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The Impact of Specific Physical Training on FGF-2 and VEGF-A Expression in Patients Post-Coronary Artery Bypass Surgery

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A B S T R A C T

Introduction: Coronary artery bypass surgery (CABG) is a common procedure for patients with coronary artery disease. This study aimed to investigate the effect of selected physical exercises on the expression of FGF-2 and VEGF-A in patients post-CABG.

Material & Methods: In this semi-experimental study, 20 male patients from Afshar Hospital (Yazd City) who had undergone CABG were selected and randomly divided into two groups: a combined training group and a control group. The combined training group performed an 8-week program consisting of endurance training (15-20 minutes at 50-60% of HRmax) and resistance training (10-20 minutes, including three upper limb and two lower limb movements, each with three sets of 10 repetitions at 30-60% of 1RM), conducted three times per week. Gene expression of Fibroblast Growth Factor 2 (FGF-2) and Vascular Endothelial Growth Factor A (VEGF-A) was measured using Real-Time PCR. Data were analyzed using paired t-tests and one-way ANOVA with a significance level set at $P<0.05$.

Results: An increase in the expression of VEGF and FGF-2 genes was observed in the combined training group post-test compared to pre-test ($p=0.019$). Additionally, there was a significant difference in VEGF gene expression between the control and training groups ($p=0.016$). Significant differences were also found in FGF-2 expression between the combined exercise group and both the healthy control and patient control groups ($p=0.001$ for both).

Conclusion: The results suggest that combined exercises may enhance physiological adaptations and improve the functional capacity of the heart and vessels in CABG patients by increasing growth factor expression.

Keywords: Exercise, Fibroblast Growth Factor 2, Vascular Endothelial Growth Factor A, Coronary Artery Bypass

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Introduction

Globally, cardiovascular diseases account for approximately 17.9 million deaths, of which 80% are due to coronary heart disease (CHD). Numerous factors have been identified as risk factors, including ethnicity, gender, levels of total cholesterol, triglycerides, and blood pressure, which are influenced by lifestyle (1). Coronary artery bypass surgery (CABG) is a common therapeutic intervention performed on patients with coronary artery disease. Although heart surgery is a reliable method to improve blood supply to the myocardium, it has many post-operative complications (2). Myocardial ischemic necrosis can trigger a response to improve myocardial perfusion by forming new capillaries (angiogenesis) and enlarging existing vessels (arteriogenesis). Endothelial cells, the extracellular matrix, and surrounding cells are affected by the cascade of growth factors, their receptors, and intracellular signals (3). CABG is an aggressive procedure, and inflammation is the most common symptom following surgery. Despite the occurrence of these symptoms in the post-surgery period, patients experience a decline in quality of life, and evidence shows that some patients will face re-injury and even death. However, various factors, including regular physical training, can help prevent and treat this disease by regulating and modulating the inflammatory process. Studies have shown that sports interventions can be effective in managing heart disease (4). The vascular endothelial growth factor (VEGF) family controls functions such as promoting angiogenesis, promoting lymphopoiesis, regulating inflammation, resisting oxidative stress, and regulating lipid metabolism, providing potential therapeutic value for CHD (5). Angiogenesis, the formation of new blood vessels, involves multiple signaling cascades in the process of tissue revascularization. VEGF-A can regulate angiogenesis, vascular permeability, and inflammation by binding to VEGFR-1 and VEGFR-2. Studies show that the VEGF family can prevent atherosclerosis by increasing angiogenesis and regulating oxidative stress (6). These functions offer great potential for using the VEGF family in treating CHD. For example, angiogenesis can compensate for hypoxia and ischemia by growing new blood vessels, while lymphangiogenesis can reduce inflammation by providing pathways for accumulated inflammatory cytokines (6). VEGF also plays an important role in the development and maintenance of immune system unresponsiveness and has been reported to play a role in early graft failure in CABG patients (7). The pathway of VEGF expression in the body can lead to different roles. Since this expression can occur through both physiological and pathological pathways, it can have varying effects. In one study, it was shown that the injection of the VEGF gene in mice with heart attacks reduced its expression, thereby reducing infarct size, increasing myocardial perfusion, and effectively improving the disease (8).

On the other hand, fibroblast growth factor-2 (FGF-2) is a member of the fibroblast growth factor family. This factor is present in normal tissue, the basement membrane, and the subendothelial extracellular matrix of blood vessels. In the absence of a messenger peptide, it remains bound to the membrane (9). FGF-2 is also a potent mitogen for various cell types, including vascular endothelial cells and fibroblasts. FGF-2 induces an angiogenic phenotype characterized by increased proliferation, migration, proteinase production, and integrin expression (9).

