



Upregulation of Ryanodine Receptor Calcium Channels (RyR2) in Rats with Induced Diabetes after 4 Weeks of High Intensity Interval Training

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ABSTRACT

Background: To date, not sufficient information is available regarding the effect of High Intensity Interval Training (HIIT) on diabetes-induced myocardial dysfunctions.

Objectives: The present study aimed to evaluate the effect of 4-week HIIT on change in expression levels of ryanodine receptor calcium channel (RyR2) and ATPase calcium pump (SERCA2a) in diabetic rats.

Materials and Methods: This study was conducted on 24 Wistar rats with average weight of 245 ± 10 g. The rats were randomly divided into a sedentary diabetic group and a trained diabetic group. Training was started two weeks after diabetes induction by Streptozotocin (STZ) injection. The training program consisted of 4 weeks running on a treadmill and was considered to be intense for the two groups' diabetic rats. After all, the animals' characteristics and myocardial gene expression were compared using independent t-test.

Results: Measurement of gene expression by Real Time-PCR revealed that cardiac mRNA expression of RyR2 was enhanced in the HIIT group. The results also revealed a significant ($P = 0.03$) difference between the hearts of the sedentary controls and the trained group regarding RyR2 levels. However, no significant difference was observed between the two groups with respect to SERCA2a levels ($P = 0.14$).

Conclusions: The study results showed that treatment with HIIT could prevent and/or minimize the loss in expression of RyR2 and SERCA2a.

► Implication for health policy/practice/research/medical education:

The present study aimed to evaluate the relationship between change in RyR2 gene expression level and SERCA2a after 4 weeks of high intensity interval training in rats with induced diabetes. The results provided a possible explanation for the decrease in diabetic cardiomyopathy seen among the diabetics involved in high intensity interval training.

1. Background

Studies have shown that coronary atherosclerosis and cardiomyopathy are caused by diabetes-based unnatural metabolism (1). Diabetic cardiomyopathy has been diagnosed in clinical and animal diabetic models without any symptom of vascular damage (2). At molecular level, these changes are probably derived from changes in

expression and/or activity of various proteins involved in maintaining or regulating intracellular calcium homeostasis (3, 4). Ryanodine receptors (RyR2) are among these groups of proteins. They include intracellular calcium-releasing channels in the sarcoplasmic reticulum membrane (5). Returning to diastolic mode for cardiac expansion in calcium cycle is done by calcium ATPase pump of sarcoplasmic reticulum (SERCA2a) (6). Cardiac expansion begins following the process of calcium delay. This event is controlled by calcium release and collection

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mechanism. SERCA pump pumps calcium from cytosol to sarco-endoplasmic reticulum in the process of calcium collection (7). Any change in the sensitivity of RyR2, SERCA2a, and calcium-based activities (derangement of two protein) in chronic diabetes is partly responsible for reduction in myocardial speed and contraction (8). In addition to decrease in gene expression and proteins regulating myocardial calcium cycles, integration and intact activity of ryanodine was decreased in rats with Streptozotocin (STZ)-induced diabetes. In these rats, a significant decrease was reported in expression of genes and regulator proteins, such as phospholamban, RyR2, and SERCA2a (3).

Training protocols are one of the most effective methods to reduce the progress of cardiomyopathy, cardiovascular disorders, and death due to diabetes. Studies on rates with type 1 diabetes have shown that most training protocols affected factors, such as ejection fraction and stroke volume, in rats with diabetes-induced cardiac disorders (9, 10). High Intensity Interval Training (HIIT) is more effective and more useful than intermediate intensity continuous training in increasing aerobic capacity and cardiovascular functions in healthy individuals and patients with cardiovascular diseases (11-13). Also, in comparison to traditional exercises with low to intermediate intensity, these types of training were more effective in cardiac revival, controlling blood sugar and most other clinical symptoms of diabetes, and the risk factors affecting cardiac patients in the long run (14, 15).

In general, RyR2 receptors become more sensitive to calcium function due to diabetes and more calcium is required in order to inactivate the aforementioned channels. Training protocols slow down the changes in the amounts of calcium. However, a limited number of studies have been conducted on the underlying mechanisms affecting RyR2 inactivity or sensitivity to calcium function (8). Moreover, covert mechanisms of the effect of exercise on diabetic heart are not known yet. Furthermore, although it has been confirmed that myocardial adaptation with exercise depends on the intensity of training schedules in healthy animals, few comprehensive studies have examined the duration and intensity of exercises and their regulating effects on the function of diabetic heart (16).

2. Objectives

The present study aims to assess the mechanisms involved in damaging, modifying, and regulating gene expression of RyR2 and SERCA2a in diabetic hearts in response to these types of exercises.

