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Original Article

The effect of eight weeks of high-intensity interval training on the expression of mir-126, EMAP-II, and levels of TGF-β in the heart tissue of young rats with type 2 diabetes

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Abstract

Background and aims: Diabetes affects some cardiac angiogenic and antiangiogenic markers. Thus, the aim of this study was to investigate the effect of eight weeks of high-intensity interval training (HIIT) on mir-126, endothelial monocyte-activating polypeptide II (EMAP-II), and transforming growth factor beta (TGF-β) in young rats with type 2 diabetes (T2D).

Methods: The statistical population of this experimental study included young rats (8 weeks old) with T2D. In this research, 24 young rats (8 weeks old) were divided into healthy + HIIT, diabetic + HIIT, and healthy control groups. The enzyme-linked immunosorbent assay method was used to measure TGF-β and the real-time polymerase chain reaction for mir-126 and EMAP-II. A one-way analysis of variance and the least significant difference post hoc test were used to compare the two groups.

Results: The results showed diabetes + HIIT and healthy + HIIT had more mir-126 compared to healthy control ($P=0.001$). Healthy + HIIT, in comparison to diabetes + HIIT, had higher mir-126 expression ($P=0.001$). In addition, diabetes + HIIT, compared to the healthy control, had a higher EMAP-II expression ($P=0.006$). On the other hand, healthy + HIIT had lower EMAP-II in comparison to healthy control ($P=0.001$). Healthy + HIIT had a lower EMAP-II compared to diabetes + HIIT ($P=0.001$). Diabetes + HIIT, in comparison to the healthy control, had higher TGF-β levels ($P=0.005$). However, healthy + HIIT, compared to healthy control, had a lower expression of TGF-β ($P=0.001$). Finally, the healthy + HIIT groups, compared to the diabetes + HIIT group, had a lower TGF-β expression ($P=0.001$).

Conclusion: Interval exercise could increase angiogenesis in diabetic samples through the increase of mir-126 and the reduction of TGF-β1 and EMAP-II.

Keywords: High-intensity interval training, Diabetes, Angiogenesis, MicroRNA

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Introduction

Diabetes has an important effect on cardiovascular angiogenesis (1). In this regard, Balakrishnan et al, in their study on 410 people with coronary artery disease (diabetic and non-diabetic), observed that diabetic patients have a lower amount of coronary collateral vessels (2). Ishibashi et al also performed a study on 50 patients and reported that the growth and development of coronary collateral vessels were reduced in diabetic patients (3). Some studies have shown that changes in microRNAs are associated with cardiovascular diseases, such as myocardial infarction, coronary artery disease, and cardiac arrest, as well as autoimmune diseases (4). One of the important microRNAs is mir126 (5), and some studies have demonstrated that diabetes decreases mir-126 in the heart tissue (6, 7). However, diabetes causes an increase in anti-angiogenic factors, along with a reduction in factors affecting cardiac angiogenesis. Endothelial monocyte-activating polypeptide II (EMAP-II) and transforming growth factor beta (TGF-β) can be mentioned among these factors. EMAP-II is an anti-inflammatory cytokine that has anti-angiogenic effects, inhibits vascular endothelial growth factor (VEGF)-related angiogenic signaling in human umbilical vein endothelial cells, and inhibits tumor progression (8,9). Yuan et al found that the inhibition of EMAP-II causes angiogenesis and cardiac function after chronic myocardial infarction (10). Studies have indicated that the amount of EMAP-II is higher in diabetic people than in healthy people. A significant relationship has been reported between blood sugar and body fat with EMAP-II (11). However, no results have been found about its values in the heart tissue of diabetics. On the other hand, cardiac fibrosis resulting from increased gene expression of extracellular matrix compounds, such as collagen and TGF-β1, leads to increased cardiac stiffness and, as a result, diastolic disorder (12). Different results have been reported regarding the role of TGF-ß in cardiac angiogenesis. Ferrari et al (13) and Fathi et al (14) concluded that TGF-β is effective in cardiac angiogenesis and VEGF regulation. However, considering that the amount of cardiac angiogenesis decreases in diabetes, and some studies have shown that diabetes increases TGF-β, it

is impossible to be sure about the role of TGF-β in cardiac angiogenesis. In this regard, Sadeghi Moheb et al (15) and Ko et al (16) found that diabetes causes a significant increase in TGF-β. Khosravi et al also reported a significant increase in TGF-β in the heart tissue of diabetic rats (17).

