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RESEARCH ARTICLE

Micro RNA-126 promoting angiogenesis in diabetic heart by VEGF/Spred-1/Raf-1 pathway: effects of high-intensity interval training

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Abstract

Purpose This study aims to investigate the effect of high-intensity interval training (HIIT) on gene expression of *MicroRNA-126* (*miR-126*) and serum concentration of vascular endothelial growth factor/ sprouty related EVH1 domain containing 1/ rapidly accelerated fibrosarcoma 1 (VEGF/Spred-1/Raf-1) proteins effective in cardiac tissue angiogenesis of diabetic rats.

Methods Forty male Wistar rats were randomly divided into four groups of healthy control (HC), diabetic control (DC), diabetic with HIIT training (DT), and healthy with HIIT training (HT). HIIT was performed 6 days per week for 6 weeks (with the overload). Diabetes was induced via the combination of intraperitoneal injection of streptozotocin and high-fat foods.

Results Diabetes remarkably diminished the expressions of *miR-126*, VEGF and Raf-1 proteins, and augmented Spred-1 expression. Meanwhile, the implementation of HIIT gave rise to a significant enhancement in expression of *miR-126* heart tissue ($P < 0.01$), and subsequently increased the expression of VEGF and Raf-1 proteins ($P < 0.01$), and declined Spred-1 expression ($P < 0.01$) in the training group compared to the control group.

Conclusion The results of this study show that HIIT increases the expression of *miR-126* by activating the angiogenesis pathway of the heart tissue. Increased angiogenesis through the *miR-126* pathway is vital to compensate for heart destruction induced by diabetes. Thus, the use of standard interval exercise can be introduced as a novel therapeutic target for diabetic cardiomyopathy.

Keywords Exercise · Diabetes mellitus · Heart muscle · *miR-126* · VEGF

Introduction

Type 2 Diabetes mellitus (T2DM) is one of the most common metabolic disorders throughout the world, which can cause cardiovascular disease leading to cardiovascular deterioration [1]. According to the international organization for diabetes, 365 million people were diagnosed with diabetes in 2011, and this population is estimated to boost up to 552 million by 2030 [2]. Diabetes mellitus in 68% of diabetic patients causes heart disease or ultimately leads to death. There is a linear relationship between diabetes and cardiovascular disease [3]. Also, hyperglycemia, high blood pressure, dyslipidemia, and insulin resistance affect myocardial infarction of the diabetic population, leading to myocardial, endothelial coronary events, left ventricular, and structural and molecular disorders [4]. Diabetes is considered a paradox in terms of angiogenesis and vascularization due to the onset of increased angiogenesis in organs such as the eyes and kidneys, and on the other hand, it declines the process of angiogenesis in the cardiovascular tissue [5]. Based on studies, the reduction of angiogenesis and lateral arteries formation in the heart have been observed in humans and animal models of diabetes mellitus [6–8].

It has been observed that diabetes leads to a decrease in blood perfusion to myocardial tissue which can enhance mortality [9]. Despite this adverse effect of diabetes on angiogenesis, the molecular mechanisms involved in this phenomenon are not well known. Recent studies have shown the important role of microRNAs in responding to cardiovascular system to

