Journal of Sports Physiology and Athletic Conditioning (J.S.P.A.C) 2024; 4 (11): 37-44

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Research Article

The effect of eight weeks of crocetin consumption and aerobic exercise on the expression of Myod in the heart tissue of elderly prediabetic rats

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Received: 25 March 2024
Revised: 20 April 2024
Accepted: 24 April 2024

Abstract

Background: Diabetic cardiomyopathy refers to changes in the heart as a result of impaired glucose homeostasis, leading to ventricular dysfunction and associated with mitochondrial dysfunction. Since sports activity is known to protect the heart, the aim of the present study was to investigate aerobic exercise and the effective substance crocetin on the expression of Myod gene in the heart tissue of an experimental model of elderly prediabetic rats.

Materials and Methods: In this experimental trial, 25 elderly female c57bl6 male mice with prediabetes (20 mg/kg peritoneal injection) aged 14-16 weeks and weighing 30-35 grams were divided into 5 healthy control groups, prediabetic, prediabetic + aerobic exercise, pre-diabetic + crocetin, pre-diabetic + aerobic exercise + crocetin were divided. aerobic training was performed for eight weeks, five sessions per week. Mice received crocetin 30 mg/kg/day by intraperitoneal injection. Myod expression was measured by Real Time PCR method. To analyze the data, one-way analysis of variance and Tukey's post hoc test were used (P≥0.05).

Results: Myod gene expression was significantly decreased in the prediabetic group compared to the healthy group (P=0.01). Myod gene expression was significantly higher in the pre-diabetes group + aerobic exercise + crocetin supplement and diabetes group + aerobic exercise + crocetin supplement than other groups (P=0.01).

Conclusion: It seems that intermittent aerobic exercise and crocetin both alone and simultaneously are effective in improving Myod gene expression in prediabetic heart tissue. Therefore, the use of periodic aerobics and crocetin n is recommended in pre-diabetes conditions of old age.

Keywords:
Aerobic exercise, crocetin, Myod, heart, elderly, pre-diabetes

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1. Introduction

Diabetic cardiomyopathy is a distinct phenomenon related to cardiac dysfunction. This condition leads to the loss of heart cells and diseases such as atherosclerosis and high blood pressure. However, the exact mechanism of cell death in diabetic cardiomyopathy is still unknown (1). In the hyperglycemic state, it disrupts the pro-oxidant/oxidant balance and causes an increase in free radicals and a decrease in the antioxidant level, which in addition to damaging the muscle metabolism, causes a decrease in muscle MRFs. Myod is one of the essential markers in myogenesis and a key protein in the regulation of muscle differentiation, so that it is necessary for the initiation of muscle hypertrophy signaling, the activation and proliferation of satellite cells (2). Research shows that before satellite cells are activated, the level of Myod protein increases. Also, in hyperglycemic conditions, the expression level of this gene decreases (3). A dose of 5 mg of streptozotocin per kilogram of body weight was diagnosed with type 1 diabetes after 21 days, they experienced a 50% decrease in Myod levels (4). Some authors have also shown that myogenin gene and MyoD protein levels were not affected by HF. Despite the conflicting results, the above-cited studies suggest that MRFs, particularly MyoD and myogenin, might be involved in controlling muscle phenotype during HF conditions (5).Physical activity, which is considered a practical solution against cardiovascular diseases, reduces cardiac risk factors, prevents myocardial destruction, and increases heart function. In recent years, exercise training has been recognized as an effective exercise intervention that can bring similar and greater benefits with different intensities, and is also considered as a strong stimulus for cardiovascular adaptations (6).The benefits of exercise on metabolic, cardiovascular, anti-inflammatory factors, etc., have led many researchers to suggest exercise as a very important non-pharmacological tool in the prevention and treatment of cardiovascular diseases, effective as a powerful tool in the treatment of abnormalities(7). Cardiac problems have been reported in clinical and experimental settings. Also, considering the nature of exercise and its challenging effect on the heart, it seems that the use of natural antioxidants along with exercise can have a better effect on the health of these people.(8). Meanwhile, saffron and its compounds have shown significant medical-biological activities. It has been reported that compounds derived from saffron, such as crocetin, have strong anti-inflammatory and antioxidant effects by inhibiting lipoprotein oxidation, significant effects on coronary heart disease, reducing (9). It has high blood pressure, neurodegenerative diseases and cancer. It has been found that crocetin has a protective effect in cardiac hypertrophy induced by norepinephrine by inhibiting lipid peroxidation and improving the activity of antioxidant enzymes catalase, superoxide desmutase and glutathione peroxidase (10). Therefore, considering the increasing prevalence of diabetes and its harmful effects on health and the occurrence of complications arising from it, and the effect of taking antioxidant supplements and a specific type of physical activity on its control, and considering that there are probably enough studies related to the effect of the type of exercise and consumption crocetin supplementation has not been done on Myod gene expression in pre-diabetic rats, therefore, the present study was conducted with the aim of investigating the effect of eight weeks of aerobic training with crocetin extract on Myod gene expression in heart tissue of elderly pre-diabetic rats.