Increasing sports activity, especially aerobic activities, can improve physical performance and cardiovascular health by reducing plasma lipid levels, blood glucose, and oxidative stress. One of the current challenges is identifying the effects of different exercise methods on physiological factors that influence physical function (10). Few studies have examined the impact of sports training on the variables investigated in this study.

Gurbanzadeh et al. (2016) explored the effects of crocin and voluntary exercise on blood glucose control and VEGF-A levels in heart tissue. They

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found that the VEGF-A level in the diabetic group was significantly lower than in the control group, and voluntary exercise significantly increased the VEGF-A protein level (11). Conversely, Danzig et al. (2010) evaluated the response of VEGF to exercise in coronary artery disease (CAD) patients and healthy individuals, finding no difference in VEGF levels between the groups either at baseline or after exercise (12). Hoier et al. (2012) demonstrated that acute training and four weeks of aerobic training affected gene and protein expression in skeletal muscle and anti-angiogenic factors in 14 trained young men. Their results showed an increase in VEGF concentration in response to acute exercise, but VEGF and VEGF receptor levels did not change after training (13). Hamidi et al. (2019) investigated the effect of aerobic and resistance exercises on plasma levels of basic fibroblast growth factor (BFGF) in coronary artery bypass graft patients. They found that eight weeks of aerobic resistance training caused a significant increase in BFGF levels in the experimental group compared to the control group (9).

Cardiovascular diseases currently affect many people worldwide, and CHD-related medical care costs billions of dollars annually. Addressing these public health emergencies requires new approaches based on a deeper understanding of the tissues and pathways involved in patient recovery. Physical activity is recognized as an integral part of preventing and treating cardiovascular diseases. However, the effect of combined exercises on angiogenesis indices in CHD patients after bypass surgery remains unclear. Given the importance of sports activity and its relationship to health, especially in CABG patients, the present research was conducted. This study aims to investigate the effect of selected physical exercises on the expression of genes for fibroblast growth factor-2 and vascular endothelial growth factor affecting blood vessels in patients after coronary artery bypass surgery.

Materials and methods

The current research is of an applied type and employs a semi-experimental method, conducted as a pre-test-post-test design. The statistical population consists of male patients from Afshar Hospital (Yazd, Iran) who have undergone CABG. After interviewing the volunteers and obtaining their consent, 66 subjects who met the necessary health conditions were selected. Sixteen subjects were then randomly chosen to participate in the interventions after completing the necessary forms and voluntarily agreeing.

The sample size for this study was determined based on the results of previous research, using a significance level of 5% (type 1 error) and a statistical power of 95% (type 2 error), calculated with Medcalc 18.2.1 software (10 subjects in each group). To familiarize the candidates with the implementation of the study, the subjects were invited to a briefing session. Following this session, the subjects were randomly divided into two groups: a combined exercises group and a control group, ensuring homogeneity based on the collected information.

Exercise protocol

In this research, aerobic and resistance exercises were performed based on the exercise programs recommended by the American Heart and Lung Association, the American Heart Association, and the American College of Sports Medicine. The combined program included aerobic and resistance training for 8 weeks (24 sessions), with three sessions per week. Exercises were performed according to the overload principle. All training sessions began with 5-10 minutes of warm-up and ended with 5-10 minutes of cool-down.

Endurance training was conducted for 15 to 20 minutes at an intensity of 20% to 30% of HRmax at the beginning, increasing to 50% to 60% of HRmax as the sessions progressed (14). The resistance training protocol, lasting 10-20 minutes, included three upper limb movements (forearm curls with dumbbells, lateral raises with dumbbells while

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standing, and hammer curls) and two lower limb movements (knee flexion and extension in a sitting position with an elastic band). Each movement consisted of three sets of 10 repetitions. The intensity of training in these movements increased gradually. In the first week, the training intensity was initially based on 30% of a one-repetition maximum (1RM) and gradually increased to 60% by the last week.

The measurement of 1RM for the subjects was calculated using the Berzyski equation. The method for determining 1RM involved the subject warming up with a light weight, then selecting a weight that could be lifted for up to 10 repetitions. If the weight was too light and more than 10 repetitions were completed, a heavier weight was chosen after a short rest until fewer than 10 repetitions could be performed. The weight and the number of repetitions were recorded for each movement and then placed into the following formula:

$1RM$=weight moved (kg)×(0.0278×number of repetitions until fatigue+1.0278)1RM=weight moved (kg)×(0.0278×number of repetitions until fatigue+1.0278).