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damage and stress [10]. MicroRNAs are small RNA molecules that act as regulators in the post-transcriptional or translation process [11]. Humans have approximately 2000 annotated MiRNA genes and the total number of microRNA loci annotated is 24, 521 loci in 206 species [12]. Studies show *miR-126* can appear in endothelial cells and regulate angiogenesis in different tissues [13]. It has been shown that plasma expression of *miR-126* reduced in diabetic patients. It is therefore utilized as a new diagnostic marker for patients with heart failure (HF) and those with type 2 diabetes [14]. It has been shown that the expression of *miR-126* is reduced in cardiovascular patients with atherosclerosis [15]. *MiR-126* directly suppresses the two negative regulators of the vascular endothelial growth factor (VEGF) pathway. These two pathways include sprouty-related protein1 (Spred-1), an intracellular suppressor of the Ras/MAPK pathway, and the second regulatory phosphate-inositol 3kinase (PIK3R2) subunit negatively affecting the PIK3/Akt/eNOS pathway [16]. Increasing the expression of *miR-126* through the inhibition (PIK3R2) and Spred-1 leads to an increase in the pre-angiogenic VEGF protein [16]. Exercise potentially plays an important role in enhancing cardiac angiogenesis by stimulating the expression of *miR-126* and VEGF proteins. In support of this, a five-day swimming program for 10 weeks significantly augmented myocardial capillary density in rats. This beneficial effect has been shown to alter the pathways of VEGF/Raf-1/ERK and VEGF/P13K/AKT [17]. Exercise also suppresses the Spred-1, PIK3R2 proteins by up-regulation of the expression of *miR-126* [18]. It can, therefore, be stated that exercise as a valuable non-pharmacologic agent potentially causes up-regulation of angiogenesis and also improves both coronary blood flow and the function of patients with diabetic heart disease (DHD) [17]. It has been found that high-intensity interval training (HIIT) affects the development of aerobic power and cardiovascular function of healthy subjects. Also, it has been displayed that in patients with cardiovascular disease, HIIT has a better effect compared to moderate-intensity continuous training (MICT) [19]. HIIT leads to reduced glucose response after meals, as well as hyperglycemia in diabetic patients [20]. Therefore, high-intensity exercise training may be better and more effective in controlling blood glucose in diabetics [21]. There are studies on consideration of these factors in the diabetic heart. However, in this study, we consider the effects of HIIT on the pathology of diabetes with these factors to see how the HIIT can affect this signal pathway. Hence, the purpose of this research is to investigate the effects of HIIT on the expression of *miR-126* and serum concentration of VEGF/Spred-1/Raf-1 proteins as the effective markers for the angiogenesis of cardiac tissue in the diabetic rat's model.

Methods

Animal

In this study, 40 Wistar male rats (194.6 ± 9.6 g, 8 weeks) were purchased from Pasteur Institute in Iran. All animal experiments were conducted according to the guidelines of the National Institute of Health Guide for the care and use of laboratory animals care and use (NIH Publications No. 8023, revised 1978) and were prepared by Baqiyatallah University of Medical Sciences (ethical with code reference: IR.BMSU.1398.077). The animals were transferred to the laboratory 24 h prior tothe beginning of the study in order to adapt to the environment. Animals were kept at 22 ± 2 °C, 50% humidity with 12 h/12-h light/dark cycles, and low noise. The rats were randomly divided into four groups of healthy control (HC), diabetic control (DC), Diabetic Training (DT), and Healthy Training (HT), and were observed for 6 weeks. The weight and blood glucose levels of all animals were measured at the beginning and the end of the experiment.

Diabetes induction procedure

In the present study, 40 rats were allocated into two dietary regimens and fed on either normal pellet diet (NPD; 4.1% fat, 22.2% protein, and 12.1% carbohydrates, as a percentage of total kcal) or high-fat diet (HFD) ($n = 20$; 58% fat, 17% carbohydrate and 27%protein, as a percentage of total kcal) ad libitum, for an initial period of 2 weeks. The composition (Table 1) and preparation of HFD have been described earlier [22]. After 2 weeks, a single intraperitoneal injection of 35 mg/kg streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) was utilized [23]. In order to confirm type 2 diabetes, a blood sample was collected from the eyes of the rats and the glucose concentration was measured by enzymatic glucose oxidase assay kit (Pars Tests Company, Iran).

Table 1 Composition of HFD

Ingredients	Diet (g/100 g)
Powdered NPD[a]	36.5
Lard	31.0
Casein	25.0
Cholesterol	1.0
Sodium cholate	0.5
Vitamin and mineral mix	6.0
DL-methionine	0.3
Yeast powder	0.1
Sodium chloride	0.1

The composition of normal pellet diet (NPD): 4.1% of fat, 22.2% protein, and 12.1% carbohydrates, as a percentage of total kcal

Forty-eight hours after STZ injection, animals with the blood glucose level higher than 300 mg/dl were considered as type 2 diabetic and were included in the study [23].

