The Effect of Interval and Continuous Training on Angiogenesis Factor in Type2 Diabetic Wistar Rats

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Abstract

Objective: The equilibrium of angiogenesis stimulus agents and angiogenesis inhibitory agents is an important factor in the increase of diabetic cardiomyopathy. This research aimed to survey the result of eight weeks of high-intensity interval training (HIIT) and moderate continuous (MICT) on the myocardium angiogenesis agent and histological alterations within male diabetic rats.

Materials and Methods: 32 male Wistar rats did casually selected within 4 groups: health without exercise control, diabetic without exercise (D), D + HIIT, and D+ MICT groups. Diabetes type 2 produced with high-fat food for two weeks and an only dose of STZ. After approval of type 2 diabetes, subjects did direct to HIIT (90 -95 percent of VO2max), and MICT (50 to 65 percent of VO2max) exercise program five times per week during eight weeks. Western blotting methods were utilized for the exposure of protein synthesis of Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor-beta (TGF-β1), and Matrix Metalloproteinase-9 (MMP9) in the left ventricle. Besides, the base and ultimate blood glucose were estimated. Histological alterations assessed utilizing H&E and Masson's trichrome staining.

Results: The installation of diabetes develops TGF-β1 (*P*-value= 0.001) and reduces MMP9 (*P*-value= 0.002) and VEGF (*P*-value= 0.002). But, eight weeks of MICT enhanced MMP9 (*P*-value= 0.002) and VEGF (*P*-value= 0.002), but the volume of TGF-β1 (*P*-value= 0.001) diminished significantly. Furthermore, the MICT did improve better than of HIIT in improving angiogenesis agents and adjust body mass, plasma glucose in the rats with diabetes.

Conclusion: Not only eight weeks of interval and continuous exercise enhanced levels of MMP9 and VEGF, but also revealed a notable reduction in TGF-β1. Additionally, both training diminishes body weight and blood sugar.

Keywords: Diabetes type 2, Diabetic cardiomyopathy, Fibrosis, MICT training

Introduction

Diabetes correlated by some difficulties, like retinopathy, nephropathy, neuropathy, and cardiovascular dysfunction. Cardiovascular diseases are a vital death problem correlated with diabetes (1). Diabetes is a universal metabolic

dysfunction realized by a high content of glucose blood due to a resistance to insulin, rarity in the secretion of insulin, or both. Type2 diabetes mellitus (T2DM) is a very common epidemic comprehensive, and considered reports exhibit that 642 million people in the world will have of T2DM by 2040 (2). Diabetic cardiomyopathy (DCM) is specified by the metabolically, functional, and morphological variations in the heart represented as a complication of T2DM. This cardiac dysfunction is defined by constant high levels of blood glucose and also lipid content that finally produces poor calcium handling, oxidative stress, infection, changed mitochondrial function, and fibrosis (3).

Furthermore, biological mechanisms of diabetes play a fully confident role in developing the risk of myocardial infarction and DCM by the production of critical metabolic dysfunction (4). DCM is related to microvascular shortage and also damage in angiogenic response to chronic ischemia, which reduces the myocardium perfusion and finally leads to interstitial fibrosis, tissue hypoxia, and heart failure (5,6). Myocardial angiogenesis is generally defined as a level of the extension of new blood vessels from pre-existing vessels, causing an increase in myocardial perfusion. Angiogenesis is the activation process of vascular endothelial cells through angiogenic agents; the endothelial cells movement, the extracellular matrix degradation, and endothelial cells differentiation into micro-vessels (7,8).