On the other hand, the role of exercise in diabetes, and the regulation of cardiac angiogenesis, and its effect on angiogenic and antiangiogenic factors are highly important. In this regard, Hadi et al found a significant increase in mir-126 due to aerobic exercise (18). In the case of EMAP-II, no clear results were found regarding the effect of exercise. Kwak et al indicated that 12 weeks of aerobic training for 45 minutes per session and 5 days per week leads to a decrease in TGF-β levels in the left ventricular tissue of old rats (19). Considering that hypoxia is extremely essential in angiogenesis, high-intensity interval training (HIIT), which is somehow effective in causing hypoxia by causing hypoxia (19), could be highly important in causing angiogenesis. The review of past studies has shown the evaluation of the effect of HIIT on angiogenesis and anti-angiogenesis indicators in diabetic people. Therefore, this research aimed to investigate the effect of 8 weeks of HIIT on mir-126, EMAP-II, and TGF-β in young rats with type 2 diabetes (T2D).

Materials and Methods

The current research is of an experimental type, the statistical population of which consists of 8-week-old young rats suffering from T2D. In this research, 24 healthy 8-week-old young rats were purchased from the Pasteur Institute. The rats were kept in the cage at Qom University of Medical Sciences under standard conditions (average temperature of 20-23 degrees Celsius, light-dark cycle of 12:12 hours, and relative humidity of 50%) with free access to water and special rat food. After familiarization with the new environment, the rats were randomly divided into diabetic-interval exercise, healthy + interval exercise, and healthy control (without exercise) groups.

Overall, 8 rats were selected and made diabetic by injecting a single dose of streptozotocin (STZ). After 8 hours of food deprivation, the animals were made diabetic by intraperitoneal injection of STZ dissolved in sodium citrate buffer (pH = 5.4 at the rate of 50 mg/kg). Frothy-eight hours after injection, the rats were examined for diabetes. To confirm diabetes, 4 days after STZ injection, by making a small wound in the tail of the animals, a drop of blood was placed on the glucometer strip, and the strip was read by the glucometer device. Blood sugar above 250 mg/dL was considered an indicator of diabetes.

The training groups performed their respective activities, including 5 minutes of low-intensity warm-up, then the main exercise of running on a treadmill for rodents (consisting of 12 repetitions of an exercise with an intensity of 90%-95% of VO_2max for 1 minute and active rest periods with an intensity of 50% VO_2max for 1 minute). In addition, low-intensity cooling was performed for 5 minutes after finishing the main exercise. It should be noted that all biological conditions for the control group, except for the main exercise protocols on the test day, were similar to those of the exercise groups. To measure the maximum oxygen consumption, due to the lack of access to direct tools (e.g., respiratory gas analyzers), and according to the conducted research, the indirect protocol was used with great accuracy. Therefore, after 10 minutes at low intensity, the rats ran at 15 m/min for 2 minutes. Then, the speed was increased by 0.3 m/s (1.8–2 m/min) every 2 minutes until the animals could no longer run.

The speed at which the rats reached a standstill was taken as the maximum speed (20). Twenty-four hours later, from the last training session, the rats were anesthetized using a combination of ketamine (75 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. After ensuring that the animals were anesthetized, the chest of the animal was opened, and blood was taken directly from the heart of the animal, and the heart of the animal was extracted as well. Then, the samples were immediately transferred to a microtube, placed in liquid nitrogen, and transferred to a freezer with a temperature of -80 degrees Celsius for further measurements. The Rat TGF-β1 enzyme-linked immunosorbent assay kit (Buster Company) was used to check the amount of TGF-β1.

RNA extraction and cDNA synthesis in the heart tissue to investigate MicroRNA expression

The RNA extraction steps were performed based on the Trizol protocol. For microRNA extraction, after adding isopropanol, the supernatant was kept at -20 ˚C for 1 night, and the next steps of extraction were performed accordingly. A Stratagene kit was utilized to make cDNA according to the company's protocol.

Real-time polymerase chain reaction

First, the desired concentration of cDNA and primer related to microRNA was determined using a concentration serial test. The RT-PCR program on the Corbett machine included 95 degrees for 10 minutes and 45 cycles, 95 degrees for 10 seconds, 60 degrees for 15 seconds, and 72 degrees for 20 seconds. U6 was employed as an internal control gene. The designed and used primers are listed in Table 1 (21, 22).

