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Effects of Lipoic Acid and High-Intensity Interval Training (HIIT) on Pancreatic VEGFR-3 Levels in Diabetic Rat Model

Seyed Ramin Hashemian Esfahani[1], Minoo Dadban Shahamat[1*], Asra Askari[2] , Abbas Nezhadebrahimi[3]

[1]Department of Physical Education, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran.
[2]Department of Physical Education, Gorgan Branch, Islamic Azad University, Gorgan, Iran.
[3]Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

Abstract

Objective: The purpose of this experimental research is to investigate the effects of High-Intensity Interval Training (HIIT) and Lipoic Acid (ALA) supplementation on VEGFR-3 of pancreatic in diabetic Wistar rats model.

Materials and Methods: 20 male Wistar rats weighing 159 ± 3 gr and aged3 weeks, were randomly assigned into 4 groups:1) diabetes/sham, 2)diabetes/ ALA, 3)diabetes/exercise/sham, and 4) diabetes /exercise/ ALA. Diabetes was induced with streptozotocin (65 mg/kg dose) and Nicotinamide (120 mg/kg dose). After two weeks of familiarization with interval training, the rats started their main training, included 10 repetitions of 4 minutes of running on the treadmill with an intensity of 85-90% VO2max and 2 minutes of active rest between repetitions (5-10 m/min) for 5 sessions per week for 6 weeks. ALA supplement was taken at a dose of 20 mg/kg/day for 6 weeks. One-way ANOVA test used and Tukey's post hoc test at for analysis ($P \leq 0.05$).

Results: HIIT has a significant effect on blood glucose ($P= 0.004$) and insulin ($P= 0.001$) and VEGFR3 ($P= 0.001$) of pancreatic tissue of diabetic rats.

Conclusion: Lymphatic vessels play an important role in the pancreas and treat diabetes.The results of this research showed that HIIT and ALA increased lymphangiogenesis in the diabetic rat model.

Keywords: ALA, HIIT, Lymph angiogenesis, Diabetic model rats

QR Code:

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Corresponding Author:

Minoo Dadban Shahamat, Department of Physical Education, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran.

Tel: (98) 173 217 3741
Email: m_dadban@yahoo.com
Orcid ID: 0000-0003-2811-9874

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Introduction

Vascular damage is one of the most important diabetes complications. It is the first cause of non-traumatic amputation of the lower limb (1). In diabetes, the endothelial cells of the lymphatic vessels are defective and become increasingly permeable, and with the severity of this condition, lymph and antigens do not reach the lymph nodes (2). Lymphatic vessels are one-way conduits parallel to blood vessels that return arterial interstitial fluid (3). The molecular characteristics of the development of lymphatic vessels and the role of these vessels in pathophysiological conditions have been greatly improved in recent years (4,5). The endocrine and exocrine functions of the human pancreas depend on the efficient transport of fluids through the blood and lymphatic vascular systems (6). The formation of new lymphatic vessels from existing lymphatic vessels is called lymphangitis; Studies show that abnormalities in the islet angiogenesis lead to decreased insulin levels in the vasculature, even though β -cells have normal secretory capacity, and therefore, vascular changes in the islets may underlie a novel mechanism for the islet dysfunction(7). VEGF family proteins play an essential role in lymphangiogenesis (8). VEGFR-3 receptor is specific to lymphatic vessel endothelium and binds to VEGF-C and VEGF-D ligands (9).

Regular exercise and nutrition are considered as the strategies to improve diabetes; The effect of interval exercise on the control of patients with type 2 diabetes has been confirmed in previous researches (10,11). Exercise can be considered a non-pharmacological treatment strategy for patients with diabetes mellitus and reduce fat mass and increase insulin sensitivity and reduce triglycerides and plasma cholesterol significantly (12,13).

ALA is a short-chain fatty acid with high antioxidant effect (14). Reviews of studies have provided evidences of the beneficial role of ALA in the treatment of polyneuropathy. (10) This antioxidant in the control of carbohydrate and lipid metabolism is still unclear in the observation of experiments that some studies have shown that ALA is anti-apoptotic. (13) ALA increases the expression of insulin receptor substrate 1 (IRS1) in the muscle of male obese rats. The protective or harmful effect of ALA on pancreatic cells is related to ALA's underlying pathophysiological condition (4). As a result, ALA shows protection for pancreatic islet cells and thus improves blood glucose levels (15). The studies conducted on the useful poly ALA in the treatment of neuropathy (6). This antioxidant in the control of carbohydrate and lipid metabolism in the experiment is still unclear. Exercise can significantly increase the metabolism of lipids and blood glucose. Clinical observations show that aerobic exercise improves blood fat levels in patients with hyperlipidemia (16) and enhance myokine and VEGF expression (17).