Vascular Endothelial Growth Factor (VEGF), the main agent of angiogenesis, exists in the center of the regulative network checking angiogenesis in both the pathological and physiological environments (9). Matrix Metalloproteinases (MMPs) are needed for the turnover of the extracellular matrix, leading to degradation of angiogenesis and connective tissue. Tissue Inhibitors of Metalloproteinases (TIMPs) have a proteolytic action hindering protease action of MMPs via binding to the catalytic site of activated MMPs (10). In addition, transforming growth factor-beta

(TGF-β) is a powerful inhibitor with a pro-fibrotic characteristic that inhibits the proliferation of endothelial cells (11). Exercise training is defined as a tool for increasing metabolism (12). Regular exercise training increases the level of glucose metabolism throughout an insulin-independent pathway, causing the improvement of oxidative capacity in muscle and the establishment of the most sufficient inducement for cardiovascular metabolism improvement (13). Moreover, regular exercise training in diabetic patients could restrict hypoglycemia, ketosis, hyperglycemia, and diabetes-associated problems (14,15). Besides, tissue hypoxia caused by exercise elevates the vascular sprouts structure composed of and proliferating stalk cells and migratory tip cells thereby angiogenesis (16). The aim of this research is to study the effects of two exercise protocols, i.e. high-intensity interval training (HIIT) and moderate-intensity contentious training (MICT), on the creation of pro-angiogenic and anti-angiogenic agents, also histological remodelings in the heart tissue of T2DM rat model.

Materials and Methods

32 pieces of male Wistar rats, scaling 181 (±22) grams, thereby 3-months age, bought of Pasteur Institute (Tehran, Iran). The animal held 4 by a cage into polyethylene cage (30*30*25 cm) by metal entries into the constant heat of 25(±5) °C and moisture 55-65 percent on a 24 day/night circle, by a simple way to rodent feed and sanitary water. Every method of this research does according to the order described within the "Guide for the Care and Use of Laboratory Animals" made with the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985).

Following one week of accommodation on the lab status, creatures randomly divided within 4 groups, consist of healthy out exercise (n=8), diabetic nonexercise (n=8) (D), diabetic and interval exercise (n=8) (D + HIIT), and diabetic and continuous exercise (n=8) (D+

MICT) groups. To produce diabetes, rats served with one high-fat feed (HDF) (58 percent lipid, 25 percent protein, 17 percent carbohydrate as a percentage of the whole kcal), equipped by commercially accessible mouse feed at two weeks and later taken an intraperitoneal infusion of 35 mg/kg streptozotocin (STZ, Sigma-Aldrich, American) soluble in citrate buffer (0.1 M) by pH=4.5 (17). Nevertheless, the healthy without exercise group served with an ordinary diet (they had not felt the situation of becoming diabetic). Forty-eight hours following the STZ dose, the samples unit was obtained of a vein to measure blood sugar. Though, the Units have taken later 10 to 12 hours of fasting. Then, the hyperglycemic state evaluated apply a glucometer (Roche, Germany), and rats by blood glucose rates higher than 250 mg/dl selected as type 2 diabetic animals (18). Subsequent evidence of diabetes, animals in the diabetic group divided into 3 subgroups (n=8/group) as regards: diabetic (D), D + HIIT, and D +MICT groups. Diabetic pets did not get any training plan while HIIT and MICT groups controlled to their separate exercise program for eight continuous weeks. Animals were accustomed to treadmill training for one week, slowly rising from ten to thirty min/day. An eight-week exercise program started, after this adjustment phase. The rats in the exercise clubs were subjected to either MICT or HIIT five days per week. The HIIT protocol comprised ten rounds of two minutes very sever running on a treadmill by 90 percent of VO2max with a 60-second stay on a velocity of twenty m/min during the commence, and the speed slowly rose to thirty m/min while the 8th week (no incline). Warm-up and cool downtime was 5 minutes. MICT group performs on a usual exercise speed to 50 ‑ 60 percent of maximal oxygen uptake (VO2max) during the exercise session (19). Animals in the control and Diabetes groups without exercise did not do any training. Training severity during any program was based on a previous statement describing the relationship between training speed and VO2max. Training capacity did estimate before and after the exercise. The process was like that -previ-ously described by Moreira and colleagues (19). Summarily, the rats ran on a graded treadmill at 15˚ inclination at the first speed of 6 m/min. Treadmill speed was finally increased near three m/min each three min till the rats cannot run. All of the time of running with any rat regarded exercise capacity. Forty-eight hours following final exercise and twelve hours of fasting, the rats anesthetized by intraperitoneal infusion of xylazine (10 mg/kg) and ketamine (100 mg/kg). Rats sacrificed, and left ventricle tissues obtained. Tissue units were flooded with normal saline and immediately frozen in liquid nitrogen and stored at -80 ° C for further analysis (20). The frosted left ventricle membranes lysed in 1 ml of RIPA lysis buffer containing 1% Protease inhibitor (Sigma) and centrifuged at 13000 rpm for 15 min, and then the supernatant was obtained. The mass of proteins in the received supernatant fixed using the Bradford assay kit. Finally, an equivalent quantity of protein (60 μg) was separated on 10% polyacrylamide gel under the controlled situation and carried on the PVDF membrane (Roche, United Kingdom). Besides, the tissue incubated late by primary antibodies including at 4° C. Measurement of biomarkers of angiogenesis consists of VEGF, TGF-β, MMP9, and β-Actinin the heart homog-enate discovered by western blotting method (21). The membrane washed 3 rates with PBS then incubated by horseradish peroxidase-conjugated (HRP) goat anti-rabbit IgG secondary antibody for 1.5 h with shaking at place temperature. Magnified chemiluminescence (ECL) discovery kit utilized for the exposure of antigen-antibody networks. Images of the protein collections received and measured using Image J 1.62 software (22).