2. Materials and Methods

In the experimental work, 25 c57bl6 male mice with an age range of 16-14 weeks and a weight range of 30-35 grams were obtained from the laboratory animal breeding and propagation center of Isfahan Royan Center and after being transferred to a specialized laboratory for one week to acclimatize in This environment was maintained. It is noteworthy that during the entire period of this research, the animals were kept under standard conditions, including a 12-hour light-dark cycle, ambient temperature of 20-22 degrees Celsius, relative humidity of 55%, and free access to food and water. Also, all the ethical principles of working with animals in this research were done according to the Helsinki Treaty and the code of ethics was obtained from the ethics committee of Islamic Azad University of Isfahan (Khorasgan).

Induction of Prediabetes

Elderly prediabetic c57bl6 mice were subjected to a single peritoneal injection of 20 mg/kg Streptozotocin (STZ) dissolved in citrate buffer after fasting for 12 hours. (15). It is worth mentioning that 3 rats died after the induction of diabetes due to individual reaction to STZ and finally 25 rats entered the research groups (11).

Grouping and research design

25 rats with pre-diabetes were randomly divided into pre-diabetic, pre-diabetic + aerobic exercise, pre-diabetic + crocetin, pre-diabetic + aerobic + crocetin groups.It is worth mentioning that in order to investigate the effect of prediabetes induction, 5 healthy rats were included in the healthy control group.

Aerobic training protocol

Aerobic interval training consisted of running with different speed intervals of 7, 10, 13, 10, 7 inthe first week and 10, 13, 16, 25, 19, 16, 13, 10 speed on the treadmill in the eighth week. The speed during the aerobic interval was gradually increased from 7 to 25 m/min over 8 weeks. Aerobic interval training was done for eight weeks, five sessions a week, (12).

Table 1. Aerobic exercise program

Number of sessions	Time(Min)	speed(M/min)	week
5	10,3,19,3,10	7,10,13,10,7	1
5	5,3,3,3,3,3,3,5	7,10,13,15,12,10,7	2
5	5,3,3,3,3,3,3,5	10,13,16,17,16,13,10	3
5	5,3,3,3,3,3,3,5	10,13,16,18,16,13,10	4
5	5,3,3,3,3,3,3,5	10,13,16,19,16,13,10	5
5	5,3,3,3,3,3,3,5	10,13,16,20,16,13,10	6
5	5,3,3,3,3,3,35	10,13,16,21,18,15,12,10	7
5	5,3,3,3,3,3,35	10,13,16,25,19,16,13,10	8

Preparation of crocetin supplement

Crocetin supplement (product number: 6-881-255) was purchased from Sigma-Aldrich (USA) and 30 mg/kg per day was given by gavage to each mouse(13).

