Effect of Black Grape Seed(Vitis Vinifera) Extract Consumption with Moderate-Intensity Aerobic Training on the Expression of MicroRNAs in Type 1 Diabetic Cardiac Tissue Rats

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Abstract

Objective: This study aimed to evaluate the effect of consuming grape seed extract with moderate-intensity aerobic training on the expression of miR-126 and miR-29 in the cardiac tissue in type 1 diabetic male rats.

Materials and Methods: 40 rats with an initial weight range of 160-220 g were divided into five groups: Training + Extract, Training, Extract, Diabetic / Control, and Healthy / Control. Aerobic training program was moderate intensity and rats performed aerobic training for 60 minutes a day with the intensity 70 to 75% of maximum oxygen consumption (28 meters per minute). Grape seed extract was also administered by gavage at a dose of 40 mg/kg per day.

Results: Expression of both miRNAs in the three groups of training + extract, healthy training and control was significantly higher than the two groups of extract and diabetic control (*P*-value= 0.001). The difference between the three groups of training + extract, healthy training and control and also the difference between the two groups of extract and diabetic control were not significant (*P*-value> 0.05).

Conclusion: Aerobic training may be able to prevent cardiac disease caused by type 1 diabetes.

Keywords: Diabetes, Aerobic training, Grape Seed, miR-126, miR-29a

Introduction

Diabetes, as a common disease (1), has chronic complications in the function of blood vessels and structure in various tissues of the body, which can be divided into two categories: microvascular (including retinopathy, neuropathy and nephropathy) and macrovascular (including peripheral vascular disease and cardiovascular disease) (2).

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To date, more than 1000 microRNAs have been identified in the human genome that play a key role in skeletal muscle function and regulating cardiovascular (3). miR-126 is a special microRNA known as AngiomiR and is expressed only in endothelial cells. miR-126 plays the most important and best role in vascular integrity and controlling angiogenesis (4). It directly suppresses the two negative regulators of the VEGF pathway (5). On the other hand, recent studies have focused on the miR-29 family and its associated cardiovascular and renal impairment (6). The miR-29 family in humans includes miR-29b, miR-29c, and miR-29a. The miR-29 family targets at least 16 genes involved in the extracellular matrix of the heart and is the best regulator of extracellular matrix synthesis (7). Given the positive effect of physical activity, it seems that research on cellular and molecular functions resulting from training can lead to the use of physical activity as a targeted treatment without complications in the future. If training is combined with nutritional interventions, it will undoubtedly have more effects, and in this regard, in recent years, much attention has been paid to herbs. Among these, black grape seed extract is one of the supplements that has flavonoid compounds, has very high antioxidant effects (8). The biological properties of polyphenols include antioxidant, anti-cancer and anti-inflammatory effects (9). Most studies have shown strong biological effects of this supplement in reducing the amount of cardiac risk factors caused by some diseases such as diabetes (10). However, it is better to focus studies on the underlying mechanisms of cardiac function in diabetic patients through various interventions, including training and nutrition, in which miR-126 plays an important role in angiogenesis and vascular integration and decreases in diabetic patients. On the other hand, miR-29 plays an important role in the response of cardiac hypertrophy to training. But, the effect of grape seed extract on genes related to angiogenesis and cardiac hypertrophy in diabetic patients such as miR-126 and miR-29 has not been studied.

The aim of this study was to evaluate the effect of consuming grape seed extract with moderate-intensity aerobic training on the expression of miR-126 and miR-29 genes in the cardiac tissue in type 1 diabetic male rats.