According to the results of various studies, it seems that the lymphatic system can play a significant role in improving type 2 and since exercise and ALA supplementation can effectively regulate lymphatic function. In this research, we seek to find the question of whether HIIT and ALA supplements have an effect on VEGFR-3 of pancreatic tissue in Wistar rats or not?

Material and methods

The current research is an experimental type and has been carried out by the post-test method with multiple control groups

Animals

20 male Wistar rats with initial weight159 (±3) gr and aged 3 weeks were kept in a cage under controlled humidity (60%) and temperature (25± 2°C) with a light-dark cycle of 12 hours. The rats had access to pellets 10 gr/100 gr BW/day (Behparvar Company, Iran)

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and tap water ad libitum throughout the whole study.

Induction of diabetes

After a week of acclimatization to the laboratory conditions, the rats were given an intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg (Sigma Aldrich, USA), dissolved in 0.1 M citrate buffer (Ph=4.5) and 15 minutes after fasting. followed by Nicotinamide (NA) with a dose of 120 mg/kg (Sigma Aldrich, USA) dissolved in normal saline (18).

Animal study design

20 male Wistar rats with an average age of 3 weeks and means (159 ±3 g) were divided into four groups, 5 rats in each group, including; 1) diabetes/sham, 2) diabetes/ ALA, 3) diabetes/ exercise/ sham, 4) diabetes/ ALA/ exercise. In this study, streptozotocin was used in a single dose (50 ml/kg) to make rats diabetic. And sugar above 250 mg/dl in 48 hours after injection was considered as induced diabetes (19). The alpha-lipoic acid supplement was administered orally at 20 mg/kg once a day for 6 consecutive weeks (20).

HIIT training protocol

Before implementing the training protocol, the animals of the training and training and supplementary groups were familiarized with how to perform the activity on a special treadmill for a week. The exercise program (Table 1**)** was based on the study of Costigan et al (2015), which included; 6 weeks of interval running on a treadmill with zero inclines, 5 sessions a week, each session, 10 intervals of 4 minutes with VO2max intensity of 90-85%; Active rest between intervals was 2-minute runs with an intensity of 5-10 m/min (21).

Warming and cooling of the rats were done with an intensity of 10-5 m/min for 5-10 minutes before and after the exercises. The principle of overload by measuring VO2max with the increasing Bedford test standardized by Landrow et al. (2007) was performed every two weeks. The test consists of 10 steps of 3 minutes. The speed in the first stage is 5 m/min, and in the next stages, 5 m/min was added to the speed of the turntable. VO2max is the speed at which VO2 reaches a plateau (22).

Tissue sampling and protein measurement

The rats were anesthetized and sacrificed by ketamine (50 mg/kg) along with xylazine (5 mg/kg) in a fasting state and after 72 hours from the last training session, and pancreatic tissue was removed. Examining the expression of VEGFR-3 protein by the Fluorescent immune-histochemical technique using the kit of Invitrogen company, made in America (code number PA5-16871).

In the present study, descriptive statistics were used to describe the variables, and inferential statistics were used to analyze the findings, one-way analysis of variance, and Tukey's post hoc test was used to compare the groups two by two. SPSS version 19 software was used for data analysis and at a significance level of $P\leq 0.05$. Also, ImageJ software was used to quantify the qualitative data obtained from immunohistochemistry.

Ethical considerations

All animal interventions were carried out according to the ethical guidelines of the

Table 1. Details of interval training protocol

Practice sections	Variables	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Warm up	Speed (m/min)	5-10	5-10	5-10	5-10	5-10	5-10
	duration (min)	5-10	5-10	5-10	5-10	5-10	5-10
High intensity interval	duration (min)	4	4	4	4	4	4
	intensity (vo2max)	85%	85%	85%	90%	90%	90%
Low-intensity Interval	Speed (m/min)	5-10	5-10	5-10	5-10	5-10	5-10
	duration (min)	2	2	2	2	2	2
Cool down	Speed (m/min)	5-10	5-10	5-10	5-10	5-10	5-10
	duration (min)	5-10	5-10	5-10	5-10	5-10	5-10

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National Institutions for the Care and Use of Laboratory Animals approved by the Ethics Committee of the Islamic Azad University Aliabad Katul (Ethical code: IR.IAU.AK. REC.1399.019).

