BRIEF REPORT

The Effect of Continuous Aerobic Training on Bax/Bcl2 Ratio in Pancreatic Tissue Diabetic Rats

Mehdi Bostani[1*], Seyed Aenollah Noaein[1]

1. Department of Physical Education, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

***Correspondence:**
Mehdi Bostani, PhD, Department of Physical Education, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.
Tel: (98) 916 701 7668
Email: bostanim@yahoo.com
ORCID ID: (0000-0002-3284-6356)

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Abstract

Objective: Apoptosis of pancreatic beta cells plays an important role in the pathogenesis of type 1 diabetes. the purpose of this study was to investigate the effect of continuous aerobic training on Bax/Bcl-2 ratio in pancreatic tissue of streptozotocin (STZ)-induced diabetic rats.

Materials and Methods: A total number of 40 male Wistar rats, were divided into healthy control (HC), healthy trained (HT), diabetic control (DC), and diabetic trained (DT) groups. Diabetes was also induced by a single intraperitoneally injection of STZ (45 mg/kg). the training groups performed the exercise on the treadmill for five consecutive days within six weeks with 10-18 m/min intensity and 10-30 minute duration based on the principle of overload. the pancreatic tissue levels of the Bax and the Bcl-2 proteins were further determined via the ELISA method. The analysis of variance test (ANOVA) with Tukey's post hoc test was used for analyzing the data.

Results: The results showed that the induction of diabetes had significantly decreased the levels of Bcl-2 protein and increased the levels of Bax protein and Bax/Bcl-2 ratio in the pancreatic tissue (*P*-value=0.001,Effect Size=0.29). As well, the findings showed that six weeks of aerobic exercise training had significantly increased the levels of Bcl-2 and significantly decreased the levels of Bax and Bax/Bcl-2 ratio in the DT group (*P*-value=0.012, Effect Size=0.23).

Conclusion: According to the results of this study, exercise can be considered an effective strategy to reduce the rate of diabetic-induced apoptosis and control its complications.

Keywords: Diabetes, Apoptosis, Bax, Bcl-2, Exercise, Rehabilitation

Introduction

Pancreatic beta-cell apoptosis plays an essential role in the pathogenesis and development of Type 1 Diabetes mellitus (T1DM) (1,2). Bax and Bcl-2 are two essential proteins are involved in the process of apoptosis through the formation of the apoptosome complex the activator and inhibitor of caspases. Bax/Bcl-2 ratio is known

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to determine the survival and death of the cell (3). The Bcl-2 family proteins play a vital role in the transmission of intracellular apoptotic signals by regulating the permeability of the mitochondrial membrane. These proteins are divided into two categories: Anti-apoptotic proteins such as Bcl-2 and pro-apoptotic proteins such as Bax (4). The purpose of this study was to examine the levels of Bax protein, Bcl-2 protein and Bax/Bcl-2 ratio and studying the effect of aerobic exercise training in pancreas tissue of diabetic rats.

Materials and Methods

In this quasi-experimental study, 40 adult male Wistar rats were supplied from Razi Institute through the Cochran's formula (Karaj, Iran) and housed four-per-cage in an animal lab under standard conditions (12-hour light/dark cycle in a room at a temperature of 20-25°C) with access to food and water ad libitum. The rats were fed a standard diet prepared from Pars animal feed. All the institutional and animal research health guidelines were also observed. The animals were randomly divided into four groups: 1. healthy control (HC, N=10), 2. healthy trained (HT, N=10), 3. diabetic control (DC, N=10), and 4. diabetic trained (DT, N=10) followed by inducing diabetes in DT and DC groups. This project was carried out in Razi Laboratory of Lorestan University of Medical Sciences.

Following overnight fasting, diabetes was induced through a single intraperitoneal injection of STZ (45 mg/kg; Sigma, St. Louis, MO) solution (dissolved in 0.5 mol/l citrate buffer at pH 4.0). Two days later, diabetes was confirmed through measuring tail vein blood glucose level (>350 mg/ld.) and blood glucose levels were controlled all along the study course, once every week.

Treadmill training protocol

The treadmill training protocol was developed based on previous protocols which consisted of 6 weeks of moderate-intensity endurance aerobic exercise on a leveled motor-driven treadmill (Model T510E, Diagnostic and Research, Taoyuan, Taiwan). To stabilize the obtained adaptations, training speed and duration were kept constant at the sixth week (5).