Materials and Methods

This project is an experimental study that was performed with a post-test design with a control group. The statistical population of this study was adult male Wistar rats from the laboratory animal center of Shiraz University of Medical Sciences with an initial weight range of 160-220 g. The sample size was 40 rats, which was selected based on related articles. After selection, 1- Diabetic / training + Extract, 2- Diabetic / training, 3- Diabetic / Extract, 4- Diabetic / Control and 5- Healthy / Control (8 heads per group). Induction of type 1 diabetes with a single intraperitoneal injection of streptozotocin (STZ) solution prepared from Sigma-Aldrich, Germany, dissolved in citrate buffer (pH= 4.5 and concentration of 0.1 M) at a rate of 55 mg performed per kilogram of animal body weight. 14 days after STZ injection, blood glucose concentrations were measured in blood samples collected from animal tails using a glucometer (manufactured by Medisign, South Korea). The criterion for being diabetic was fasting blood glucose concentration higher than 250 mg/dl (11). Glucose oxidase method was used to measure glucose. For the control group, in order to equalize the effect of injection, 0.1 M citrate buffer with the same volume was injected intraperitoneally (11). All mice were kept in controlled environmental conditions with an average temperature of 22±3 ℃, light cycle to dark 12:12 hours and with free access to water and food for rats (prepared in the form of pellets from Behparvar Iran Company).

The stage of familiarity and aerobic training was such that in the stage of familiarity with the treadmill (the first week) the rats walked for 15 minutes on a 10-channel treadmill for

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rodents for 15 consecutive days at a speed of 10 meters per minute with a zero degree slope. Training started between 7 and 11 in the morning every day. During the second and third weeks, the treadmill speed and duration of training gradually increased. The rats performed aerobic training for 60 minutes a day at an average speed of 28 meters per minute (intensity equivalent to 70 to 75% of maximum oxygen consumption and training volume of 8.4 km per week). At the end of the training program, in order to perform cooling, the speed of the device was reduced inversely until the speed of the device reached zero. This procedure continued until the end of the eighth week of training (12).

Grapes used by the family of Vitis vinifera, genus amplidase, Genus Vitis, subgenus uveitis, and Iranian grape species were selected and approved by the cultivation and development group of the Tehran Institute of medicinal Plants. After purchasing red grapes, the seeds were separated from red grape pulp manually. The seeds were then washed in the open air and dried in direct sunlight at 50 ° C for 30 minutes. The dried grains were crushed and ground to the stage of powder formation. Separation of grain fat was done by soxhlet method using hexane solvent. In this method, first 500 mg of grape seed powder was poured into filter paper and placed in a Soxhlet apparatus and the suction operation was performed on 500 ml of hexane solvent for one hour. The hexane extract prepared with a rotary apparatus was concentrated at 50 °C and then dried in an arc at the same temperature. The resulting powder was processed by Soxhlet method using methanol solvent twice and filtered through filter paper. Finally, the methanol solution containing the extract was dried in a vacuum at 40 °C by an evaporator to separate the extract from the methanol solvent. After evaporation of methanol, the pure extract remained in a container which, after collection, was kept away from light and moisture (13). Grape seed extract was consumed by gavage at a dose of 40 mg/kg per day.

All groups 48 hours after the last training session in the same condition and the fasting state of animals by intraperitoneal injection of ketamine (30-50 mg/kg body weight) and xylazine (3-5 mg/kg body weight) fainted and the chest was dissected and heart tissue was collected, gene analysis was performed, and body weight, heart, and left ventricle were measured.

Expression of miR-126 was assessed by qRT–PCR. Triplicate assays were performed for each RNA sample. MicroRNA was extracted from the heart tissue using the miRCURYTM RNA Isolation Kit (Exiqon, Denmark) according to the manufacturer's protocol. The procedure was performed based on spin column using a proprietary resin as a separation matrix for RNA from other cell components. RNA content and purity were measured at a wavelength of 260–280 nm using Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington DE 19810 USA). cDNA synthesis was done according to LNA universal RT miRNA PCR kit (Exiqon, Denmark). Briefly, total RNA containing microRNA was polyadenylated and cDNA was synthesized using a poly (T) primer with a 3' degenerate anchor and a 5' universal tag. Syber Green qPCR Mix purchased from Exiqon (denmark) and used for real time PCR. Real time PCR was done using Rotor-Gene 6000 Corbett. The 2-(ΔΔCt) method was used to determine relative quantitative levels of miR-126. The results were expressed as the fold-difference to the control group. Mir-1 was used as the endogenous control miRNA.