This article is taken from a master's thesis without financial support. All ethical principles have been observed in this study and have been approved by the Ethics Committee of Aliabad Katul University (IR.IAU.AK.REC.1399.019).

Results

In this study, a total of 20 male Wistar rats participated in 4 groups, and the descriptive information of the indicators studied in the research, including the mean and standard deviation, is presented in Table 2.

The VEGFR-3 of pancreatic tissue was significantly increased in all three groups of diabetes/ ALA, diabetes/ HIIT/ sham, and diabetes/ HIIT/ ALA compared to the diabetic control group (*P*= 0.001). Histological images of VEGFR-3 protein expression are shown in

Table 2. Descriptive information of research indicators

Groups	Blood glucose (mg/dl) Mean±SD	Serum insulin (ng/ml) Mean±SD	VEGFR-3 (Pixle/um^2) Mean±SD
Diabetes/Sham	266 (±19.97)	4.84 (±0.65)	6.95 (±1.7)
Diabetes/LAL	269.7 (±30.66)	4.57 (±0.32)	15.51 (±0.58)
Diabetes/HIIT/Sham	200.3 (±10.6)	6.86 (±0.28)	24.33 (±1.08)
Diabetes/HIIT/LAL	146.7 (±6.8)	8.16 (±0.39)	34.16 (±2.43)
***P*-value**	0.004	0.01	0.001

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Figure 1. Comparison of VEGFR-3 in different research groups. Immunohistochemical images show the amount of angiogenesis with VEGF-r3 marker in pancreatic tissue with brown color; which is due to the reaction of the antigen with the corresponding antibody. The presence of this protein is evident in the walls of blood vessels and in the tissue layers of the pancreas. As can be seen, the level of protein expression has increased in the tissue of the groups that had sports activity (D/T) or supplement use (D/S). The images clearly show that the highest expression of protein was in the exercise foliar group with supplements (D/S/T). Histological images were evaluated by Image J software and presented in the form of graphs, which are presented in quantitative results.

Figure 1.

Discussion

Angiogenesis is the process by which the new blood vessel is formed from a pre-existing blood vessel (23). However, angiogenesis is only one of the mechanisms responsible for vessel formation. Since blood vessels feed almost every cell in the body, a decrease or increase of angiogenesis can affect the body's function. Then, angiogenesis is useful for tissue growth and regeneration, but it can also trigger an inflammatory or malignant response. Also it can contribute to cancer metastasis leading to mortality (24). Stimulation of insulin receptor(s) has pleiotropic effects on endothelial cells (25). These actions, together with the capacity of insulin to increase the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) as well as increase the survival of pericytes and decrease the expression of anti-angiogenic proteins, suggest the role of insulin in physiological and pathological angiogenesis (26).

Several environmental factors play a role in the prevention and treatment of diabetes, the most important of which is exercise and controlling the way patients eat. The results of this study showed that HIIT decreased serum glucose and increased pancreatic tissue VEGFR-3 and serum insulin in diabetic rats. Madsen SM, et al. (2015) research results show that HIIT improves overall blood sugar control and pancreatic β -cell function in T2D patients (10). Studies show that impaired lymphatic angiogenesis of pancreatic islets causes a decrease in blood insulin, even if beta cells have normal secretory capacity (7). Lymphatic vessels are the main route of transport of high-density lipoprotein (HDL) particles into the bloodstream (reverse cholesterol transport), and defective lymphatic function severely affects reverse cholesterol transport (27); These results indicate that lymphatic vessels play an effective role in reducing cholesterol and arteriosclerosis, which are major complications of diabetes (28). In another study, the effect of 10 weeks of endurance training with 75% VO2max intensity on the expression of VEGF and VEGFR-2 genes in the heart tissue of diabetic Wistar rats was investigated, and the results showed that endurance training leads to an increase in VEGF protein expression, but does not have a change in VEGFR-2 protein expression (13). Studies have shown that the angiogenic response in diabetic rats is lower than in healthy rats; Diabetes increases the expression of angiogenesis inhibitor TSP-1. After STZ induction and increased blood glucose, FTY720 which is known as VEGFR-3 inhibitor is increased to prevent blood glucose increase. ALK1 is a member of the growth factor family receptors that is expressed in lymphangiogenesis; Blockade of ALK1 signaling leads to defects in lymphatic vessel development (29) On the other hand, ALK1-Fc prevents diabetes in mice by inhibiting lymphangiogenesis caused by type 2 diabetes (30). The research results also showed that ALA supplementation had an increasing effect on VEGFR-3 in pancreatic tissue of diabetic rats.