Exercise training protocol

Exercise training familiarizing with running on a treadmill consists of 5 days, 10 min daily at a speed of 10 m / min, and with a zero gradient [24]. HIIT was utilized for 6 weeks, 6 days a week (95–100% VO_2max), and implemented with the principle of overload [25]. The summary of the HIIT protocol is described in Table 2.

Sampling

After 48 h of the last exercise session and fasting at night, the rats were anesthetized by intraperitoneal injection of a combination of xylazine (100 mg/kg) and ketamine (70 mg/kg) (Sigma Chemical Co., St. Louis, MO, USA); the heart tissue samples were immediately frozen in liquid nitrogen and kept in a freezer at −70 °C for gene expression studies.

Real-time PCR

The blood was removed from the cavities prior to extractions; in other words, we flushed the hearts before freezing. MiR-126 accession number was rno-miR-126a-5p (MIMAT0000831). To analyze the expression of *miR-126* gene, Real Time PCR method was employed. To this end, 50–100 mg heart tissue was homogenized and 1 ml of the Invitrogen Trizol buffer (invitrogen, USA) was added to the tissues. Then, 200 μl of chloroform was added and incubated at room temperature for 15 min, after centrifuges (at 4000 g. RPM for 15 min), a liquid containing mRNA was extracted and 600 μl of absolute ethanol was added and stored overnight at −20 °C. Following centrifugation, 600 μl of 70% ethanol was added and centrifuged (at 12000 rpm for 20 min). In the end, 20 μl of distilled water was added to the precipitate, and the RNA was kept at −70 °C.

For the synthesis of cDNA, we used the cDNA transgene kit)China) (accession number E-MEXP-3118). One μg of each RNA sample was added to a reaction buffer containing: affinity script RT buffer, 2 μl, polyadenylated RNA, 4 μl, dNTP mix, 0.8 μl, RT adapter primer, 1 μl, affinity script RT/RNase block enzyme mixture, 1 μl, and water, until 20 μl. The final volume of the product reached 28.8 μL. The

Table 2 High-intensity interval training protocol

Weeks	Days	Warm-up	Intense interval	Rest interval	Cool down
1	Odd	3 min (16 m/min)	2*3 min (40 m/min)	2*60 s (16 m/min)	3 min (16 m/min)
	Even		3–7*30 s (54 m/min)	3–7*60 s (16 m/min)	
2	Odd		4*3 min (40 m/min)	4*60 s (16 m/min)	
	Even		7–11*30 s (54 m/min)	7–11*60 s (16 m/min)	
3	Odd		5*3 min (40 m/min)	5*60 s (16 m/min)	
	Even		11–15*30 s (54 m/min)	11–15*60 s (16 m/min)	
4	Odd		6*3 min (40 m/min)	6*60 s (16 m/min)	
	Even		15–20*30 s (54 m/min)	15–20*60 s (16 m/min)	
5	Odd		6*3 min (40 m/min)	6*60 s (16 m/min)	
	Even		15–20*30 s (54 m/min)	15–20*60 s (16 m/min)	
6	Odd		6*3 min (40 m/min)	6*60 s (16 m/min)	
	Even		15–20*30 s (54 m/min)	15–20*60 s (16 m/min)	

thermal program in thermocycler machine was 5 min at 55 °C, 15 min at 25 °C, 30 min at 42 °C (production of cDNA by RT enzyme), and 5 min at 95 °C (to deactivate the RT enzyme). Then, the sequence of PCR was 10 min at 95 °C, 10 s at 95 °C, 15 s at 60 °C, 20 s at 72 °C. We repeated this reaction for 40 cycles.

