Introduction
Diabetes mellitus (DM) is a chronic metabolic disorder of the endocrine glands that has affected millions of people around the world and often associated with the functional and structural complications in various organs [1]. Pancreatic beta cell apoptosis plays an important role in the pathogenesis of type 1 diabetes. Exercise as a non-pharmacological strategy to reduce the diabetic-induced complications has always been of interest to researchers. Therefore, the purpose of this study was to investigate the effect of aerobic exercise on levels of Bax, Bcl-2 and Bax/Bcl-2 ratio in pancreatic tissue of streptozotocin (STZ)-induced diabetic rats. Materials and methods. A total number of 40 male Wistar rats (10 weeks old, 200–250 gr weight) were randomly 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 streptozocin (45 mg/kg). The training groups performed the exercise on the treadmill for five consecutive days within six weeks. The pancreatic tissue levels of the Bax and the Bcl-2 proteins were further determined via ELISA method. 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 < 0.05). 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 protein in DT group. Also, the Bax/Bcl-2 ratio reduced significantly in DT group (p < 0.05). The increase in displacement and transmission of apoptosis inducing factor (AIF) that have seen in oxidative stress status, is reduced in the tissues of trained individuals which indicating of the inhibition in the apoptotic signaling. Conclusions. According to the results of this study, exercise can be considered as an effective strategy to reduce the rate of diabetic-induced apoptosis and control its complications. Keywords: exercise; Bax; Bcl-2; apoptosis; diabetes mellitus
Exercise and physical activities have been recommended for the decrease of complications in patients with diabetes [9–11]. Cheng et al. [12] in their study showed that exercise training with increases the levels of Bcl-2 protein, enhances the cell survival in the heart of diabetic mice. N. Ramezani et al. [13] in their study reported that high-intensity interval training it caused a significant increase in Bcl-2 and decreased significantly in Bax after the four-week training intervention in heart of diabetic rats.

According to various studies, the effect of physical activity on apoptotic factors in diabetic pancreatic tissue has not been studied, therefore the aim 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 streptozotocin (STZ)-induced diabetic rats.

Materials and methods

Animals

A total number of 40 adult male Wistar rats were supplied from Razi Institute (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. All the institutional (as registered under the code LUNS. REC.1395.170 at Lorestan University of Medical Sciences) 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.

Diabetes induction

For the purpose of acclimatization and reaching optimal weight (at least 250 gr), all of the rats were kept in an animal lab for two weeks prior to the experiments. Subsequently, following an overnight fasting, diabetes was induced through a single intraperitoneal injection of streptozotocin (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/dl.) by Accu-Chek Compact Plus blood glucometer (Roche Diagnostics K.K., Tokyo, Japan). In this respect, the animals without any trace of hyperglycemic were excluded from the rest of the samples. In addition, blood glucose levels were controlled all along the study course, once every week [29].

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). The aerobic power of the animals in terms of VO2max was further obtained based on the relationship between VO2max to speed and treadmill slope [14]. Within the first week, the speed and the duration of the treadmill running were 10 m/min and 10 min per day, respectively. The figures were then gradually increased until the fifth week, ending up with training speed and duration of 18 m/min and 30 min per day, respectively. To stabilize the obtained adaptations, training speed and duration were kept constant at the sixth week [15].

### Tissue extraction

Two days after the last exercise session in the sixth week of training, the animals were anesthetized by inhalation of 2 % halothane in a mixture of 20 % O2 and 80 % CO2. For the analysis of the levels of Bax and the Bcl-2 pancreatic tissue, total rats in four-groups were removed immediately and separated pancreatic tissue were stored at −80 °C until the analysis process was completed.

### 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 10 Mm, 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). Normality and homogeneity of the data were also assessed via Shapiro-Wilk test and Levene’s test, respectively. 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 error of measurement (SEM) values and the statistical significance level was set by p < 0.05.

### Results

Figure 1 illustrated the average of blood glucose levels during the six-week endurance training intervention in the diabetic groups. As shown, blood glucose levels within six weeks in the diabetic group were more than 300 mg/dl which was considered as the threshold for diabetes. In the diabetic control group, the blood glucose levels had been also rising since the injection of the STZ by the end of the sixth week; but in the diabetic trained group, a decreasing trend was observed which revealed a significant reduction started at the beginning of the fourth week of the study (p < 0.05).