The units of the left ventricle of heart tissues involved in 10% buffered formalin in 0.1 M PBS for a week at 4°C then washed in PBS, dehydrated in a series of sorted ethanol, profited in xylene, and fixed in paraffin wax.

Paraffin-embedded tissues were cut at 5 µm thick transverse parts utilizing a microtome and then attached on slides. Beside, slides stained by Hematoxylin-Eosin (H&E) for the discovery of capillary density or Masson's trichrome for detecting collagen deposits based on standard protocols. Three random parts from each animal photographed through a 20x objective lens, applying a light microscope (Leica, Germany) and histological alterations discovered using Image J software (23).

Ethical considerations

The study was approved by the ethics committee of Tabriz University, Tabriz, Iran (IR.TBZMED.VCR.REC.1398.426).

Results

The results of ANOVA test revealed a significantly enhancement in the protein synthesis of TGF-β1 (*P*-value< 0.001, Figure 1B) and a significantly reduction in protein synthesis of VEGF (*P*-value< 0.001, Figure. 1C) in the left cardiac ventricle of diabetic rats associated to the control group. But, HIIT and MICT protocols could especially down-regulate TGF-β protein synthesis during up-regulating VEGF protein rats.

The analysis of Western blotting showed that diabetic animals had higher volumes of TGF-β1 (*P*-value< 0.01) protein and lower volumes of MMP9 (*P*-value< 0.01 to both) proteins than the control group (Figure 2). Nonetheless,

MICT significantly reduced TGF-β (*P*-value< 0.05) volumes while risen MMP9 (*P*-value< 0.001) in the diabetic animals. But, HIIT only significantly improved MMP9 (*P*-value< 0.01) in the D+HIIT group related to the D group.

We additionally examined the bodyweight differences at the base, the fourth, and the eighth week in the trial groups. The data displayed no notable variation in the first bodyweight between different groups. The control animals significantly raised body weight from the origin to the end of the test, while D+HIIT and D+MICT groups experienced a slight weight increase through the fourth week and then revealed weight loss during the eighth week. Nonetheless, diabetic animals presented lower weight increase than the control group, and end the eighth weight demonstrated weight loss (Table 1). The body weight (gr) alteration of four groups through the study (M±SD).