Reactions CTs were also extracted and recorded utilizing Real-Time PCR software, and the $2-\Delta\Delta CT$ formula was used to quantify the expression of the desired gene (reference gene: β-actin). A negative control sample was also provided as NTC (NO template control). A positive control sample with the UR6 snRNA primer was utilized as internal control. Primers' sequences for *miR-126* gene include:

F; 5-TATGGTTGTTCTCGACTCCTTCAC-3, R; 5-TCGTCTGTCGTACCGTGAGTAAT-3. Forward sequences were derived from MiRBase (http://www.mirbase.org).

Measurement of serum concentrations of VEGF and Spred-1, Raf1 proteins via ELISA test

ELISA test was employed to measure the serum concentration of VEGF, Spred-1 and Raf1 proteins (spred-1 ELISA kit, catalog no: ABIN1232768, online antibodies, Raf1 ELISA Kit, catalog no: ABIN1125759, online antibodies and Vascular Endothelial Growth Factor (VEGF) ELISA Kit, Catalog no: ABIN772617, online antibodies). In summary, 100 μl of serum samples, standards with concentrations of 1000, 400, 160, 64, 25.6, 10.2, and 4.1 pg/ml and PBS-BSA 1% as blank was added to separate wells, and the plates were gently shaken for 2 h at room temperature. Next, 100 μl Streptoavidin-HRP was added to each well and the plates were incubated at room temperature for 1 h under dark conditions. The washing steps were performed according to the kit protocol. Then, 100 μl substrate solutions were added to each well and the plates were incubated in the dark for about 30 min. Finally, 100 μl stopping solution was added to each well, and the optical density of the wells was immediately recorded at 450 nm using (Bio-Tech Instruments, USA). According to the standard curve obtained from the OD standard wells, the amount of protein was calculated.

Statistics

In this study, we used a statistic package for social science (SPSS) software (18.0, SPSS Inc., Chicago, IL). The normality of the data was confirmed with Kolmogorov-Smirnov test. Comparisons between groups were analyzed using one-way ANOVA and Tukey's post hoc test ($p < 0.05$).

Results

Body weight

According to Table 3, in the second week and after the induction of diabetes, a slight decrease in body weight was evident in diabetic groups ($P < 0.05$). Afterward, the weight gain in all groups continued naturally. After 4 weeks from the onset of HFD in diabetic groups, there was a significant discrepancy between the weight of the diabetic training group with that of diabetic control ($P < 0.05$) and this difference was still observed until the end of the study for the control group and 2 weeks before the end of the training for the diabetic training group continued.

Serum glucose

In Fig. 1, serum glucose levels of rats after diabetes induction and after 6 weeks of HIIT training are depicted. The induction of diabetes resulted in a significant increase in plasma glucose both in diabetic control and diabetic training groups ($P < 0.001$). Six weeks of HIIT training also led to a significant enhancement in plasma glucose in the diabetic training group compared to DC ($P < 0.001$).

MiR-126

Figure 2 shows the relative mRNA expression of *miR-126* among different groups. The expression of *miR-126* heart in the healthy exercise group was significantly higher (2.5-fold) than the control group ($P < 0.01$) and the control group of diabetes ($P < 0.01$) (5-fold). Meanwhile, in the healthy control group, it was significantly higher than the control group of diabetes ($P < 0.03$) (2-fold) and in diabetic training group it was higher than the control group of diabetes ($P < 0.01$) (2-fold).

Serum VEGF

Figure 3 shows changes in serum VEGF concentration among different groups. The results of one-way ANOVA for serum VEGF levels showed a significant difference between the study groups ($P < 0.01$, $F = 135.985$). The results of Tukey's post hoc test showed that VEGF serum concentrations were significantly higher in the healthy exercise group than the control group ($P < 0.01$) (147.9 pg/ml) and diabetes control group ($P < 0.01$) (306.9 pg/ml). Meanwhile, in the healthy control group they were significantly higher than the control group of diabetes,) $P < 0.01$) (159 pg/ml), and in diabetic training group were higher than the diabetic control group ($P < 0.01$) (159 pg/ml).

