis in cardiac tissue is affected by

several factors, including hypoxia, hemodynamic forces, metabolites, left artery descending (LDA), dilation of blood vessels, muscle contraction, some cytokines and elasticity, making blood vessels again in heart tissue to improve cardiovascular function of patients is an adapted advantage (1). Therefore, assessing the interaction between factors involved in angiogenesis and angiostatic process in different situations can help to find an effective way to increase angiogenesis process and ultimately help improve the quality of patients with myocardial infarction. Vascular endothelial growth factor or VEGF, as the most powerful and the most important factor affecting

angiogenesis, increases migration and proliferation of endothelial cells and forms vascular network (1). VEGF is a glycoprotein with a molecular weight of 4,500 Dalton which is essential to distinguish endothelial cells and to sprout new capillaries from previous coronary (angiogenesis) in the growth and development of the capillary network (1). When the VEGF binds to its special receptors on endothelial cell, it activates messages resulting in proliferation and migration of endothelial cells and increased vascular permeability. This factor can play its role by the gene expression of proteases and receptors that are vital for tissue regeneration and preventing cell death in endothelial cells. This glycoprotein continues signaling by binding to VEGFR- 1 and VEGFR- 2 receptors in endothelial cells (2, 3). Next, VEGF does DNA synthesis through up-regulation of anti-apoptotic components. The destruction of the basement membrane and intracellular phosphorylation of endothelial adhesion components and firm attaches provide the condition for survival, proliferation, migration and permeability of endothelial cells (4). Also, endostatin, as the strongest angiogenesis inhibitor, reduces proliferation and apoptosis of endothelial cells. This compound has been identified as a factor inhibiting the growth of endothelial cells and inhibition of angiogenesis (5). Endostatin is one of the most important angiogenesis factors that is isolated from collagen 18 and has a molecular volume of 20 KDa. This inhibiting factor is produced by different tissues in the body. Endostatin binds with VEGF receptor and inhibits its function. Also, endostatin inhibits the degradation of the basement membrane of capillaries which would ultimately prevent the migration of endothelial cells. In fact, endostatin is an obstacle to the growth of capillary network by inhibiting the proliferation and migration of endothelial cells. The main role of endostatin is the activation of intracellular messages that are mainly opposed to VEGF messages for

proliferation and migration of endothelial cells. Endostatin is generally secreted in the basement membrane area of blood vessels and is affected by several enzymes including elastase and matrix metallo proteinase (MMP) so that increasing MMP amounts will increase endostatin (6). Different training methods have been considered as one of the factors in establishing cardiac angiogenesis. In the meantime, the low intensity interval training to overcome fatigue increase physical activity and the health of individuals with regard to the nature of the training method (which is according to past research effective in the creation of factors affecting angiogenesis including shear stress and hypoxia and metabolites, etc.) and their effect on angiogenesis process of heart tissue have received less attention and the need to conduct these studies is strongly felt. Previous studies have shown that endurance training significantly increased the serum level of VEGF (1). Gavin et al. showed that short-term training exercises significantly increased the rate of mRNA VEGF; But the results of this study were not consistent with the results of Brixius (7). Brixius et al. (2008) showed that serum levels of VEGF in obese men 50- 60 years old does not change after 6 months of regular aerobic exercise. The reason for this difference may be due to the type of participants and duration of activity (7). Animal studies (8, 9) and human studies (10) showed changes in gene expression of angiogenic factors of fibroblast growth factor and VEGF following exercise. Rullman et al., showed that changes in VEGF amount of serum was due to changes in its gene expression in different tissues such as activated skeletal muscle (11). Also Frisbee showed that 10 weeks of endurance training increased the capillary density of active muscles by increasing nitric oxide (12). They stated that increasing the produced nitric oxide (NO) in response to exercise training increases the VEGF secretion by endothelial cells and this leads to increased capillary density. On

