Evaluation of Bcl-2 and Bax Expression in the Heart of Diabetic Rats after Four Weeks of High Intensity Interval Training

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ABSTRACT

Background and Objectives: Diabetes is one of the most common diseases and a major risk factor for cardiovascular disease. Studies have shown that regular exercise can affect apoptosis in cardiomyocytes. The purpose of this study was to investigate the effect of high-intensity interval training on the expression of Bcl-2 and Bax as important apoptosis factors in diabetic rats.

Methods: After inducing diabetes in 20 male Wistar rats (weighing 250 ± 1.15 g), the rats were randomly divided into a control group and a training group. The training group performed high-intensity interval training five days a week for four weeks, and the control group did not perform any training. After the intervention, RNA was extracted and TCF mRNA was subjected to real time RT-PCR for measuring Bax and Bcl-2 expression in the heart tissue of diabetic rats. Data were analyzed by IBM SPSS Statistics V22 using independent t-test. P-values less than 0.05 were considered as statistically significant.

Results: The expression of Bcl-2 increased significantly and the expression of Bax decreased significantly after the four-week training intervention.

Conclusion: The high-intensity interval training can have beneficial effects on the expression of apoptotic genes in rats with type 2 diabetes.

Keywords: Bax, Bcl-2, High intensity interval training, Heart, Type 2 diabetes.

INTRODUCTION

The prevalence of type 2 diabetes is increasing worldwide (1). Diabetes is also a major risk factor for cardiovascular disease (2). Studies have shown that cardiomyopathy and coronary atherosclerosis are caused by an abnormal diabetes-related metabolism (3). In these conditions, cardiomyocytes use fat as source of energy due to a defect in the transfer and oxidation of glucose. Accumulation of fat oxidation and abnormal metabolism byproducts causes cell death and myocardial infarction (4). Increased rate of apoptosis in pathological conditions may also cause myocardial dysfunction (5). Normally, the rate of apoptosis in myocardium is very low (0.001 to 0.002%). However, this rate increases in acute and chronic heart diseases as well as in diabetes. Apoptotic messages in the early stages of apoptosis are intensified through three complex signaling pathways: 1) cytokine/Fas receptor-driven pathway, 2) mitochondrial-mediated pathway, and 3) endoplasmic reticulum/Ca^{2+}-driven pathway. Among these pathways, the mitochondrial-mediated pathway, including the Bcl-2 family is well-characterized and believed to be critical in apoptosis regulation (6). The balance between pre-apoptotic and anti-apoptotic signals controls the fate of cells and future apoptosis programs (5). The Bcl-2 family proteins have anti-apoptotic members (such as Bcl-2 and Bcl-XL) and a subset of pro-apoptotic members (such as Bax, Bid and Bad) (7). The ratio between the apoptotic and anti-apoptotic proteins determines cellular life cycle through mitochondrial regulation (8). It has been shown that Bax uses its NH_2 terminus after moving to mitochondria to induce apoptosis. Due to this adaptation, Bax enters the outer layer of the mitochondria and subsequently apoptotic factors are released from the mitochondrial membrane (e.g., cytochrome c). It seems that Bax oligomerisation is also important in the permeabilization of the mitochondria membrane, while Bcl-2 counteracts the pre-apoptotic activity of Bax by preventing the oligomerisation of Bax (9, 10). Therefore, it is important to seek strategies that can protect the heart against the risk of apoptosis.

The risk of developing cardiovascular disease and diabetes can be reduced by regular exercise (2, 11). Recently, researchers have shown great interest in the effect of exercise on apoptosis (6). Some studies reported that moderate-intensity exercise can reduce apoptosis (5, 6, 12). Peterson et al. showed that nine weeks of moderate exercise training reduces the amount of Bax protein, caspase activity and DNA damage in the heart tissue of obese rats (13). Quindry et al. confirmed these findings by revealing the effect of treadmill exercise on anti-apoptotic markers in the heart tissue of rats with ischemic heart disease (14). However, Xixin et al. showed that long-term endurance exercise alters the expression of Bcl-2 and Bax in the heart, and induces apoptosis via oxidative stress (15). A study showed that low and moderate intensity training are more beneficial for type 2 diabetics compared to high intensity interval training (HIIT) (16), while another study reported that HIIT is more effective in controlling diabetes and its complications (17). Given the contradictory results of previous studies, we aimed to study the effect of HIIT on the expression of Bcl-2 and Bax in diabetic rats.