Evaluation of Bax and Bcl-2

To measure the expression of the Bax and the Bcl-2 proteins, the ELISA kits were used (Cusabio-Japan). At the first step, the Bax and the Bcl-2 levels of the pancreatic were homogenized (1:10 in PBS 10Mm, pH 7.4 in 4°C) and then centrifuged (20,000 rpm in 45 min). After that, using special kits of rats (Bax; 15.6 pg/mL and Bcl-2; 0.078 pg/mL), the levels of proteins were measured and spectrophotometrically read by an Autobio Elisa Reader (China).

Statistical analysis

Statistical analyses were conducted using SPSS statistics software (Version 21, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test was further employed to compare the Bax and the Bcl-2 levels in the HC, HT, DC, and DT groups. The data were reported as mean (±standard deviation) values and the statistical significance level was set by *P*-value< 0.05.

Ethical considerations

The study was approved by Lorestan University of Medical Sciences, Iran (Ethic code: LUNS.REC.1395.170).

Results

As shown in figure 1, the Bax protein levels in the DC group significantly increased compared to the HC group. There was also a significant reduction in Bcl-2 protein levels in the diabetic control (DC) group compared to the healthy group (HC). Also, the Bax/Bcl-2 ratio in diabetic rats increased significantly (*P*-value= 0.001).

In the diabetic group that performed aerobic exercise on a treadmill for six weeks, there was a significant decrease in the levels of Bax

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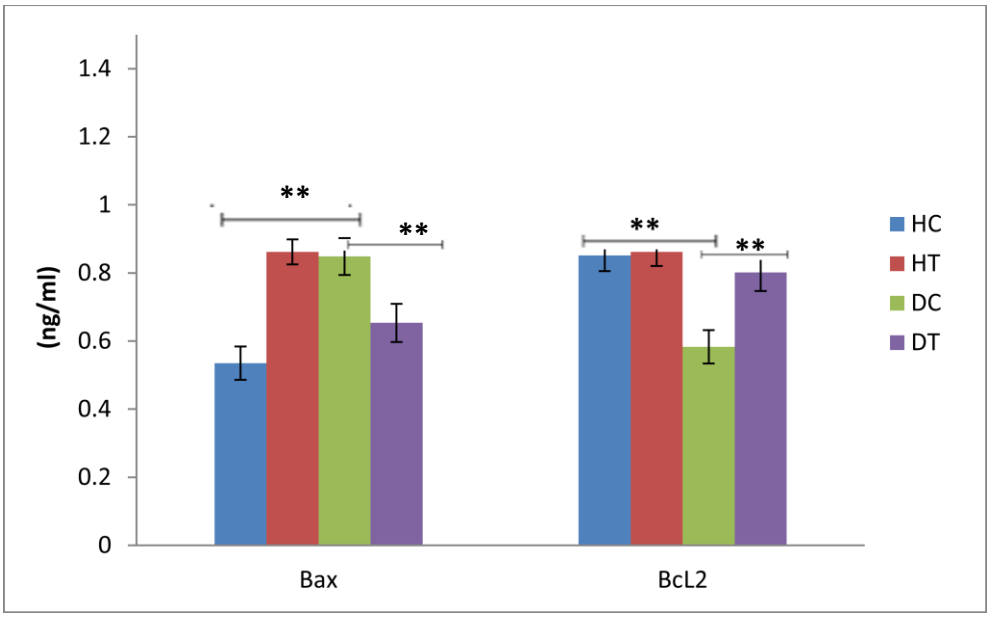

Figure 1. Diabetes decreased the levels of the Bcl-2 protein and increased the Bax protein in the pancreatic tissue, while aerobic exercise training for six weeks significantly increased the Bcl-2 and decreased the Bax protein. **Significantly different, *P*-value≤ 0.01.

protein, significant increase in Bcl-2 protein and significant reduction in Bax/Bcl-2 ratio compared to the diabetic control group (*P*-value= 0.012).

Discussion

Physical activity, depending on the intensity and duration, can lead to inflammation and oxidative stress in the body. Of course, light to moderate exercise has been shown to reduce the risk of inflammation, but high-intensity exercise, due to open window theory, may increase the risk of inflammation and therefore oxidative stress (6,7).