Furthermore, the biochemical investigation revealed no significantly variance in base FBS between groups. But, after diabetes induction, diabetic animals demonstrated higher blood glucose than the control group. But HIIT slightly reduced blood glucose, and there were no significant differences between D and D+HIIT groups. Nevertheless, MICT could decrease FBS in diabetic rats significantly (Table 2).
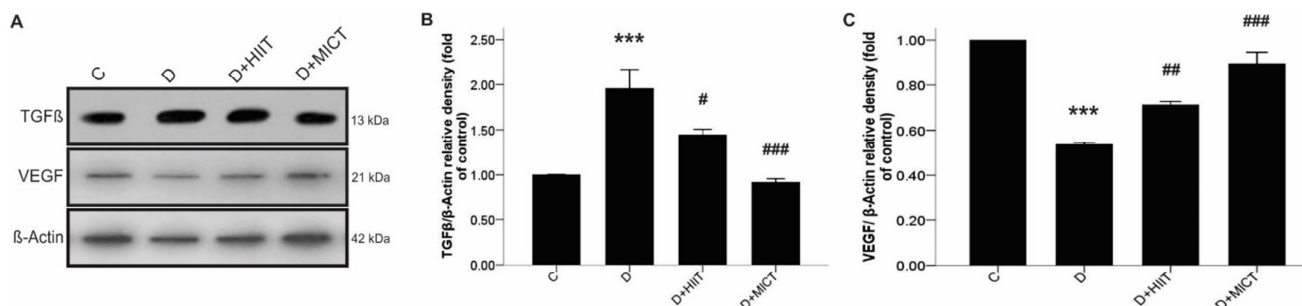
Discussion

Figure 1. Results of MICT and HIIT program on the protein synthesis of TGF-β1, an inhibitor angiogenesis factor, and VEGF, as a stimulator angiogenesis factor, in the trial groups. (A) Protein synthesis of TGF-β1, VEGF, and β-Actin. Densitometric report of B) TGF-β1 and C) VEGF proteins in various groups. The result did normalize on the control group and showed as the M± SD (n=3/group). *** *P*-value<0.001 vs. control (C) group. # *P*-value<0.05, ## *P*-value<0.01, ### *P*-value<0.001 vs. diabetic (D) animals. HIIT: high-intensity interval training MICT: continuous intensity moderate training.

This work approved that diabetes increases the level of TGF-β 1 but, interval and continuous exercise reduces the level of TGF-β 1 in groups. In addition, VEGF and MMP9 levels were decreased in diabetic condition but increased significantly after eight weeks of tow type exercise (interval& continuous). Besides, consistent exercise training such as

Table 1. The body weight (gr) alteration of 4 groups through the research (M±SD)

Group	Baseline	4th week	8th week	P-value
Healthy control no exercise	185 (±12)	194 (±15)	213 (±11)	0.001
Diabetic control no exercise	188 (±13)	227 (±16)	278 (±20)	0.000
Diabetic continuous exercise	189 (±14)	207 (±14)	218 (±18)	0.002
Diabetic interval exercise	188 (±12)	209 (±15)	228 (±18)	0.001
P-value	0.211	0.317	0.345	--

The results displayed as mean±SEM (n=10/group). HIIT: high-intensity interval training MICT: continuous intensity moderate training.

Table 2. The FBS (mg/dl) in the trial groups (Mean±SD)

Group	Baseline	4th week	8th week	P-value
Healthy control no exercise	121 (±7.54)	123 (±6.9)	126 (±9.5)	0.000
Diabetic control no exercise	116 (±3.63)	251 (±8.88)	273 (±6.8)	0.001
Diabetic continuous exercise	117 (±3.88)	253 (±7.3)	258 (±5.9)	0.001
Diabetic interval exercise	118 (±2.91)	255 (±7.1)	258 (±6.5)	0.002
P-value	0.313	0.129	0.212	--