Table 3 Changes in rat's body weight in health control (HC), health training (HT), diabetic control (DC), and diabetic training (DT) groups. The results are expressed as mean ± SEM

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
HC	192.5 ± 11.9	208 ± 4.52	215 ± 9.55	221 ± 3.55	229 ± 7.53	238 ± 6.4
HT	193 ± 10.85	207 ± 3.44	213 ± 11.55	220 ± 8.29	224 ± 6.24	231 ± 6.43
DC	194 ± 7.65	201 ± 6.34	224 ± 9.32	252 ± 5.78[a]	259 ± 11.29 [a]	271 ± 7.11 [a]
DT	192 ± 6.55	201 ± 6.77	225 ± 7.73	238 ± 8.44 [ab]	248 ± 7.39 [ab]	262 ± 11.54 [ab]

[a] $P < 0.01$ significant sign is compared to healthy groups

[b] $P < 0.01$ significant sign compared to DC group

Serum Raf-1

Figure 4 shows the changes in serum concentration of Raf-1 in four groups: control, exercise control, diabetes, and diabetic training. The results of one-way ANOVA for serum Raf-1 showed a significant difference between the study groups) $P < 0.01$, F = 103.617). The results of Tukey's post hoc test showed that changes in serum concentration of Raf-1 in the healthy exercise group were significantly higher than the control group ($P < 0.01$) (199.3 pg/ml) and diabetes control group ($P < 0.01$) (256.8 pg/ml). Also, in the healthy control group, Raf-1 concentration was significantly higher than the control group of diabetes ($P < 0.05$) (57.5 pg/ml) and in the diabetic training group was higher than the control group of diabetes ($P < 0.32$) (45.7 pg/ml).

Serum spred-1

Figure 5 displays the changes in serum Spred-1 concentration in health control (HC), health training (HT), diabetic control (DC), and diabetic training (DT) groups. Results from one-way ANOVA for Spred-1 serum values showed a significant difference between study groups $P < 0.01$, F = 212.86). The results of Tukey's post hoc test showed that the changes in serum Spred-1 concentration in the healthy control group were significantly higher than the healthy exercise group ($P < 0.01$) (81.5 pg/ml) but less than the control group for diabetes ($P < 0.01$) (203.3 pg/ml). Meanwhile, in the control group diabetes was significantly higher than both the healthy exercise group ($P < 0.01$) (203.3 pg/ml) and diabetic training group ($P < 0.01$) (138.1 pg/ml).

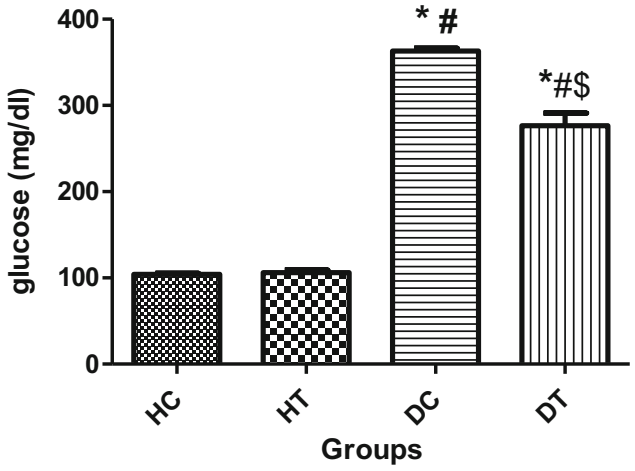

Fig. 1 Change of serum glucose after diabetes and exercise training in different groups of study. Diabetes significantly increases serum glucose compared to healthy groups. However, DT group significantly decreases blood glucose compared to DC group. The results are expressed as mean ± SEM. * significant sign compared to HC group, # significant sign compared to HT group, $ significant sign compared to DC group ($P < 0.05$). HC; health control, HT; health training, DC; diabetic control, DT; diabetic training