Evaluation of Variables

The blood samples of the subjects were collected before and after the commencement of the training period. All stages of the test were conducted under uniform and standard conditions between 8 to 10 in the morning. Expression analysis of the target genes was performed on peripheral blood mononuclear cells of the patients. Blood sampling occurred at specified times and followed a time-dependent protocol. To prepare PBMNCs, 3 to 5 ml of whole blood was added to the RNA Blood tube containing Ficoll solution. Subsequently, centrifugation was carried out according to the protocol, resulting in the separation of the outer layer containing peripheral blood mononuclear cells. Following multiple washes with PBS, the samples were stored at -80°C for further analysis. Total RNA was extracted from peripheral blood mononuclear cells using specialized kits, followed by cDNA synthesis using specific protocols. Following the RT reaction, polymerase chain reaction (RT-PCR) was conducted on the synthesized cDNAs. Subsequently, the products underwent gel electrophoresis, and for quantitative analysis, Q-RT-PCR was utilized to report gene expression results quantitatively. After analyzing the data and comparing the expression levels of the studied genes among the different groups, the changes in gene expression were presented. The collected data were analyzed using SPSS software and GraphPad Professional Prism (Table 1).

Table 1. List of oligonucleotide primers used in this study.

Genes	Forward primers	Reverse primers
FGF-2	AGCGGCTGTACTGCAAAAACGG	CCTTTGATAGACACAACTCCTCTC
VEGF-A	5′-GAGGAGTTCAACATCGCCAT-3′	5′-GAGGAGTTCAACATCGCCA-3'

Statistical Analysis

The normality of data distribution was assessed using the Shapiro-Wilk test. Subsequently, paired t-tests and analysis of covariance were employed to compare the groups. All calculations were performed using SPSS version 26 statistical software, with significance set at $p<0.05$ for the tests.

Results

Table 2 displays the mean and standard deviation of the subjects. Results from the dependent t-test assessing intra-group changes in VEGF levels within the control and experimental groups revealed a significant increase in the desired factor during the post-test phase compared to the pre-test phase within

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the patient control group (p=0.019), indicating an upward trend. Conversely, no significant change was observed in the healthy group (p=0.849). Additionally, a notable increase in VEGF gene expression was noted in the combined training group during the post-test phase compared to the pre-test (p=0.019).

Furthermore, analysis of FGF2 expression index within the control and experimental groups demonstrated a significant rise in the desired factor during the post-test phase compared to the pre-test phase solely within the combined training group (p=0.007). While an upward trend in FGF2 expression level was observed in the patient control group during the post-test phase compared to the pre-test, it did not reach statistical significance (p=0.238).

Covariance analysis results indicated a significant disparity in VEGF gene expression between the control and training groups (p=0.016) (Table 3). Moreover, significant differences were observed in the FGF2 expression index between the combined training group and both the healthy control and patient control groups (p=0.001, p=0.001, respectively) (Table 4).

Table 2. Mean and Standard Deviation of Subjects' Personal Characteristics.

Variable	Group	Control	Patient	Exercise
Age (years)	Pre-test	61.62 ± 8.22	57.25 ± 9.46	62.25 ± 8.82
Height (meters)	Pre-test	1.68 ± 0.058	1.69 ± 0.087	1.7 ± 0.073
Weight (kg)	Pre-test	74.87 ± 14.33	72.25 ± 12.81	76.18 ± 15.83
	Post-test	75.31 ± 13.95	12.03 ± 0.74	74.62 ± 14.80
Heart rate at rest (numbers per minute)	Pre-test	82.60 ± 5.07	77.60 ± 6.65	77.60 ± 6.65
	Post-test	80 ± 4.89	72 ± 7.51	72 ± 7.51
Systolic blood pressure (mmHg)	Pre-test	123.1 ± 2.9	127.40 ± 4.66	134.2 ± 5.47
	Post-test	122.5 ± 1.8	128.7 ± 8.7	129.8 ± 6.12
Diastolic blood pressure (mmHg)	Pre-test	80.2 ± 7.95	79.2 ± 6.41	79.2 ± 6.41
	Post-test	79.4 ± 5.54	73.8 ± 9.23	73.8 ± 9.23
Body mass index (kilograms per square meter)	Pre-test	26.26 ± 3.94	25.31 ± 3.34	26.13 ± 3.68
	Post-test	26.42 ± 3.73	25.6 ± 3.16	25.62 ± 3.42

Table 3. The Results of Covariance Analysis Related to VEGF Index in Different Groups.