ALA is a fatty acid with a strong antioxidant capacity that improves mitochondrial function; the results of research in diabetic samples have shown that the decrease in lipoic acid levels in these people is compensated by supplementation and causes a decrease in glucose levels and glycosylation. protein and HbA1c (14). Chronic ALA treatment significantly reduces body weight gain by reducing food intake, and this effect is mediated by the effect of ALA to reduce AMP-activated protein kinase (AMPK) activity in the hypothalamus. AMPK is the major regulator of cellular energy metabolism (31,32), and when it is activated, it increases the absorption of glucose and the oxidation of fatty acids and prevents the accumulation of fat in the tissue (33). The results of this research showed that the interactive effect of HIIT and ALA supplementation caused a significant increase in VEGFR-3 in the pancreatic tissue of diabetic rats. In fact, when

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exercise and supplement consumption was done together, it had very favorable effects on the indicator. In diabetic people, hyperglycemia inhibits reNOS and increases oxidative stress, causing disruption in the production of NO in endothelial cells and vascular smooth muscle, which causes the production of ROS, which leads to the inhibition of angiogenesis (34) And probably, by reducing insulin and glucose resistance, increasing AKT phosphorylation and eNOS protein expression, which are important signaling pathways for endothelial cell migration and proliferation, exercise increases gene expression of VEGF receptors and angiogenesis (13,35). In previous studies, ALA was shown to normalize body weight in mice; In addition, all metabolic changes in Free Fatty Acid, triglycerides, and insulin were observed in them and improved vascular function (36). AMPK activity in endothelial cells is an important regulator for endothelial function; Studies have shown that ALA modulated AMPK activities in endothelial cells and improved vascular disorders in mice (34). The defect of endothelium-dependent vasodilation is an early event in the development of atherosclerosis. One of the key features of endothelium-dependent vasodilation is the bioavailability of NO (37). Endothelium-dependent vasodilation is impaired in OLETF obese mice, which is associated with increased fat accumulation and apoptosis, decreased NO synthesis, and decreased AMPK activities in endothelial cells; All these changes in endothelial cells and vascular dysfunction were significantly improved by ALA treatment (36). Physical activity in itself improves glucose homeostasis, the cornerstone of regulating overall blood sugar control in patients (38). Since 2013, it is recommended that T2DM patients should do at least 150 minutes of moderate aerobic exercise per week. Recently, there has been more focus on the beneficial health effects of high-intensity interval training, which has beneficial and effective effects on blood sugar reduction and pancreatic function. Studies have shown that 8 weeks of low-volume HIIT on an Ergometer bike improves pancreatic β-cell function and insulin sensitivity in T2D patients. Karstoft et al. (2014) reported that interval walking (5 sessions of 60 minutes per week, over 4 months with 3 minutes of intense intervals greater than 70% of VO2max compared to continuous slow walking of 40% of VO2max) significantly improved pancreatic β-cell function in adults with T2D (39).

Studies have shown that 8 weeks of low-volume HIIT training on a bicycle ergometer improves pancreatic β-cell function and insulin sensitivity in T2D patients. Carstaff et al. (2014) reported that interval walking (5 sessions of 60 minutes per week, over 4 months with 3 minutes of intense intervals greater than 70% VO2max compared to continuous slow walking at 40% VO2max) significantly improved performance. Improve pancreatic β-cells Adults with T2D (32).

The most important limitation of this study is that the researcher could not measure inflammatory factors and other effective growth factors due to the high cost of research. Few studies have been conducted on the effect of interval training on vascular angiogenesis, and this study was one of the first studies that investigated the mutual effects of HIIT and ALA supplementation on pancreatic tissue lymphangiogenesis. With the results obtained from this study, high-intensity intermittent exercise with ALA supplementation increased lymphatic angiogenesis, which is an important component in insulin secretion. However, more studies are needed in this area.

Conclusion

Angiogenesis through intense aerobic exercise and taking lipoic acid supplement is a method to improve blood supply in tissues including pancreas of diabetic people. It plays an important role in blood glucose control and metabolic homeostasis. Therefore, exercise can improve insulin resistance in diabetic patients.

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Conflict of Interest

Research participants do not have any conflicts or interests.

Authors' contributions

SR. HE: Statistical analysis and interpretation of data.

M. DSh: Study concept and design, Analysis and interpretation of data and writing initial draft of the manuscript.

A. A and A. N: Study concept and design and critical revision of the manuscript for important intellectual content.

All authors have accepted responsibility for the entire content of this manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and approved the version to be published.

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