3. Materials and Methods

3.1. Experimental Models and Injection of Streptozotocin

This study was performed on 24 Wistar rats with average weight of 245 ± 10 g. At first, 50 mg/kg body weight of STZ in buffer citrate solution (pH: 4.5) was injected

intraperitoneally to the rats in a single dose. Four days after STZ injection, blood sugar level was measured by 0 - 1 glucometer (made in Japan) in order to confirm diabetes. Blood sugar level above 300 mg/mL was considered to be the indicator of diabetes. Two weeks after diabetes induction, the rats were randomly divided into two groups (sedentary control and HIIT) each containing 12 animals. At this stage, the rats got familiar with treadmill (four rats were selected as pilot protocols). Training protocol of the HIIT group consisted of 24 minutes of running on treadmill. However, the control group did not participate in any training schedules.

3.2. Training Protocol

Two weeks after diabetes induction by STZ injection, the rats started their training schedule as follows: five minutes warm-up with 30 - 40% VO₂max intensity, 3 minutes with 58 - 90% VO₂max intensity, one minute recovery with 3 - 35% VO₂max intensity, and five minutes cool-down with 30 - 40% VO₂max intensity. The HIIT group performed this protocol five days a week for four weeks.

3.3. Real-Time-PCR Analysis

Real-time PCR was used to quantitatively measure the messenger RNA (mRNA) levels of RyR2 and SERCA2a. In doing so, the cells' RNA was extracted and treated following DNase I treatment. In this method, extra DNA was removed from the sample. Ultimately, cDNA was made and PCR-qRT reactions were done. The extent of expression of the intended mRNAs was measured according to $2^{-\Delta\Delta Ct}$.

3.4. Statistical Analysis

All the analyses were performed using the SPSS statistical software, version 16. The animals' characteristics and myocardial gene expression were compared using independent t-test. All the values were expressed as mean \pm SEM and $P < 0.05$ was considered to be statistically significant.

4. Results

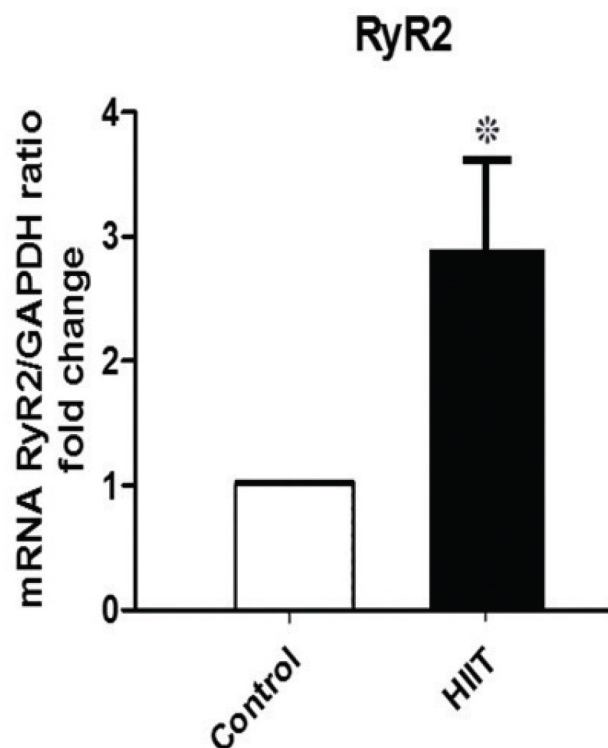
The general characteristics of the rats at the time of sacrifice have been presented in Table 1. As expected, the level of blood sugar was higher in the sedentary diabetic rats than in the training group. Thus, training reduced the blood sugar levels. Besides, body weight reduced in the sedentary group, but slightly increased in the training group (Figures 1 and 2).

5. Discussion

Previous clinical studies demonstrated that exercise training slowed down and/or delayed the progression of myocardial contractility loss induced by both type 1 and type 2 diabetes. However, molecular mechanisms underlying this beneficial effect have remained incompletely characterized.

Table 1. General Characteristics of the Animals Used in the Study ($P \leq 0.05$ Indicated Significant Difference Between the two Groups.)

Parameter	Sedentary Control Group	Training Group
Weight (g)	238.17 ± 17.9	276.4 ± 16.5
Blood sugar (mg/dm)	577.6 ± 11.5	502.8 ± 13.4

Figure 1. Measurement of RyR2 Gene Expression Fold Change by RT-PCR

The effect of HIIT on the expression of RyR2 gene in the rats. Experiments on the control rats were performed in triplicate and PCR quantification was done by densitometry analysis.

* $P \leq 0.05$ indicated significant difference between the two groups.