Molecular Analysis of Myocardial Tissue by Real Time PCR

QReal Time PCR method was used to measure Myod gene expression levels. For this purpose, first, 50 mg of tissue was separated from the heart, and RNA extraction was performed from the tissues in all investigated groups, according to the manufacturer's protocol (Qiagen, Germany). To ensure the quality of RNA, it was electrophoresed using agarose gel and using the property of optical absorption at a wavelength of 260 nm with a Sigma PicoDrop device (made in America).

In addition, the formula (C (µg/µl) = A260 × ε × d/1000) was used to check the quality of RNA. Next, after the synthesis of cDNA using the manufacturer's protocol in the fermentase kit (K1621) and using the designed primers based on the Myod gene guide on the PUBMED site, the reverse transcription reaction was performed. To determine the efficiency and specificity of the primers, the pre-primers were evaluated using the software available on the NCBI website, and to measure the gene expression levels of the research variables, the TBP internal control gene was used and after ensuring the completion of the work The qReal Time PCR machine and after the samples reached the expression threshold (Cycle Threshold) was used to quantify the ratio of the target gene to the reference gene using the formula 2-ΔΔCT (Table 2).

Table 2. Primer sequence of research variables

Gene	Primer sequence	Temperature (°C)
B2m	*F: ACAGTTCCACCCGCCTCACATT*	60
	R: TAGAAAGACCAGTCCTTGCTGAAG	
Myod	*F: ATTGAGAAGTTGAAAGGAATCCATG*	53
	R: GAACATGTCTGCGTATCTC	

Statistical analysis

In order to analyze the data in this research, the Shapiro-Wilk test was used to check the normality of the distribution of the findings. Next, due to the normal distribution of the findings, one-way analysis of variance was used to investigate the difference between the groups, and Tukey's post hoc test was used to determine the location of the difference between the groups All statistical operations were performed with SPSS software version 26 (version 26, IBM Corporation, Armonk, NY, US).

3. Results

The results of one-way analysis of variance showed a significant difference in glucose (P=0.01) and insulin (P=0.01) and Myod gene expression (P=0.001) in the heart tissue of rats with prediabetes.. Also, the results of Tukey's post hoc test showed that glucose and insulin values in prediabetic groups (P=0.05) were significantly increased compared to the healthy group.

Also, the results of Tukey's post hoc test showed that the Myod values in the prediabetic groups (P=0.01) had a significant decrease compared to the healthy group, but in the prediabetic + aerobic exercise (P = 0.01) and prediabetic + herbal supplement groups (P=0.01) and pre-diabetes + aerobic exercise + herbal supplement (P = 0.01) showed a significant increase compared to the pre-diabetes group.

Table 3. The level of Myod gene expression in heart tissue of elderly with pre-diabetes using one-way analysis of variance test

	sum of squares	df	average of squares	F	P
between groups	2/083	8	0/260	478/898	0.000
within the group	0/008	27	0.000		
Total	2/091	35			

4. Discussion

The results of this study showed that crocetin consumption and aerobic exercise caused a significant decrease in insulin and glucose and increased Myod gene expression in the pre-diabetic group, and this reduction was more in the simultaneous exercise and crocetin supplement group. Diabetes has a wide range of complications, the most important of which are cardiovascular diseases and diabetic cardiomyopathy (14). Studies have shown that some positive lifestyle changes, such as a healthy diet and exercise, can improve metabolic state abnormalities such as prediabetes and its complications through various mechanisms (15,16). The purpose of this study was the effect of eight weeks of aerobic training with consumption of crocetin extract on expression of Myod in the heart tissue of prediabetic rats. According to the obtained results, diabetes caused by STZ led to an increase in serum glucose concentration and an increase in insulin level (17).