the other hand, increasing shear stress during exercise may be one of the causes of increasing angiogenic factors (12). Studies have shown that shear stress has a key role in increasing VEGF serum by increasing NO and subsequently activation of HIF-1 (13, 14). It should be noted that HIF-1 is the most important factor in regulating VEGF transcription process. Few studies on endostatin revealed that the rate of endostatin in serum significantly increased in healthy subjects in response to a single activity session (10). However, results obtained from this study and other studies have shown that exercise training, has a different effect, unlike acute activity. Brixius et al. showed that endostatin is reduced in obese men in response to long-term aerobic activity (7). In addition, Suhr et al. demonstrated that 6 months of endurance training significantly reduced the amount of endostatin in male runners (15). Seida et al. demonstrated that exercise training does not change endostatin levels in sedentary men. The results showed that endostatin's response to exercise training is dependent on anthropometric characteristics and preparation level of subjects (16). Recent studies have shown that endostatin levels has a reverse relationship with capillary density and tissue metabolic characteristics (10). Endostatin reduction mechanism in response to exercise training is not yet clear. However, it is likely that exercise training reduces the amount of change in the extracellular matrix and this may prevent the separating of endostatin from collagen (17). Little research has been conducted in connection with the effect of endurance training on angiogenesis of cardiac muscle. In a study Iemitsu et al. asserted that physical activity increased VEGF gene expression in cardiac muscle through activation of kinase pathways, protein kinase B (PKB), also known as Akt (serine- threonine kinase) and increased levels of and endothelial NOS (eNOS) (18). Due to the fact that the concomitant stimuli and inhibitors have not been investigated simultaneously in this

process and also the possible mechanism of LIIT's influence on angiogenesis not yet clear, also, the creation of chronic adaptation with exercise requires at least six weeks and the purpose of this research is to find the minimum impact time; therefore, it seems that the production of information on this subject will help the medical community and the scientific community of sports. Based on the above research, the present study was aimed at improving the quality of life of patients suffering from myocardial infarction. In this regard, the study investigated the effect of six weeks of low intensity interval training on VEGF, Fms-related tyrosine kinase-1 (Flt-1), collagen 18 in rats with myocardial infarction.

## Methods

In this experimental research, 12 10-week-old male Wistar rats were randomly divided into two groups of 6 rats including control and experimental. The rats were kept in separate cages with free access to water and food parcels according to the principles of Laboratory Animal Care (D NIH-publication). Then, rats underwent surgery and their left descending coronary artery (LAD) was blocked and the rats were MI patients. Later, the rats passed the recovery period after surgery for four weeks. In the third and fourth weeks of the recovery period, rats were familiar with the treadmill by walking slowly on it (at a speed of 5 meters per minute for five minutes a day, 4 days a week). At the end of the fourth week,  $VO_{2max}$ , was measured by maximal exercise test in rats. The running speed of each rat on the treadmill was calculated according to their  $VO_{2max}$  individually. Rats then rested for two days. Then, to ensure catching MI, echocardiography was used for measuring hemodynamic parameters. During this process, hemodynamic parameters such as left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs), injection fraction (EF), left ventricular shortening fraction (EFS), right ventricle and

left ventricle weight and all the cardiac muscle were measured. Rats with FS $\geq$ 35 percent were assigned as rats with MI and assigned for this study. Finally, the surviving rats with MI were randomly divided into two groups of low intensity interval training (LIIT) and control, and the training protocol was implemented after two days. Finally, remaining rats were re-anesthetized for echocardiography after two days of resting and samples of cardiac muscle tissue were taken to measure RNA levels of VEGF genes and collagen Type XVIII (COL-18) as a strong precursor to endostatin and also their common receptor Flt-1 by qRT-PCR method. The studied primer sequence for VEGF and collagen 18 were as follows:

**VEGF**

forward: 5'-TGAGACCCTGGTGGACATCTT-3'

reverse: 5'-CACACAGGACGGCTGAAGA-3'

**collagen 18**

forward: 5'-TGACAAGTTCCAGGGAATGA-3'

reverse: 5'-CGGTCGTCATCATCATCTTC-3'

**Flt-1**

forward:5'-

ACCCTCTGTACCTGGTCAATTGATGCAAAG-3'

reverse:5'-

TGCAAACCTCCCACTTGCTGGCATCATAG-3'

The training protocol consisted of 60 minutes of interval running on treadmill. Each cycle consists of 4 minutes of running with 60-75% of VO<sub>2max</sub> and 2 minutes of recovery with 40-

50% of VO<sub>2max</sub>, four days a week, for six weeks. Before starting the main phase of training, the rats did warm up for 8 minutes with 5 meters per minute. The collected data were analyzed using the SPSS18 statistical software. Kolmogorov-Smirnov test for normality of data was used and for statistical analysis of data, independent samples t- test was used (p $\leq$ 0.05).