Table 1. Primers

	Forward Primer	Reverse Primer
Mir-126	5-TATGGTTGTTCTCGACTCCTTCAC-3	5-TCGTCTGTCGTACCGTGAGTAAT-3
EMAP-II	5' TAATACGACTCACTATAGGG 3'	5' TAGAAGGCACAGTCGAGG 3'
U6	GCGCGTCGTGAAGCGTTC	GTGCAGGGTCCGAGGT

Note. EMAP-II: Endothelial monocyte-activating polypeptide II.

Statistical Method

In this research, the Shapiro-Wilk test was used to check the normality of the data distribution. If the data were normal, parametric tests, including a one-way analysis of variance and the least significant difference (LSD) post hoc test, were applied to compare the two groups. All analyses were performed using SPSS software (version 22, Chicago) at a significance level of $P \leq 0.05$.

Results

The results of the ANOVA showed that there is a significant difference in the expression of mir-126 among the research groups ($P=0.001$). The LSD post hoc test also determined that the diabetes + exercise group had a higher expression of mir-126 compared to the control group ($P=0.001$). Further, the healthy + exercise group had more mir-126 in comparison to the control group ($P=0.001$). The healthy group exercise also had higher mir-126 levels compared to the diabetes + exercise group ($P=0.001$, Tables 2 and 3).

Furthermore, there was a significant difference between the research groups in terms of the EMAP-II value ($P=0.001$). The LSD post hoc test also determined that the diabetes + exercise group had a higher EMAP-II value compared to the control group ($P=0.006$). However, the healthy + training group had lower EMAP-II in comparison to the control group ($P=0.001$). The healthy + exercise group also had a lower EMAP-II value compared to the diabetes + exercise group ($P=0.001$, Tables 2 and 3).

The results of the ANOVA statistical test demonstrated that there is a significant difference in the amount of TGF-β among the research groups ($P=0.001$). The LSD post hoc test also indicated that the diabetes + exercise group had higher TGF-β levels in comparison to the control group ($P=0.005$). On the other hand, the healthy + exercise group had a lower amount of TGF-β compared to the control group ($P=0.001$). Finally, the healthy + exercise group also had a lower TGF-β amount in comparison to the diabetes + exercise group ($P=0.001$, Tables 2 and 3).

Table 2. ANOVA test results for mi-126, EMAP-II, and TGF-β

Variable	Health control	Diabetic + exercise	Healthy + exercise	*P* Value
mir-126	1 ± 0.4	2.6 ± 0.5	3.3 ± 0.3	0.001
EMAP-II	1.7 ± 0.7	2.8 ± 0.66	0.8 ± 0.4	0.001
TGF-β	300 ± 40	730 ± 80	220 ± 22	0.001

Note. ANOVA: Analysis of variance; EMAP-II: Endothelial monocyte-activating polypeptide II; TGF-β: Transforming growth factor beta.

Discussion

The results of the ANOVA statistical test revealed that the diabetes + HIIT group, compared to the control group, had a higher amount of mir-126. Additionally, the healthy + HIIT group had more mir-126 in comparison to the control group. The healthy + exercise group also had a higher amount of mir-126 compared to the diabetes + exercise group. Mousavinezhad et al also reported that performing 8 weeks of aerobic exercise significantly increased mir-126 expression and capillary density of the heart tissue (23). Ya et al showed that mir126 is significantly reduced in cells under hypoxic conditions in comparison to normoxic conditions. Similarly, the expression of mir126 was decreased in the retinal tissue of diabetic rats (24). Zampetaki et al also found a decrease in plasma mir126 expression in patients with T2D. These researchers stated that mir126 is considered a predictor of diabetes (25). Liu et al concluded that mir126 in impaired glucose tolerance (IGT) or impaired fasting glucose (IGT/IFG) subjects and patients with T2D were significantly lower than healthy subjects. After 6 months of treatment (diet and exercise control in IGT/IFG subjects, insulin control, and diet and exercise activity in T2D patients), serum mir126 increased significantly (26). Mir126 directly suppresses two negative regulators affecting the VEGF pathway. These two pathways are Sprouty-related protein 1 (Spred-1), and phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2); the negative side regulates the activity of the PIK3/Akt/eNOS pathway (27). It seems that interval exercise increases VEGF and ultimately leads to angiogenesis through the increase of mir-126. Along with factors that increase angiogenesis, anti-angiogenesis factors are also affected by diabetes and exercise.

Moreover, the results showed that the diabetes + exercise group has a higher expression of EMAP-II compared to the control group. Additionally, the healthy + exercise group had more EMAP-II in comparison to the control group.