Gen all kit with Cat # 3505151 was used to extract mRNA and Exiqon kit with Cat # 253351 was used to extract micro RNA. Mir-29 and U6 as internal control gene expression levels were determined using a device (Light Cycler 96) and determined with Cyber Green I color. A standard curve gradient was used to evaluate the efficiency of each gene. In the next step, to analyze the data, first the ct delta gene in each sample was calculated by differentiating the corresponding ct gene and ct U6 gene as the reference gene. Gene

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expression at baseline relative to reference genes was calculated by ferrule 2- Δ ΔCT. Then the final results of expression of each gene (expression rtio) were analyzed by software.

$Ct_{Target\ Gene(Tumor)} - Ct_{Housekeeping\ Gene(Tumor)} = \Delta CT$

$\Delta CT_{(Margin)} = Ct_{Target\ Gene\ (Margun)} - Ct_{Housekeeping\ Gene\ (margin)}$ $2^{-\Delta\ \Delta CT}$ = expression ratio

To measure the desired parameters, first the cardiac tissue was pulverized using nitrogen liquid and then 0.1 g (100 mg) of the powder made was homogenized with 1 ml buffer (PBS). The extracted solution was then centrifuged for 15 minutes at 5000 rpm and its supernatant was used to measure the desired parameters. The expression of miRNAs was assessed by real-time PCR.

One-way analysis of variance was used to compare the groups in the studied variables and Bonferroni post hoc test was used to perform additional tests. Significance level was considered *P*-value< 0.05 and SPSS software version 19 was used for statistical analysis.

Ethical considerations

This study with the identification code 15021423971006 was reviewed and approved by the Ethics Committee in Biomedical Research of Islamic Azad University, Najafabad Branch.

Results

There was a significant difference between miR-126 expression in the five groups (*P*-value= 0.001). This was the difference between the groups of training + extract, training and healthy control with the two groups of extract and diabetic control (*P*-value< 0.05). There was no significant difference between miR-126 expression in the three groups of training + extract, training and healthy control (*P*-value> 0.05). Also, there was no significant difference in miR-126 expression between the two groups of extract and diabetic control (*P*-value> 0.05). There was a significant difference between miR-29a expression in the five groups (*P*-value= 0.001). This was the difference between the groups of training + extract, training and healthy control with the two groups of extract and diabetic control (*P*-value< 0.05). There was no significant difference between of miR-29a expression in the three groups of training + extract, training and healthy control (*P*-value> 0.05). Also, there was no significant difference in miR-29a expression between the two groups of extract and diabetic control (*P*-value> 0.05).

Discussion

Based on the findings of the present study, the expression of miR-126 and miR-29a genes increased significantly in the aerobic training + grape seed extract and aerobic training groups, but no significant change was observed in the grape seed extract group. In fact, eight weeks of moderate-intensity aerobic training significantly increased the expression of miR-126 and miR-29a genes. However, consumption of grape seed extract did not have a significant effect in this regard and adding it to training did not increase the effect of training. Yang liu et al. (2014) in their study concluded that urinary miR-126 was significantly higher in patients with type 2 diabetes mellitus with diabetic nephropathy. The researchers also found that medication and training reduced urinary miR-126 in patients with type 2 diabetes and with diabetic nephropathy. Therefore, these researchers stated that miR-126 is stable in urine and can be used as an indicator of diabetic nephropathy as well as a marker of therapeutic response (14). One possible reason for this discrepancy could be the presence of diabetic nephropathy in addition to type 2 diabetes. The second reason for the discrepancy between the findings of Yang et al and the present study can be related to the type of sampling. Also, in confirmation of the present findings, Nekouei et al. (2016) observed a significant increase in miR-29a expression in the hearts of healthy rats following training (15).