**Results**

Weight changes in rats during 10 weeks (two weeks recovery after surgery and eight weeks training) is shown in Table 1. Results of independent samples t-test showed that despite the fact that COL-18 index values was higher in the LIIT group than the control group, as shown in table 2, but this difference was not statistically significant (P=0.34). but changes in FLT -1 had a significant difference in two groups (P=0.02) and based on Table 2, FIT-1 index values was more in the LIIT group than the control group. So the results of the independent samples t-test showed that the two groups of control and LIIT had a significant difference in VEGF index (P=0.01) and based on Table 2, VEGF values were more in the LIIT experimental group than the control group.

**Table 1.** weight changes of rats in 8 weeks

Group	Control	LIIT
Before surgery	265.00 $\pm$ 28.50	283.33 $\pm$ 12.90
Week1 (recovery after surgery)	243.00 $\pm$ 27.29	259.166 $\pm$ 12.41
Week2 (recovery after surgery)	258.00 $\pm$ 25.39	280.83 $\pm$ 9.70
Week3 (familiarity with the treadmill)	263.00 $\pm$ 27.74	249.16 $\pm$ 12.00
Week4 (familiarity with the treadmill)	276.00 $\pm$ 23.82	308.33 $\pm$ 2.41
Week5(training)	290.00 $\pm$ 31.42	320.83 $\pm$ 33.22
Week6(training)	301.600 $\pm$ 24.55	323.16 $\pm$ 29.66
Week7(training)	311.800 $\pm$ 18.40	337.50 $\pm$ 31.79
Week8(training)	321.00 $\pm$ 17.81	355.16 $\pm$ 28.88
Week9(training)	327.00 $\pm$ 17.53	360.00 $\pm$ 3404
Week10(training)	335.00 $\pm$ 22.36	375.00 $\pm$ 37.38

**Table 2.** Descriptive statistics and Kolmogorov-Smirnov test results(mg/ml)

Variable	Group	Minimum	Maximum	Mean	Standard Deviation	Independent t sample t-test	P
Collagen 18	Control	0.55	2.52	1.265	0.977	-1.01	0.34
	LIIT	1.23	2.36	1.724	0.518		
Flt-1	Control	0.97	2.10	1.380	0.560	-3.09	0.02
	LIIT	2.86	9.98	5.699	3.372		
VEGF	Control	0.91	1.07	1.002	0.073	-3.85	0.01
	LIIT	1.28	5.41	3.541	1.610		

## Discussion

The results of this study showed that six weeks of low intensity interval training with 60-75 percent of  $VO_{2max}$  induces the factors influential in increasing the expression of VEGF genes and Flt-1 and thereby stimulates angiogenesis. As mentioned before factors such as hypoxia, hemodynamic forces, metabolites, single-vessel dilation, cytokines and elasticity are effective on cardiac angiogenesis. The results of this study were consistent with the results of Davis et al., which showed that training significantly increased VEGF mRNA level in the cardiac muscle of rats. It also was consistent with Iemitsu et al., (2006) that showed that physical activity increased VEGF gene expression in cardiac muscle through Akt and increasing levels of eNOS and it was consistent with this study (18). It seems that exercise hypoxia has activated angiogenesis. HIF was not hydroxylated in hypoxic conditions and remained stable and migrated to the nucleus and induced factors effective in angiogenesis. Hypoxia has probably triggered the release of cytokines. Cytokines entered to the endothelial cells by NO that stimulated by fibroblast growth factor 2 (FGF- 2). It was also likely that the occurrence of acute and immediate increases in shear stress due endurance training (19, 20) was more via activation of ion channels, especially  $K^+$  channels and cause the secretion of vascular dilation LDA especially NO (21). NO caused upregulated