Discussion

Despite extensive research on diabetes and angiogenesis, the molecular mechanisms involved in this phenomenon are not well known [10]. The results of this study showed that diabetes significantly reduced *miR-126* expression, decreased expression of VEGF and Raf-1 proteins, and increased Spred-1 expression. Six weeks of HIIT also led to a significant increase

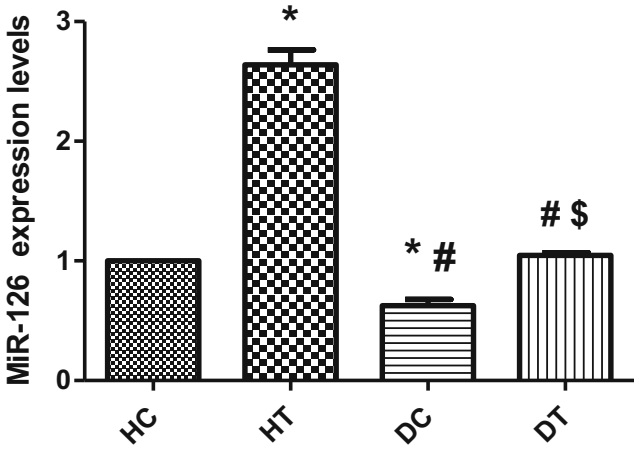

Fig. 2 Expression of cardiac *miR-126* in different groups of study. HIIT significantly increases *miR-126* in cardiac muscle; however, diabetes decreases this gene. The results are expressed as mean ± SEM. * significant sign compared to HC group, # significant sign compared to HT group, $ significant sign compared to DC group ($P < 0.05$). HC; health control, HT; health training, DC; diabetic control, DT; diabetic training

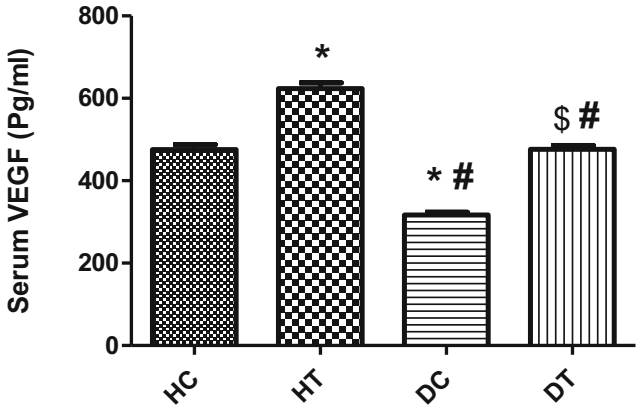

Fig. 3 Serum levels of VEGF in different groups of study. Although diabetes decreases VEGF, diabetes with HIIT significantly increases serum VEGF. The results are expressed as mean ± SEM. * significant sign compared to HC group, # significant sign compared to HT group, $ significant sign compared to DC group ($P < 0.05$). HC; health control, HT; health training, DC; diabetic control, DT; diabetic training

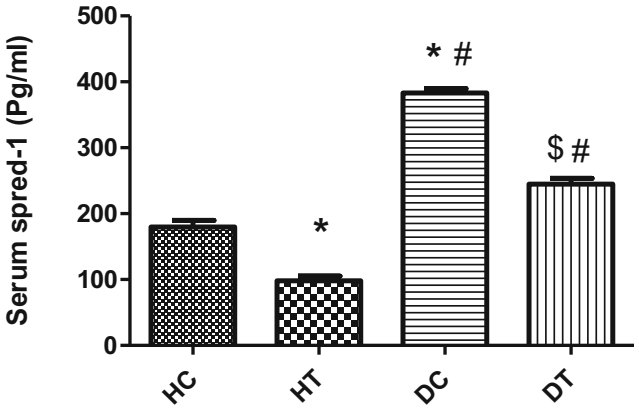

Fig. 5 Serum levels of Spred-1 in different groups of study. Serum levels of Spred-1 decrease with HT; however, diabetes significantly increases this protein and HIIT (DT) decreases this protein compared to DC group. The results are expressed as mean ± SEM. * significant sign compared to HC group, # significant sign compared to HT group, $ significant sign compared to DC group ($P < 0.05$). HC; health control, HT; health training, DC; diabetic control, DT; diabetic training