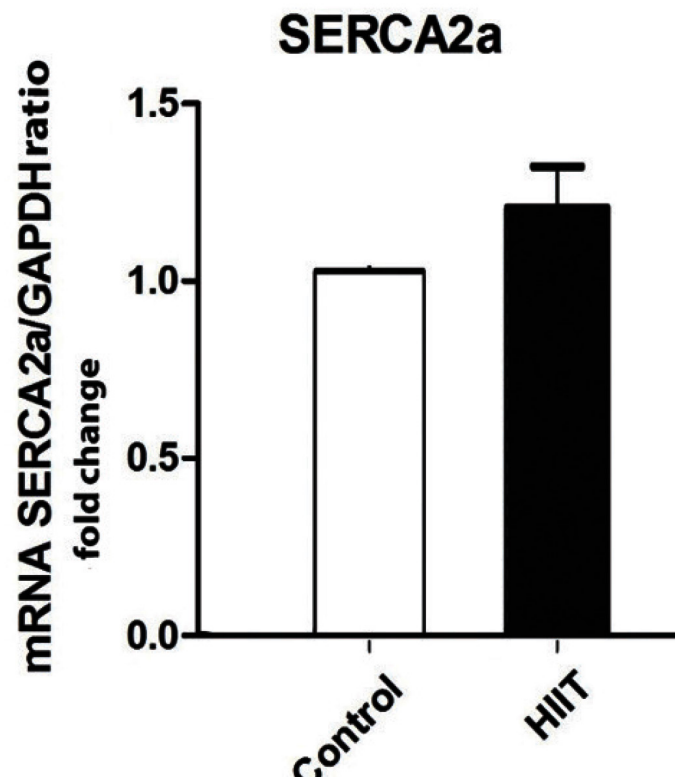
In the present study, a multifaceted approach was used that revealed for the first time that HIIT during diabetes minimized irregularity of RyR2. The results showed that HIIT normalized or reduced down-regulation of RyR2 and dysfunction of both systolic and diastolic parameters in cardiomyocytes from diabetic cardiomyopathy hearts. Patients with chronic diabetes also showed severe systolic dysfunction, which was probably due to changes in the expression and function of numerous genes and proteins involved in regulating/maintaining intracellular calcium homeostasis (3).

In this study, we focused on the effects of HIIT on expression and function of two proteins, namely RyR2 and SERCA2a. The study findings demonstrated that the cardiac mRNA expression of RyR2 (1.847 ± 0.65 , $P < 0.05$) and SERCA2a (1.206 ± 0.11 , $P < 0.05$) enhanced in the HIIT group. The results also revealed a significant ($P = 0.03$) difference between the sedentary control and trained animals regarding RyR2 levels. However, no significant difference was found between the two groups concerning SERCA2a levels ($P = 0.14$). Moreover, the increased expression of RyR2 was reversed (upregulated) in the diabetic rats after performance of HIIT. Similarly, previous studies demonstrated that different exercise training protocols led to an increase or a change in RyR2 expression at mRNA or protein levels in diabetic rats induced by STZ compared to the age-matched control rats. In the study conducted by Salem et al. (2013), daily 1-hour exercise training sessions were repeated 5 days a week for 2 - 3 months. Their program was focused on

pyramidal continuous exercise training protocol. The results of that study indicated no significant difference between sedentary Goto-Kakizaki (GK) and sedentary control rats with respect to expression of mRNA encoding intracellular Ca^{2+} transport and RyR2 regulatory protein (17). Chun Hong Shao et al. (2009) also assessed total and phosphorylation forms of RyR2 antibody in rats after 4 weeks of exercise training by ascending continuous protocol. They found that the phosphorylation form of RyR2 was increased in the exercise training group, but the total form of RyR2 remained unchanged (8).

Alterations in RyR2 and phospholamban could regulate SERCA2a function in the heart, which controls Ca^{2+} loading and magnitude of Ca^{2+} transients (8, 18). SERCA2a is the main contributor to removal of cytosolic Ca^{2+} during diastole. Similar to the previous studies, our study results showed that exercise training restored the functions of SERCA2a to levels comparable to those of the sedentary control group. This suggested that exercise training normalized diastolic function in the trained group by shifting the control of diastolic Ca^{2+} to SERCA2a and, consequently, increased the rate of Ca^{2+} removal. This also increased the SERCA2a load, which might have contributed to improvement of systolic function.

Several researches have reported that the effects of exercise on the cardiovascular system depend on the intensity or amount of exercise (19-21). The intensity and duration of the exercise used in our study were comparable with those in the majority of the previous studies conducted on diabetic cardiomyopathy. Additionally, several studies

Figure 2. Measurement of SERCA2a Gene Expression Fold Change by RT-PCR

The effect of HIIT on the expression of SERCA2a gene in the rats. Experiments on the control rats were performed in triplicate and PCR quantification was done by densitometry analysis.

* $P \leq 0.05$ indicated significant difference between the two groups.

have demonstrated the beneficial effects of exercise on myocardial muscle glucose homeostasis (9). High intensity and short duration of exercise might have played a role in our study. In this study, exercise training reduced blood sugar level to a small extent in the HIIT group compared to the age-matched controls.

In conclusion, the findings of the current study showed that HIIT could prevent and/or minimize the loss in expression of RyR2 and SERCA2a. Thus, high intensity and short duration of exercise might have played a role in prevention of decreased gene expression in diabetic rats' hearts in our study.

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Authors' Contribution

Mohammad Ali Babaei Bigi and Hossein Faramarzi funded this project and contributed to the research design. Mohammadreza Izadi was the corresponding author. Abbas Ali Gaeini, Ali Asghar Ravasi, and Delfan contributed to the research design and laboratory works. Esmail Izadi participated in pharmacological guidance.

Financial disclosure

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