increase of VEGF and VEGFR- 2 on shear stress and the release of NO in the early stages of angiogenesis. Evidence suggested that NO involvement was independent of VEGF and VEGFR- 2 in the later stages of angiogenesis and is involved in this process by regulating ERK1/2 and activation of protein kinase ERK-C and c-jun. Endurance training probably has also stimulated VEGF gene expression through increasing muscle adaptations especially reducing degradation of creatine phosphate and glycogen (22) as well as increasing anabolic hormones (23) and adenosine (21). It seems that the adenosine has an important role in angiogenesis. Probably extracellular adenosine has activated adenosine receptors and then VEGF has been released from the parenchyma cells (24). VEGF has caused the proliferation and migration of these cells by binding to its receptors on the surface of endothelial cells. Also, adenosine can stimulate proliferation of vascular endothelial cells by regulating pro and anti-angiogenic growth factors or be effective in the development and reconstruction of new vessels by causing vasodilatation. In some circumstances. Evidence suggests that adenosine may be necessary as mediation for 50 to 70% of induced angiogenesis by hypoxia (25). Stretching of endurance training holds muscles on a length longer than the rest time and this stretching has increased MMP levels. Metalloproteinase are secreted by the



mentioned endothelial cells and analyzes the basement membrane in the above mentioned area through the entrance of calcium into the cell and its depolarization and activation of voltage-dependent potassium channel and calcium entry into the cell and cell hyperpolarization and endothelial cells migrated and proliferated. This has been a negative feedback mechanism to limit depolarization activity, VGCC activity, and consequently muscle contraction has been smooth. In the later stages of angiogenesis, MMP have been produced for extracellular matrix degradation starting the rebuild. Next, the process of pipe forming has begun by the interaction of Angiopoietin-2. Finally, pericytes and smooth muscle cells have been added to the structure to stabilize the newly formed blood vessel (26). As a result of all these changes leading to increased expression of VEGF and its Flt-1 receptor, mural cells moved from existing vessels' branch and vessels were unstable due to angiopoietin-2 that changed the endothelial cell (EC) status from without growth stable condition to a proliferation phenotype and proteases and matrix components leaked from the vessel wall and thus began to multiply the ECs. After proliferation, ECs migration occurred and tubular structures are formed and blood flowed. Mesenchymal cells proliferated and differentiated through new blood vessels cells and emigrated to adult pericyte cells. Finally, encouraging cell interactions and building the new matrix carefully will stabilize the new vessel. Capillary density has not been measured in this study so it is suggested that in future research other stimulant and inhibitory angiogenesis factors and capillary density should be studied

### Conclusion

The results of this study indicated that the exercise protocol used in this study - 60 minutes of high-intensity interval running on a treadmill at 60-75% VO<sub>2</sub>max intensity, four days a week for six weeks - was able to

increase angiogenesis stimulants in the heart and improve heart function.

### Ethical issues

Not applicable.

### Authors Contributions

All authors equally contributed to the writing and revision of this paper.

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### References

1. Gavin TP, Stallings HW, Zwetsloot KA, Westerkamp LM, Ryan NA, Moore RA, Pofahl E, Hickner RC. Lower capillary density but no difference in VEGF expression in obese vs lean young skeletal muscle in humans. *J Appl Physiol*. 2005; 98: 315- 323.
2. Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? *J Appl Physiol*. 2004; 97: 1119-1128.
3. Lloyd PG, Prior BM, Yang HT, Terjung RL. Angiogenic growth factor expression in rat skeletal muscle in response to exercise training. *Am J Physiol Heart Circ Physiol*. 2003; 284: 1668- 1678.
4. Bates DO. Vascular endothelial growth factors and vascular permeability. *Cardiovas Res*. 2010; 87: 262- 271.
5. Sugiyama A, Hirano Y, Okada M, Yamawaki H. Endostatin stimulates proliferation and migration of myofibroblasts isolated from myocardial infarction model rats. *Int J Mol Sci*. 2018; 19 (741): 1- 15.