in the expression of *miR-126*, VEGF and Raf-1 proteins, and the reduction of Spred-1. Consistent with the results of our study, it has been shown that diabetes significantly declined the angiogenesis and lateral arteries in the heart and skeletal muscle of human and animal models [26, 27]. In this study, serum glucose was increased. It has been shown that hyperglycemia leads to a defect in the signaling of the downstream VEGFR2 pathway in endothelial cells. Therefore, an increase in glucose due to diabetes can reduce the angiogenic marker in the heart tissue. Also, exercise training by improving glucose homeostasis regulates hyperglycemia that affects VEGF and heart angiogenesis. In line with the results of this study, Iemitsu et al. showed that swimming exercise for 8 weeks, increased VEGF in the heart of the animal model [28]. However, in this study, they showed VEGF is improved by

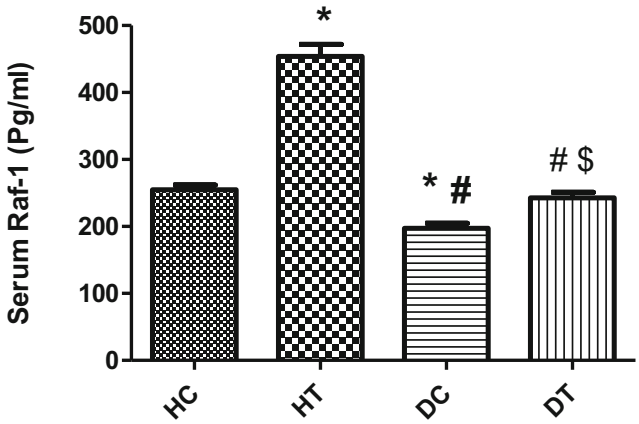

Fig. 4 Serum levels of Raf-1 in different groups of study. HT significantly increases serum Raf-1 compared to the control group. However, diabetes significantly increases serum Raf-1. The results are expressed as mean ± SEM. * significant sign compared to HC group, # significant sign compared to HT group, $ significant sign compared to DC group ($P < 0.05$). HC; health control, HT; health training, DC; diabetic control, DT; diabetic training

exercise training from AKT and eNOS signaling pathway. In our study, we just considered VEGF/Spred-1/Raf-1 Pathway. An increase in AKT along with exercise training can affect VEGF signaling. Increasing the expression of angiogenic factors with exercise intervention leads to an increase in capillary density of the heart tissue, whereby oxygen and nutrients are readily available to the heart and ultimately the phenomenon of apoptosis and fibrosis of the heart tissue is delayed [28].

Studies combined different exercise programs, and considered their effects on cardiovascular system [29, 30]. It has been shown that combination of endurance exercise training increases the expression of VEGF and its receptors, giving rise to the development of capillary density [31]. In the present study, there was a significant increase in the level of VEGF protein due to HIIT. Other mechanisms and pathways such as PGC-1a and HIF-1a may play a role in this process [32]. Quantitative studies have reported the simultaneous effects of diabetes and exercise on *miR-126*, VEGF, Raf-1 and Spred-1 expressions. Mohammad Yari et al. (2018) showed that an eight-week aerobic training led to a significant increase in the expression of *miR-126*, increased the expression of VEGF, Raf-1, and decreased in Spred-1 expression [33]. This result is in agreement with that of this study and it seems that aerobic and interval exercise pieces of training have similar effects on VEGF, Raf-1 and, Spred-1 expression in the heart tissue. Also, Matin Homaee et al. showed that eight weeks of aerobic training led to an increase in the expression of the *miR-126* gene and the capillary density of the heart tissue in diabetic male rats [34]. Our findings suggest that the HIIT also results in a significant increase in the expression of *miR-126*, VEGF, and Raf-1 expression and the reduction of Spred-1 expression, just like aerobic exercise. Contrary to the findings of this study, Karolina et al. (2011) reported an increase in *MiR144*, 192,29a in the blood of diabetic patients