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Table1. Comparison of variables between five groups (ANOVA)

Variables	Group	Mean (±Std. Deviation)	F	*P*-value
miR-126 (fold-change)	Diabetic / training + Extract	1.53 (±0.64)	7.47	0.001 *
	Diabetic / training	1.39 (±0.55)		
	Diabetic / Extract	0.57 (±0.32)		
	Diabetic / Control	0.46 (±0.40)		
	Healthy / Control	1.43 (±0.65)		
miR-29a (fold-change)	Diabetic / training + Extract	1.62 (±0.50)	10.71	0.001 *
	Diabetic / training	1.42 (±0.45)		
	Diabetic / Extract	0.68 (±0.47)		
	Diabetic / Control	0.51 (±0.55)		
	Healthy / Control	1.70 (±0.38)		

* Significantly at the level of *P*-value ≤0.05

Table 2. Pair comparison between groups (Bonferroni post hoc test)

Variables	Paired comparison	*P*-value
miR-126	Diabetic / training + Extract - Diabetic / training	1
	Diabetic / training + Extract - Diabetic / Extract	0.010 *
	Diabetic / training + Extract - Diabetic / Control	0.003 *
	Diabetic / training + Extract - Healthy / Control	1
	Diabetic / training - Diabetic / Extract	0.038 *
	Diabetic / training - Diabetic / Control	0.013 *
	Diabetic / training - Healthy / Control	1
	Diabetic / Extract - Diabetic / Control	1
	Diabetic / Extract - Healthy / Control	0.027 *
	Diabetic / Control - Healthy / Control	0.003 *
miR-29a	Diabetic / training + Extract - Diabetic / training	1
	Diabetic / training + Extract - Diabetic / Extract	0.001 *
	Diabetic / training + Extract - Diabetic / Control	0.004 *
	Diabetic / training + Extract - Healthy / Control	1
	Diabetic / training - Diabetic / Extract	0.038 *
	Diabetic / training - Diabetic / Control	0.006 *
	Diabetic / training - Healthy / Control	1
	Diabetic / Extract - Diabetic / Control	1
	Diabetic / Extract - Healthy / Control	0.001 *
	Diabetic / Control - Healthy / Control	0.001 *

* Significantly at the level of *P*-value ≤0.05

However, we could not find a study that examined the effect of training on miR-29 in diabetic rats. The exact mechanism for regulating miR-29 in response to exercise stress is not yet fully understood; however, the results of various studies have shown that transforming growth factor-beta (TGF-β), which is the main regulator of fibrosis and a stimulator of many extracellular matrix genes, can suppress miR-29. On the other hand, exercise stress stimulates the secretion of brain natriuretic peptide (BNP) from cardiac myocytes, which counteracts the effect of TGF-β. BNP signaling from cardiac myocytes to fibroblasts appears to modulate miR-29 expression. Although miR-29 plays a key role in the control of cardiac fibrosis, it should be noted that there is no direct one-to-one relationship between miR-29 and collagen levels. For example, a slight decrease in miR-29 expression after myocardial infarction leads to a 20-fold increase in collagen expression (16). Regarding grape seed extract, no research has been conducted to investigate its effect on the expression of miR-126 and miR-29a genes in diabetic rats. The results of Sanna et al. (2019) showed that treatment with grape seed proanthocyanidin extract (GSPE) and insulin altered the expression of apoptotic proteins in diabetic rats. Their findings identified GSPE as a type of insulin adjuvant therapy to reduce the complications of diabetes (17). The results of Irak et al. (2018) also indicated that when grape seed extract was given to diabetic rats, beneficial changes in histopathological changes occurred in the pancreas. Their final conclusion was that grape seed extract causes positive changes in the

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function and structure of the pancreas and the structure of the islets of Langerhans (18). However, in the present study, no significant effect was observed for grape seed extract. It seems that in this regard, further research is needed by examining different doses of grape seed extract.

Conclusions

Eight weeks of moderate-intensity aerobic training may increase the expression of miR-126 and miR-29a in type 1 diabetic rats and can lead to improved cardiovascular function through possible improvements in heart structure and angiogenesis. Therefore, moderate-intensity aerobic training may be able to prevent cardiac disease caused by type 1 diabetes. However, the consumption of grape seed extract could not have a significant effect, and adding it to training did not increase the effect of training. Moreover, because this is the first time such a study has been conducted, it is best to do more research before concluding.