6. Mooren F, Völker K. Molecular and cellular exercise physiology. *Human Kinetics*. 2004; 451.
7. 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. *Br J Sports Med*. 2008; 42: 126-129.
8. Hyuek Jong L. Exercise training regulates angiogenic gene expression in white adipose tissue. *J Exerc Rehabil*. 2018; 14 (1): 16- 23.
9. Olivier JG, Koulmann N, Peinnequin A, Xavier AB. Exercise- induced expression of vascular endothelial growth factor mRNA in rat skeletal muscle is dependent on fiber type. *J Physiol*. 2003; 552 (1): 213- 221.
10. Gu JW, Gadonski G, Wang J, Makey I, Adair TH. Exercise increases endostatin in circulation of healthy volunteers. *BMC Physiol*. 2004; 16: 2- 4.
11. Rullman E, Rundqvist H, Wågsäter D, Fischer H, Eriksson P, Sundberg CJ, et al. A single bout of exercise activates matrix metalloproteinase in human skeletal muscle. *J Appl Physiol*. 2007; 102: 2346-2351.
12. Frisbee JC, Samora JB, Peterson J, Bryner R. Exercise training blunts microvascular rarefaction in the metabolic syndrome. *Am J Physiol Heart Circ Physiol*. 2006; 291: 2483- 2492.
13. Kimura H, Weisz A, Kurashima Y, Hashimoto K, Ogura T, D'Acquisto F, et al. Hypoxia response element of the human vascular endothelial growth factor gene mediates transcriptional regulation by nitric oxide: control of hypoxia-inducible factor-1 activity by nitric oxide. *Blood J*. 2000; 95 (2): 189- 197.
14. Lloyd PG, Yang HT, Terjung RL. Arteriogenesis and angiogenesis in rat ischemic hindlimb: role of nitric oxide. *Am J Physiol Heart Circ Physiol*. 2001; 286 (6): 2528- 2538.
15. Suhr F, Rosenwick C, Vassiliadis A, Bloch W, Brixius K. Regulation of extracellular matrix compounds involved in angiogenic processes in short- and long-track elite runners. *Scand J Med Sci Sports*. 2010; 20: 441- 448.
16. 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. *Int J Obes Relat Metab Disord*. 2003; 27: 1325-1331.
17. Zhang R, Wang L, Zhang L, Chen J, Zhu Z, Zhang Z, Chopp M. Nitric oxide enhances angiogenesis via the synthesis of vascular endothelial growth factor and cGMP after stroke in the rat. *Cir Res*. 2003; 92 (3): 308- 313.
18. Iemitsu M, Maeda S, Jesmin S, Otsuki T, Miyauchi T. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *Am J Physiol Heart Circ Physiol*. 2006; 291: 1290- 1298.
19. Dinunno FA, Tanaka H, Monahan KD, Clevenger CM, Eskurza I, DeSouza CA, et al. Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *J Physiol*. 2001; 534 (1): 287- 295.
20. 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. *J Vasc Res*. 2009; 46: 504- 512.
21. Holloszy, John O, and Edward F. Adaptations of skeletal muscle to endurance exercise and their metabolic consequence. *Exe Physiol*. 2000; 56 (4): 831- 838.
22. Nakatani A, Han D, Hansen PA, Nolte LA, Host HH, Hickner RC, et al. Effect of endurance exercise training on muscle

- glycogen supercompensation in rats. *J Appl Physiol.* 1997; 82 (2): 711- 715.
23. Leslie AC, Jennifer L. Endogenous anabolic hormone responses to endurance versus resistance exercise and training in women. *J Sports Med.* 2012; 32 (1): 1- 22.
24. Koos BJ, Adenosine A. A receptors and O (2) sensing in development. *Am J Physiol Regul Integr Comp Physiol.* 2011; 301: 601- 622.
25. Ribatti D, Crivellato E. Mast cells, angiogenesis, and tumor growth. *Biochim Biophys Acta.* 2012; 1822: 2- 8.
26. Folkman J. Fundamental concepts of the angiogenic process. *Curr Mol Med.* 2003; 7 (3): 643- 651.