Table 3. LSD post hoc test for mi-126, EMAP-II, and TGF-β

Variable	Group (1)	Group (2)	Mean difference	Standard deviation	*P* value
mir-126	Health control	Diabetic + exercise	-2.31	0.11	0.001
		Healthy + exercise	-1.72	0.11	0.001
	Diabetic + exercise	Healthy + exercise	2.31	0.11	0.001
EMAP-II	Health control	Diabetic + exercise	0.92	0.21	0.006
		Healthy + exercise	-1.21	0.21	0.001
	Diabetic + exercise	Healthy + exercise	-2.12	021	0.001
TGF-β	Health control	Diabetic + exercise	88	19.82	0.005
		Healthy + exercise	445	19.82	0.001
	Diabetic + exercise	Healthy + exercise	533	19.82	0.001

Note. LSD: Least significant difference; EMAP-II: Endothelial monocyte-activating polypeptide II; TGF-β: Transforming growth factor beta.

The healthy + exercise group also had a lower expression of EMAP-II compared to the diabetes + exercise group (8). Other studies have demonstrated that EMAP-II levels are higher in diabetics than in healthy individuals. Some other studies indicated a significant relationship between blood sugar and body fat with EMAP-II (11,28). However, no results have been reported about its values in the heart tissue of diabetics. No clear results were found regarding the effect of exercise on EMAP-II. However, EMAP-II suppresses tumor growth with anti-angiogenic properties and downregulates the expression of VEGF. EMAP-II competes with VEGF to inhibit its binding to VEGFR1 and VEGFR2, and this inhibition is greater for VEGFR1 compared to VEGFR2. It has been shown that the binding affinity of VEGF to VEGFR1 is higher than that of VEGFR2 (8).

On the other hand, the results of the research revealed that the diabetes + HIIT group had more TGF-β compared to the control group. In addition, the healthy + exercise group had more TGF-β in comparison to the control group. On the other hand, the healthy group + exercise also had a lower amount of TGF-β compared to the diabetes + exercise group. Kwak et al concluded that 12 weeks of aerobic exercise led to a decrease in TGF-β levels in the left ventricular tissue of old rats (19). Silva et al also showed that the renal amounts of TGF-β1 in diabetic rats were higher compared to healthy rats, which significantly decreased after 8 weeks of resistance training (29). In the condition of diabetes, the increase in glucose levels leads to the production of angiotensin II in cardiac myocytes and fibroblasts, which is associated with the positive regulation of collagen synthesis and the production of TGF-ß in the cardiac tissue (29,30). Therefore, high levels of angiotensin II, glucose, and oxidative stress may lead to structural changes and cardiac fibrosis through the reduction of matrix metalloproteinase-2 activity and the increase of TGF-ß1 (30,31). It seems that interval exercise can lead to the reduction of TGF-ß1 through the reduction of angiotensin 2 and the reduction of oxidative stress. Baghaiee et al reported a reduction in oxidative stress due to aerobic exercise (32).

Different results have been reported regarding the role of TGF-β in cardiac angiogenesis. Ferrari et al (13) and Fathi et al (14) reported that TGF-β is effective in cardiac angiogenesis and VEGF regulation. Song et al also demonstrated that dysregulated TGF-β is commonly associated with fibrosis, unstable angiogenesis, and accelerated progression in heart failure (33). However, considering that the amount of cardiac angiogenesis decreases in diabetes and some studies have shown that diabetes increases TGF-β, it is impossible to be sure about the role of TGF-β in cardiac angiogenesis (15-17). The anti-angiogenic effect of TGF-β is specifically mediated through the TβRII and ALK-5 signaling pathways (34-36). On the other hand, the relationship between TGF-β and Let-7a is also important (37). It seems that the reduction of TGF-β, followed by the increase of Let-7a, can also increase VEGF.

Conclusion

mir-126 increases cardiac angiogenesis, and TGF-β1 and EMAPII decrease cardiac angiogenesis. The results of this research revealed that high-interval training increases angiogenesis in diabetic samples through the increase of mir-126 and the reduction of TGF-β1 and EMAPII.

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Competing Interests

The authors indicated that there is no conflict of interests.

Ethical Approval

This article is extracted from the Ph.D. thesis of Islamic Azad University, Mahallat Branch. To follow the principles of research ethics, the protocol of this study has been approved by the Medical Committee of the Qom University of Medical Sciences (Reference number IR.IAU.QOM.RES.1399.037).

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