The mechanism of the reduction in cellular death related to the increase of the body's antioxidant capacity (8), reduction in the cytokines such as TNF-α (9), the decrease in the levels of pre-apoptotic proteins such as Bax protein (10), increasing the anti-apoptotic factors (Bcl-2, Hsp70), increasing the some of DNA regeneration enzymes and reduction in (reactive oxygen species) ROS production (11). It seems that the mitochondria play an essential role in regulating apoptotic events. in this regard, the members of Bcl-2 family, including Bax and Bcl-2 proteins as the main proteins, are involved in the formation of apoptotic channels, mitochondrial permeability regulation, and mitochondrial apoptosis signaling (12). the Bax/Bcl-2 ratio is also an indicator for the determination of mitochondrial apoptosis potential, which Bcl-2 through preventing of Bax-Bax oligomerization, opposes the proapoptotic activity of Bax. As soon as the Bax protein enters to mitochondria, it forms the pores in the mitochondrial membrane, that results in the release of proteins such as cytochrome C into the cytosol and triggers the downstream apoptotic signaling caspase cascade such as caspases-3 and caspases-9 (13,14). The Bcl-2 protein by entering the mitochondrial outer membrane, maintains the integrity of the membrane and through the removing of H ions from the ion channels and via binding to apaf-1, inhibits caspase activation (15,16).

In response to exercise, the levels of oxidative stress are associated with the less phosphorylation of c-Jun-N-terminal kinase (JNK). this is accompanied by a reduction in the expression of Bax protein and its transfer to mitochondria, as well as an increase in the levels of Bcl-2 protein. Also, the increase in displacement and transmission of apoptosis-inducing factor (AIF) that have seen in

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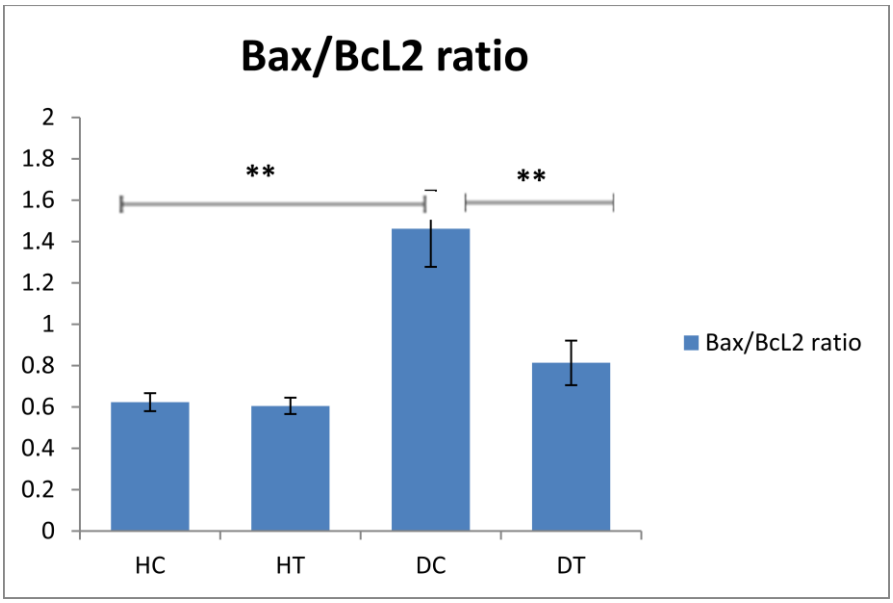

Figure 2. Diabetes increased the Bax/Bcl-2 ratio in the pancreatic tissue, however aerobic exercise training for six weeks significantly decreased this ratio. **Significantly different, *P*-value≤ 0.01.

oxidative stress status, is reduced in the tissues of trained individuals which indicating of inhibition in the apoptotic signalling (17).

Given that in this study we only examined proteins associated with apoptosis but did not perform histological and immunohistochemical survey, it is suggested that future studies also study histochemistry on pancreatic tissue, as well as the effect of different exercises, including resistance training and exercises with a longer period be be assessment.

Conclusions

According to the results of the present study, it can be concluded that aerobic exercise as a non-pharmacological, low-cost and useful strategy can be considered by reducing the levels of pro-apoptosis and increasing the amounts of anti-apoptosis factors, and can control diabetes-induced cell death and its complications.

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Conflict of Interest

Authors have no conflict of interest.

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