METHODS

This developmental-experimental research was performed on 20 8-week-old male Wistar rats (mean weight 250.6 ± 15.1 g) that were obtained from the Baqiyatallah Center for breeding and duplication of laboratory animals in Iran. The animals were kept at temperature of 22 ± 2 ℃, humidity of 25-30%, and under a 2:12 h light-dark cycle. The animals had free access to water and food. The study received approval from the ethics committee of the University of Tehran. One week after transferring rats to the laboratory, diabetes was induced and the rats were randomly divided into two weight-matched groups. Accordingly, 10 rats were included in the diabetic group and the rest were included in the control group. The control group did not perform any training during the four-week period, while all rats in the training group were subjected to a five-day a week training program for four weeks.

Diabetes was induced by intraperitoneal injection of 95 mg/Kg nicotine amide (dissolved in saline solution) and 55 mg/Kg streptozotocin (dissolved in citrate buffer solution) (18). In order to confirm diabetes, five days after the injections, a drop of blood was taken from the tail of the animals and placed on a glucometric strip. A blood glucose

level of >126 mg/dl indicated diabetes (19). The rats were also weighed weekly using a digital scale. The rats were anesthetized with ketamine (90 mg/Kg) and zylasein (10 mg/Kg) 48 hours after the last training session, and biopsy specimens were taken from the hear*t*s. The heart muscle (after isolation) and amount of food consumed were also weighed using a digital scale (Sartorius, Germany) with sensitivity of 0.1 g.

Exercise started when the rats were 17 weeks old. The training group performed HIIT for four weeks, five days a week (Table 1). The frequency of training was observed during the intervention; the rats trained on Saturdays, Sundays, Tuesdays, Wednesdays and Thursdays and rested on Mondays and Fridays. During each training session, the rats performed 10 repetitions of 1 minute with treadmill running at 30 m/min and 2 minutes of rest between the integrals. They also performed 5 minutes of warm up and 5minutes of cool down at 10 m/min at the beginning and the end of each training session, respectively. The treadmill speed reached 55 m/min in the last week of training (21).

Twenty mg of heart tissue were crushed by scalper and transferred to a microtube (22). RNA was extracted from the heart tissue using the RNeasy Protect Mini Kit (QIAGEN) according to the manufacturer's instructions.

Optical density was measured by a NanoDrop spectrophotometer to assess the extracted RNA concentration for cDNA preparation.

Real time RT-PCR of TCF mRNA was performed using the Rotor-Gene 6000 real-time rotary analyzer and the One-Step SYBR PrimeScript RT-PCR Kit (Takara BIO Inc., Japan). Melting curve analysis was performed at the end of the PCR cycle to determine the validity of the expected PCR products. Thermal cycling conditions were as follows: 42 °C for 20 minutes, 95 °C for 2 minutes, 40 cycles at 94 °C for 10 seconds, and 60 °C for 40 seconds. To study the properties of primers (Table 3), temperatures of 50 to 99 °C were used to obtain melting curve. RNA Polymrasell was used as the control for determining gene expression. Reactions' cycle thresholds (CT) were extracted and recorded using the Real Time-PCR software. The ΔΔCT method was used for quantification of TCF mRNA expression.

Data were analyzed using IBM SPSS Statistics V22. The Shapiro–Wilk test confirmed the normal distribution of all data. Descriptive statistics including mean and standard error of the mean were used to describe the findings. Independent t-test was used to compare differences in the mean of variables between the two groups. The statistical significance level was set at 0.05.