	Sum of squares	Df *	Average of squares	F **	P- value
Modified model	14.062	1	14.062	5.570	0.058
VEGF (pre-test)	14.475	1	14.475	5.850	0.057
Group	25.359	2	12.679	5.924	*0.016

*Df= Degree of Freedom , ** F= F-test, Analysis of Variance

Table 4. Results of Covariance Analysis Related to FGF2 Expression Index in Different Groups.

	Sum of squares	Df *	Average of squares	F**	P-value

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Modified model	51.312	1	51.312	28.220	0.000
FGF-2 (pre-test)	1.807	1	1.807	1.065	0.314
Group	44.955	2	22.478	13.251	*0.000

*Df= Degree of Freedom , ** F= F-test, Analysis of Variance

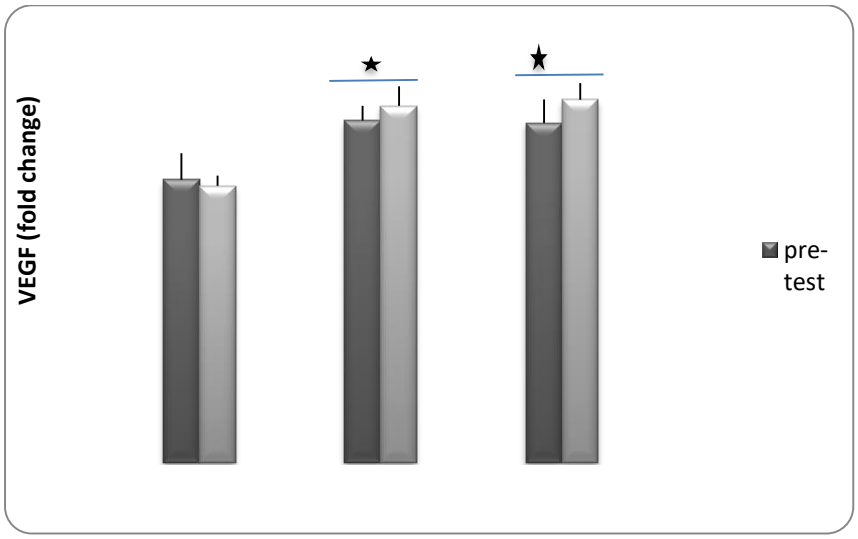


Figure 1. Comparison of VEGF level changes in control and experimental groups.
★ Significant difference with the control group
✦ Significant difference with the pre-test

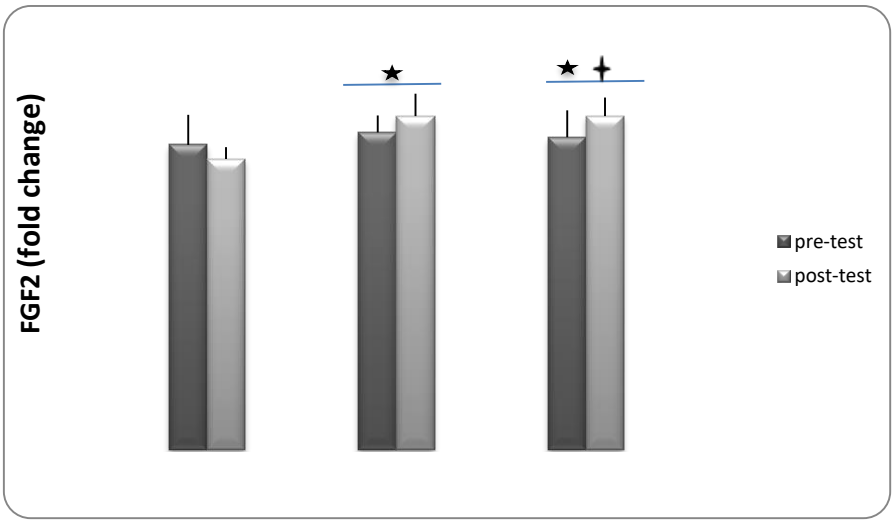


Figure 2. Comparison of FGF2 level changes in control and experimental groups.
★ Significant difference with the control group
✦ Significant difference with the pre-test

Discussion

VEGF, as the primary angiogenic molecules, govern the growth and functionality of vessels, vascular homeostasis, as well as vessel permeability and dilation. They are crucial for adult heart angiogenesis and are therefore considered novel therapeutic agents for ischemic heart and peripheral vascular disease (15). During CABG, acute ischemia is linked to

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elevated circulating levels of VEGF, potentially beneficial due to the stimulation of VEGF synthesis in human coronary arteries and aortic endothelial cells (16).