Data are represented as mean ± SEM (n=10/group). HIIT: high-intensity interval training MICT: continuous intensity moderate training.
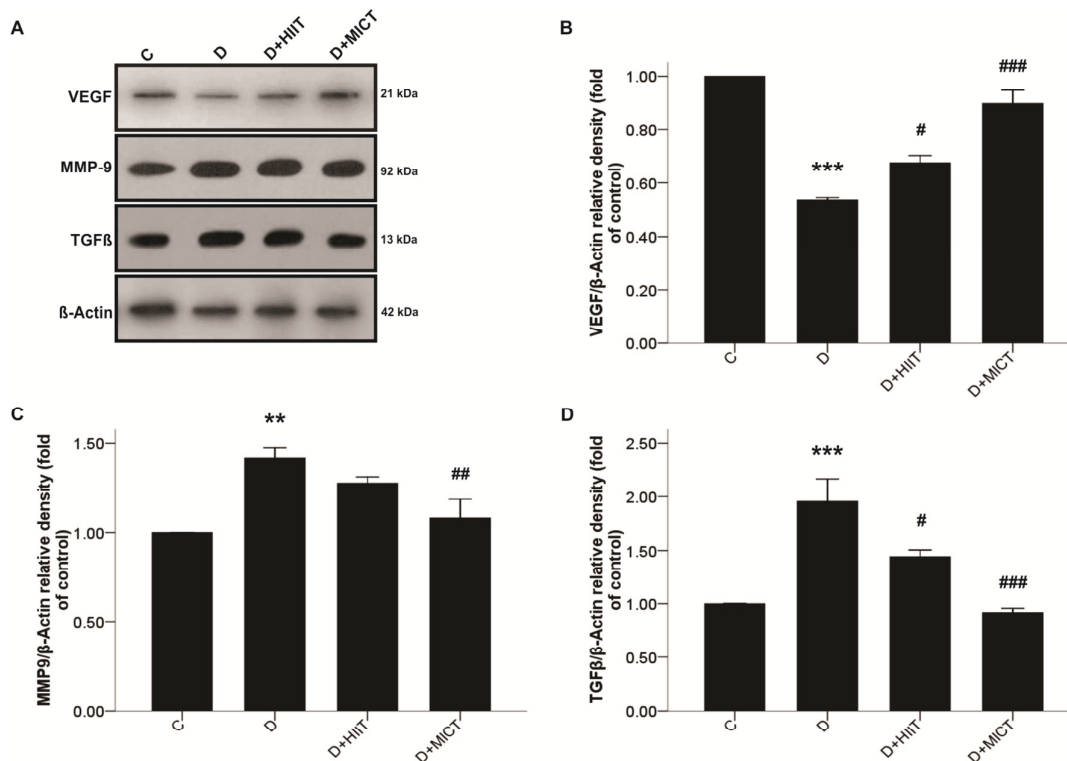

Figure 2. Consequences of MICT and HIIT program on the protein synthesis of VEGF, MMP9, and TGF-β1 in the trial groups. (A) Protein synthesis of VEGF, MMP9, and β-Actin. Densitometric review of B) VEGF, C) MMP9, and D) TGF-β protein amounts in diverse groups. The results did normalize to the control group and showed as the M± SD (n=3/group). **P-value< 0.01 vs. control (C) group. # P-value<0.05, ## P-value< 0.01, ### P-value< 0.001 vs. diabetic (D) animals. HIIT: high-intensity interval training MICT: continuous intensity moderate training.

MICT protocol adjusted all these alterations. Diabetes also extended the bodyweight of diabetic rats and the level of the blood sugar of Wistar rats after eight weeks had noticeable incremental variations that were lower in the case of continuous and intermittent exercise groups as compared to the non-exercise diabetic group. The current study has concentrated on corrective angiogenic agents such as a remedial approach for the administration of diabetes-associated cardiovascular diseases (24).

A developing collection of data has described which exercise is a non-pharmacological method in improving attenuates myocardial fibrosis and cardiovascular health in a diabetic model (13). VEGF protein causes the formation of blood vessels and angiogenesis. The decrease in VEGF isoforms is correlated with damage to myocardial angiogenesis and critical left ventricular complication (25). Many pre-clinical and clinical research have observed diminished myocardial VEGF levels in diabetic animal models or diabetic patients, exhibiting microvascular homeostasis in the myocardium and attenuation of capillary density (26-29).