After 8 weeks of aerobic exercise and crocetin treatment, glucose and insulin levels were improved compared to the healthy group. One of the acceptable reasons for the increase in glucose and insulin levels in diabetic rats can be related to the destruction of pancreatic beta cells. Possible mechanisms for glucose control in response to aerobic exercise include increased glucose uptake in skeletal muscle in insulin-dependent and insulin-independent ways, increased number of glucose transporter type 4 (GLUT-4) and increased glycogen synthase activity (18,19). Myod is one of the essential markers in muscle development and a key protein in regulating muscle differentiation, so that it is necessary for the initiation of muscle hypertrophy signaling, the activation and proliferation of satellite cells (20). The results of Jazer's research showed that Myod decreases in diabetes and prediabetes It was found and increased with exercise and consumption of crocetin in prediabetic rats (21,22).

Soltanian et al. compared the effect of two methods of circuit resistance training and intense performance on satellite cell activating factors Myod and Myf-5 in young non-athletes men. In this semi-experimental study, with a pre-test-post-test design with a control group, among non-athletic young men of Isfahan city in 1401, 45 people were selected as available and randomly divided into three groups: intense functional training, circular resistance training and control. Exercise protocols were performed in 8 weeks (three sessions of 40-50 minutes each week). Blood sampling was done to evaluate the expression of Myod and Myf-5 genes in two stages (24 hours before the start of training and 48 hours after the last training session). The expression level of the variables was estimated by ELISA method. Data were analyzed by covariance and post hoc Benferoni tests. Both training methods had a significant effect on the increase of Myod and Myf-5, but the improvement of these variables was greater in the intense functional training group than in the circular resistance training group. The results show the effect of both training methods on increasing the expression of Myod and Myf(23). In general, the findings of this research showed that intermittent aerobic activity and consumption of crocetin increased the expression of Myod and can lead to hypertrophy and improve the performance of the cardiovascular system, but it seems that the activation of Myod is for the growth and regeneration of the heart muscle and inhibition of autophagy in Prediabetic mouse heart is necessary (24). Recent studies have shown that regular physical activity not only helps the antioxidant capacity of the body, but also reduces the level of oxidative stress and probably helps reduce the severity of this disease by reducing the amount of apoptosis (25,26).

With its high antioxidant and anti-inflammatory properties, crocetin reduces the conditions of oxidative stress and inflammation, and by inhibiting apoptosis, it improves this disease. It seems that the combined use of crocetin and regular and continuous exercise protects the heart against oxidative stress by reducing fat peroxidation and activating antioxidant defenses (27).

Conclusion

The results of the present research showed that the administration of crocetin supplement and intermittent aerobic training for 8 weeks increased the expression of Myod gene, which is a confirmation of the improvement of heart hypertrophy in elderly diabetic mice. In addition, the simultaneous administration of intermittent aerobic exercise and crocetin has the greatest effect in reducing damage. In general, crocetin supplement with antioxidant properties along with periodic aerobic exercise improved heart hypertrophy in elderly diabetic rats.

Acknowledgements

This research is the results of a part of a specialized doctoral thesis. The authors thank and appreciate all the people who participated in this research.

Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: A.Sh.R, Kh.J, M.A.B, F.T; Methodology: A.Sh.R, Kh.J, M.A.B; Software: Kh.J, M.A.B, F.T; Validation: A.Sh.R, Kh.J, F.T; Formal analysis: A.Sh.R, M.A.B, F.T; Investigation: A.Sh.R, F.T; Resources: Kh.J, M.A.B; Data curation: M.A.B, F.T; Writing - original draft: A.Sh.R, Kh.J, M.A.B, F.T; Writing - review & editing: A.Sh.R, Kh.J, F.T; Visualization: A.Sh.R, Kh.J, M.A.B; Supervision: A.Sh.R, Kh.J, M.A.B, F.T; Project administration: A.Sh.R, Kh.J, M.A.B, F.T; Funding acquisition: A.Sh.R, M.A.B.

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