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Conflict of Interest

The authors of this article state that there is no conflict of interest.

References

1. Raffort J, Hinault C, Dumortier O, Van Obberghen E. Circulating microRNAs and diabetes: potential applications in medical practice. Diabetologia. 2015;58(9):1978-92.
2. Kantharidis P, Wang B, Carew RM, Lan HY. Diabetes complications: the microRNA perspective. Diabetes. 2011;60(7):1832-7.
3. Winbanks CE, Ooi JY, Nguyen SS, McMullen JR, Bernardo BC. Micro RNA s differentially regulated in cardiac and skeletal muscle in health and disease: Potential drug targets?. Clinical and Experimental Pharmacology and Physiology. 2014;41(9):727-37.
4. Rawal S, Manning P, Katare R. Cardiovascular microRNAs: as modulators and diagnostic biomarkers of diabetic heart disease. Cardiovascular diabetology. 2014;13(1):1-24.
5. nD Jr DS, Fernandes T, Soci UP, Monteiro AW, Phillips MI, EM DO. Swimming training in rats increases cardiac MicroRNA-126 expression and angiogenesis. Medicine and science in sports and exercise. 2012;44(8):1453-62.
6. Gupta S, Das B, Sen S. Cardiac hypertrophy: mechanisms and therapeutic opportunities. Antioxidants & redox signaling. 2007;9(6):623-52.
7. Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. Physiological genomics. 2012;44(4):237-44.
8. Terauchi M, Horiguchi N, Kajiyama A, Akiyoshi M, Owa Y, Kato K, et al. Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular parameters in middle-aged women: a randomized, double-blind, placebo-controlled pilot study. Menopause. 2014;21(9):990-6.
9. Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A. Plant polyphenols and their anti-cariogenic properties: a review. Molecules. 2011;16(2):1486-507.
10. Saada HN, Said UZ, Meky NH, Azime AS. Grape seed extract Vitis vinifera protects against radiation-induced oxidative damage and metabolic disorders in rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2009;23(3):434-8.
11. Bugger H, Abel ED. Rodent models of diabetic cardiomyopathy. Disease models & mechanisms. 2009;2(9-10):454-66.
12. Garekani ET, Mohebbi H, Kraemer RR, Fathi R. Exercise training intensity/volume affects plasma and tissue adiponectin concentrations in the male rat. Peptides. 2011;32(5):1008-12.
13. de Blanco EC. Review of Drugs of Natural Origin. A Treatise of Pharmacognosy. J. Nat. Prod. 2016;79:1707.
14. Liu Y, Gao G, Yang C, Zhou K, Shen B, Liang H, et al. Stability of miR-126 in urine and its potential as a biomarker for renal endothelial injury with diabetic nephropathy. International journal of endocrinology. 2014;2014.
15. Nekouei A, Kordi M, Choobineh S, Soleimani M, Shafiee A, Hadidi V. The Effect Of Eight Week Continuous Training On Expression Of Micrornas29 Mrna, In Healthy Male Rat's Cardiac Muscle. 2016;17(6):113-120.(in Persian)

[DOI: 10.18502/ijdo.v13i4.8003]

16. Fernandes T, Soci UP, Oliveira EM. Eccentric and concentric cardiac hypertrophy induced by exercise training: microRNAs and molecular determinants. Brazilian Journal of Medical and Biological Research. 2011;44:836-47.
17. Sanna RS, Muthangi S, BK CS, Devi SA. Grape seed proanthocyanidin extract and insulin prevents cognitive decline in type 1 diabetic rat by impacting Bcl-2 and Bax in the prefrontal cortex. Metabolic brain disease. 2019;34(1):103-17.
18. Irak K, Yıldırım S, Mert H, Mert N. Grape seed extract effects on serum amylase levels and immunohistochemical alterations in Streptozotocin-induced diabetic rats. Cellular and Molecular Biology. 2018;64(4):92-7.

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