Accordingly, the restoration of VEGF expression may cause the development of cardiac function and microvascular homeostasis. Furthermore, Yoon and colleagues reported that right intra-myocardial injection of the plasmid DNA encoding VEGF leads to improvement in capillary density, limited apoptosis, and fibrosis of cardiomyocytes and, as a result, developed a cardiac role in studied diabetic rats through VEGF replenishment (30). The findings in the current research also demonstrated a loss in left cardiac ventricle VEGF protein synthesis in the investigated diabetic animals. However, both the HIIT and MICT exercises can develop the levels of myocardium VEGF protein supported by enhancing attenuation capillary and density of interstitial fibrosis. Erekat and coworkers have shown that treadmill exercise increased the expression of VEGF in the investigated myocardial tissue in rats with type one diabetes (31).

Karbalaeifar and coworkers reported that six weeks of HIIT leads to improvement in cardiac VEGF mRNA expression and development of myocardial function in rats after infarction in myocardial (32). An outstanding feature of DCM is interstitial perivascular fibrosis, worsening myocardial ischemia through the creation of a barrier to myocardial perfusion via an enhancement in the distance of oxygen diffusion (33). Diabetes correlated with cardiac fibrosis is mainly correlated with high blood glucose. Hyperglycemia by the variety of extracellular matrix causes fibrosis and inflammation (34,35). Furthermore, our data approved that TGF-β has an important function in triggering cardiac fibrosis and thereby DCM (36).

In our work, the diabetic group exhibited raised expression of TGF-β, higher collagen deposition, and cardiac fibrosis. Recently, Li and coworkers also exhibited that tetrahydro curcumin relieved cardiac fibrosis and hyperglycemia-induced oxidative stress in studied diabetic rats (37). It appears that MICT by developing blood glucose levels or attenuation of TGF-β expression could attenuate cardiac fibrosis. It should be noted that MICT has a superior effect as compared to HIIT in the improvement of angiogenesis as well as in modulate of blood glucose level and fibrosis in the studied diabetic rats. Moreover, we revealed that diabetic animals have a higher TGF-β 1 protein synthesis level and lower activated MMP9 level in the myocardium as compared to control animals. Li and coworkers have also exhibited diminished myocardial MMP-9 function and synthesis and also increased TGF-β expression levels in STZ-induced diabetes in studied rats (38). Similarly, Lu and coworkers observed an increase in the rates of TIMPs in aortic intima and left ventricle myocardium and also reduced serum levels of MMP9 in STZ-decreased diabetes in investigated mini pigs (39). Evidence reveals that MMP-TIMP dysregulation in diabetes leads to left ventricle

hypertrophy and cardiac dysfunction. Besides that, it improves the collagen amount and cardiovascular fibrosis (39-41). In addition, a synergy can exist between VEGF-MMP-TIMP2 pathways. Evidence conclude that VEGF can up-regulate MMPs (42), while over the synthesis of TIMP2 can down-regulate VEGF expression (43).

However, the protocol of MICT exercise can decrease TIMP2 expression, while it can raise the cleaved MMP2 levels in studied diabetic rats. The mentioned conclusions highlight the significance of VEGF-MMPs-TIMPs pathways in exercise-induced myocardial angiogenesis. In addition, our results confirmed that MICT has a dominant effect on modifying blood glucose levels. As the intense exercise (>85% of VO2max) appears in post- exercise hyperglycemia because of the augmentation of the hepatic glucose output by epinephrine. Moreover, prolonged exercise at moderate intensity (40-60 percent of VO2max) can exhibit the patient to the risk of post-exercise hypoglycemia (44). It is a conclusive note that the current research exhibited that exercise developed fibrosis and cardiac angiogenesis via improvement in the expression of pro-angiogenic agents as well as attenuation of anti-angiogenic agents, along with improvement in glucose levels control in the investigated diabetic rat model.

Conclusions

Briefly, the data of the current study demonstrated that 8 weeks of continuous and interval exercise training can improve the sugar profile in the studied Wistar rats. Additionally, 8 weeks of continuous and interval exercise training may appear to cause improved through modulating angiogenic protein synthesis, and it should be noted that further research is needed in the future.

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Conflict of Interest

Authors declare that they have no competing interests.

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