In this study, the expression levels of VEGF and FGF2 genes in the patient control group significantly increased in the post-test phase compared to the pre-test phase, whereas no such changes were observed in the healthy control group. Additionally, the results indicated elevated expression of VEGF and FGF2 genes in the combined training group compared to the healthy control group. VEGF induces angiogenesis and enhances microvascular permeability. In vivo, VEGF directly acts on the endothelium, facilitating angiogenesis. Research suggests VEGF's significant role in inflammation (3). However, VEGFs also contribute to pathological conditions by promoting small vessel growth in tumors and atherosclerotic lesions. Consequently, excessively high VEGF levels may signal excessive inflammation, while very low levels could indicate insufficient vascular repair.

During acute coronary atherosclerotic diseases, angiogenesis may emerge as a compensatory mechanism to alleviate myocardial ischemia, indicating a potential protective role for VEGF in heart muscle disease. However, atherosclerotic plaques become unstable when angiogenesis permeates the plaque, leading to intraplaque hemorrhage and increasing cardiovascular events. Animal experiments have confirmed that exogenous administration of VEGF fosters the development of atherosclerotic plaques. The establishment of peripheral blood circulation involves endothelial cell proliferation, extracellular matrix regeneration, and leukocyte accumulation, with VEGF playing a pivotal role in these processes (17).

Therefore, the level of VEGF may have the ability to predict the formation of vessels in coronary circulation. Determining whether observed changes in growth factors are harmful or beneficial to the patient is challenging. On one hand, higher levels observed compared to the healthy control group suggest that elevated levels of growth factors could enhance the healing processes of diseased vessels. On the other hand, the association of growth factors with inflammatory processes leads to speculation that observed changes may result from the long-term anti-atherosclerotic effects of applied drugs. Several reports have indicated that cardiac bypass surgery is associated with increased growth factors post-surgery. Myocardial ischemia secondary to cardiac bypass serves as a potent stimulus for the production of growth factors, leading to increased VEGF levels up to six days after CABG (3).

However, in the present study, MCP-1 levels were not measured. Nevertheless, an increase in VEGF or FGF gene expression was observed in the control group. The serum level of VEGF is strongly regulated by monocyte chemoattractant protein (MCP)-1, a member of the C-C chemokine family. MCP-1 is produced by monocytes/macrophages and smooth muscle within atherosclerotic plaques. Under physiological conditions, VEGF-A and its receptors are not expressed in normal coronary arteries. However, in the presence of atherosclerotic lesions, VEGF-A expression increases in vascular endothelial cells, macrophages, and partially differentiated smooth muscle cells. Animal experiments have indicated that VEGF can serve as a marker of atherosclerosis (19).

Therefore, VEGF is believed to have a dual role in coronary heart disease. Additionally, in the current study, engaging in combined exercises for 10 weeks resulted in a significant increase in growth factors in the exercise group compared to the control groups. Perhaps one reason for the rise in VEGF levels is the activation of MCP-1 due to oxidative and inflammatory stress conditions caused by heart failure. This occurrence typically arises in patients due to the weakness of the antioxidant system and the increase in reactive oxygen species and inflammatory molecules (20).

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In Ellison et al.'s research, they demonstrated that exercise prompts heart muscle regeneration through pathways activated by growth factors. Physical activity appears to enhance blood supply to muscles, particularly the heart muscle. Consequently, the heart secretes growth factors, including VEGF, in response to arterial pressure to enhance blood supply and angiogenesis (21).

Among other factors contributing to the increase in growth factors, the effect of hypoxic conditions induced by physical activity can be mentioned. In the absence of oxygen, hypoxia-inducible factor (HIF-1) accumulates and increases PDGF. Moreover, PDGF, through its mitogenic activity, acts in the heart by binding to tyrosine kinase receptors, inducing the migration of mesenchymal cells (22). PDGF also stimulates VEGF and regulates adhesive junctions in the inner part of the myocardium, facilitating the angiogenesis process (23).

In this study, moderate-intensity combined exercise was utilized for CABG patients. Generally, various studies have demonstrated the safety and efficacy of light to moderate-intensity exercises. Gainini et al. investigated the effects of eight weeks of combined and endurance training on functional capacity, body composition, and heart functional strength in post-CABG patients, reporting improvements in the functional capacity of heart patients with both programs (24).

Conclusion

The findings of this study suggest that combined exercises may potentially enhance physiological adaptation and improve the functional capacity of the heart and blood vessels in CABG patients by increasing growth factors.

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Conflict of interest

The authors declare no conflicts of interest regarding this research.

Authors' contributions

AB conceived and designed the study. MM contributed to data acquisition and interpretation, and drafted the manuscript. AB and AA critically revised the manuscript.

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