Table1- Details of the high-intensity interval-training program used in the study

Week	First	Second	Third	Fourth
Speed (m/min)	30	40	55	55
Duration (min)	1	1	1	1
Repeat	10	10	10	10
Rest/min	2	2	2	2

Table 2- Sequence of the primers used in the study

Genes	Primer sequence		Code Number
Bax	For:	ACAGGGTTTCATCCAGGATCGAG	NM-017059.2
	Rev:	AGCTCCATGTTGTTGTCCAGTTC	
Bcl-2	For:	GGATTGTGGCCTTCTTTGAGTTC	NM-016993.1
	Rev:	AGAGCGATGTTGTCCACCAG	
GAPDH	For:	AAGTTCAACGGCACAGTCAAGG	NM-017008.4
	Rev:	CATACTCAGCACCAGCATCACC	

RESULTS

Diabetes caused a significant increase in the mean weight of rats in both groups after two months (P=0.001) (Table 3). After the four-week training program, the mean weight of rats in the training group decreased significantly compared to the control group.

The weight of the heart muscle was significantly higher in the training group than in the control group (P=0.000). The expression of Bax (P=0.002) decreased significantly, while the expression of Bcl-2 (P=0.009) increased significantly in the training group.

DISCUSSION

Diabetes is one of the main causes of cardiovascular disease. The prevalence of diabetes in patients with heart disease has been reported to be three times higher (23). Different pharmacological and non-pharmacological interventions have been proposed for the prevention and treatment of diabetes. Recently, regular physical activity was introduced as an effective method for the prevention and treatment of type 2 diabetes (24). In this regard, most studies have investigated the therapeutic effects of continuous low-intensity training (such as walking, jogging and cycling) for 30 minutes or more in a single session. However, this method of training has not been well established. On the other hand, HIIT may be more effective in controlling blood glucose in diabetic patients (24). Studies have shown that this type of training improves insulin sensitivity in the liver and muscle tissue (25). However, the subcellular mechanisms involving this effect are not well understood in patients at risk of metabolic disorders and chronic heart disease. In this study, we investigated the effects of HIIT on the expression of Bcl-2 and Bax genes in the heart tissue of type 2 diabetic rats.

In the present study, the induction of diabetes increased the weight of rats, which was continued until the end of the intervention, indicating that diabetes induces weight gain and obesity in rats. After the four-week training intervention, the weight of rats in the training group was significantly lower than the control group. Bcl-2 and Bax gene expression increased and decreased, respectively in the heart tissue of diabetic rats. Increased expression of Bax and decreased expression of Bcl-2 in endothelial cells is thought to be associated with an increase in glucose and A1C levels (26). Molly showed that hyperglycemia increases p53 expression and decreases glucose transport regulators, thus stimulating mitochondrial-mediated apoptosis (27). Similar to our findings, many studies have shown that physical activity modifies apoptosis factors (6, 28). In a study by Jafari et al., 12 weeks of treadmill running at speed of 24-33 m/min (15% slope) for 10-60 minutes decreased Bcl-2/Bax ratio significantly (6), which is in line with our findings. Inconsistent with our study, they found no significant difference in Bcl-2 expression between the training group and the control group. This could be attributed to the difference in the intensity and type of training used in the two studies. A study claimed that only HIIT could effectively regulate superoxide dismutase activity in ventricular myocardial infarction (29). Another study showed that HIIT might lower the level of superoxide anion radical and malondialdehyde, which could prevent apoptosis and cell death. Lu et al. studied the effects of HIIT and moderate-intensity aerobic training on apoptosis, oxidative stress and myocardial metabolism of rats. They found that only HIIT reduced oxidative stress by lowering levels of malondialdehyde and increasing levels of superoxide dismutase and glutathione peroxidase. In addition, Marefati et al. reported that moderate-intensity interval training could improve cardiac function indices and prevent cell death by reducing TNFα levels in cardiac tissue (30).

CONCLUSION

Our results suggest that HIIT can have similar effects to aerobic exercise in lowering oxidative and inflammatory factors and modifying amount of apoptotic and anti-apoptotic proteins. This type of exercise can also reduce blood glucose level and the subsequent oxidation and inflammation in diabetic patients, thus reducing the risk of diabetic complications. We recommend this type of exercise as an effective method for preventing cell death in the heart tissue of patients with type 2 diabetes.

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CONFLICT OF INTEREST

All contributing authors declare that there is no conflict of interest.

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