while the amount of *miR-126* did not change. The reason for these inconsistencies may be due to their blood-type sampling compared to the heart tissue as well as the type of induced diabetic rats (STZ injection alone against STZ injection plus high fat intake in our study) [35]. Liu et al. (2014) reported that serum *miR-126* levels were significantly lower in subjects with IGT/IFG and T2DM compared to healthy subjects [36]. The important point of this study was that after 6 months of treatment, including exercise intervention, diet therapy in IGT / IFG subjects as well as exercise intervention, insulin control and regimens in patients with type 2 diabetes mellitus (T2DM), there was a significant increase in serum *miR-126* and the use of The *miR-126* serum was identified as a golden indicator for early diagnosis of diabetic patients and diagnosis of treatment responses [36]. Uhlemann et al. (2014) showed that a maximum exercise test resulted in an increase in *miR-126* at peak power (2.1-fold increase) while the concentration of *miR-133* remained unchanged. Also, four cycles of cycling increased the *miR-126* plasma concentration by 4.6 times, while this type of exercise did not affect the *MiR-133* concentration. Eventually, a marathon race also led to an increase in *MiR-133* and *miR-126*. Conversely, these eccentric resistance exercises increased *MiR-123* (2.1 fold) resulting in a non-change in *miR-126*. Finally, the researchers concluded that different protocols of endurance exercises lead to damage to the endothelial cell layers (which was determined by the enhancement of *miR-126*). On the other hand, resistance exercise activity does not affect endothelial cells but the destruction of muscle cells leads to hypertrophy [37].

Although it has been shown that exercise improves angiogenesis through various mechanisms, these studies have reported limited effects of aerobic training on MiRNA involved in angiogenesis (*miR-126*). In agreement with the results of this study, Silva et al. (2005) studied the role of swimming aerobic exercises on *miR-126* expression, which is relevant to angiogenesis [17]. This study showed that aerobic exercise increases the expression of *miR-126*, and this can be due to cardiac angiogenesis owing to exercise activity by indirectly regulating the VEGF pathway and directly regulating its goals by increasing angiogenesis pathways such as MAPK, PI3K/ Akt/eNOS. Yoo et al. (2016) [38] reported that HIIT has beneficial effects on the improvement of serum levels of VEGF and NO and blood pressure control in postmenopausal women [38]. In agreement with the results of this study, Little et al. (2011) have proven that the implementation of HIIT reduces hyperglycemia in diabetic patients [39]. In another study, Gillen et al. (2012) found that HIIT performed glucose response after meals and also reduced hyperglycemia in diabetic patients [20].

Conclusion

The results of this study showed that 6 weeks of HIIT resulted in a significant increase of expression of *miR-126*, increased serum concentration of VEGF and Raf-1 proteins, and reduction of Spred-1 serum concentration in cardiac tissue in diabetic rats. The findings of this study give us a real insight into angiogenesis mechanisms created by HIIT in diabetes and show that VEGF and its associated pathway (*miR-126*/ Spred-1 / Raf-1) are a potential therapeutic target for pathological conditions involved in the angiogenesis process.

Limitations

Some limitations should be considered when interpreting the data presented in this study. The present study did not assess the morphology and histology of heart tissues. It is suggested that future studies consider fixed tissues by IHC method to study angiogenesis in heart tissue.

Availability of data and material Data is available by contacting the corresponding author.

Authors' contributions All authors equally contributed to the preparation of this manuscript.

Compliance with ethical standards

Ethics approval and consent to participate All animal experiments were conducted according to the guidelines of the National Institute of Health Guide for laboratory animal care and use (NIH Publications No. 8023, revised 1978) and prepared by Baqiyatallah University of Medical Sciences (ethical code: IR.BMSU.1398.077).

Consent for publication Not applicable.

Conflict of interests None of the authors had any conflicts of interest to declare.

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