As shown in Figure 2, the levels of the Bax protein in the DC group significantly increased compared to the HC group (p < 0.05). There was also a significant reduction in Bcl-2 protein levels in the diabetic control (DC) group com-

<table>
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<th>Table 1. Endurance Training Protocol</th>
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<td>Training duration (minute)</td>
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<td>Treadmill speed (m/min)</td>
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pared to the healthy group (HC). Also, the Bax/Bcl-2 ratio in the diabetic rats increased significantly (p < 0.05).

In the diabetic group that performed aerobic exercise on a treadmill for six weeks, there was a significant decrease in the levels of Bax protein, significant increase in Bcl-2 protein and significant reduction in the Bax/Bcl-2 ratio compared to the diabetic control group (p < 0.05).

**Discussion**

In the present study, we found that in the streptozotocin (STZ)-induced diabetic rats, the levels of the Bax pro-apoptotic protein increased, but the levels of the Bcl-2 anti-apoptotic protein decreased. In addition, Bax/Bcl-2 ratio was increased also. This means that pro-apoptosis processes have overcome anti-apoptosis processes in diabetic pancreas tissue.

However, six-weeks of aerobic exercise led to an increase in Bcl-2 anti-apoptosis protein and a decrease in the Bax pro-apoptosis protein and the Bax/Bcl-2 ratio.

The mechanism of the relationship between cell death and hyperglycemia is not yet well understood. Diabetes-induced oxidative stress may play an important role in the development of apoptosis in hyperglycemic state. Oxidative stress is created by an imbalance between the production of reactive oxygen species (ROS) and antioxidant factors. The role of the free radicals in the dysfunction of beta cells has been well established, in fact increment of the oxidative stress plays an important role in the spread of diabetic-induced cell death in most tissues [16, 17]. It is believed that the oxidative stress to be a major factor of the adverse effects of diabetes on tissues, which can trigger or accelerate apoptosis in affected cells [18].

The results of the various studies have shown that physical activity can reduce the amount and speed of cellular apoptosis. Some of these studies have suggested that the mechanism of the reduction in cellular death related to the increase in body’s antioxidant capacity [19], reduction in the cytokines such as TNF-α [20], the decrease in the levels of pro-apoptotic proteins such as Bax protein [21], increasing the anti-apoptotic factors (Bcl-2, Hsp70), increasing the some of DNA regeneration enzymes and reduction in ROS production [22]. It seems that the mitochondria play an essential role in regulating of the 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 [23].

The Bax/Bcl-2 ratio is also an indicator for the determination of the mitochondrial apoptosis potential, which Bcl-2 through preventing of Bax-Bax oligomerization, opposes the pro-apoptotic 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 cystosol and triggers the downstream apoptotic signaling caspase cascade such as caspases-3 and caspases-9 [24]. The importance of the mitochondrial permeability has been explained in the J. Fang et al. study in 2008. They showed that the blocking of the mitochondrial permeability pores reduces the rate of apoptosis [25]. Moreover, 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 [26, 27].

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.

![Figure 1. Blood glucose levels during the six weeks](image1.png)

**Figure 1. Blood glucose levels during the six weeks**

Note: * — P < 0.05 significant decrease in blood glucose levels in diabetic trained (DT) versus the diabetic control (DC) group.

![Figure 2. 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](image2.png)

**Figure 2. 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**

Note: * — Significantly different, p ≤ 0.01.

![Figure 3. Diabetes increased the Bax/Bcl-2 ratio in the pancreatic tissue, however aerobic exercise training for six weeks significantly decreased this ratio](image3.png)

**Figure 3. Diabetes increased the Bax/Bcl-2 ratio in the pancreatic tissue, however aerobic exercise training for six weeks significantly decreased this ratio**

Note: * — Significantly different, p ≤ 0.01.
In addition, the increase in displacement and transmission of apoptosis inducing factor (AIF) that have seen in oxidative stress status, is reduced in the tissues of trained individuals which indicating of the inhibition in the apoptotic signaling [28, 29].

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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Фізична активність як нефармакологічна та ефективна стратегія в контролі симптомів апоптозу у щурів з експериментальним діабетом

Резюме. Актуальність. За останні роки цукровий діабет (ЦД) став глобальною проблемою охорони здоров’я. Апоптоз бета-клітин підшлункової залози відіграє важливу роль у патогенезі ЦД 1-го типу. Фізична активність як нефармакологічна стратегія для зменшення частоти ускладнень, спричинених ЦД, завжди цікавила дослідників. Тому метою цього дослідження було встановлення впливу аеробних вправ на рівні апоптозу у білках Bax та Bcl-2 у тканині підшлункової залози. Матеріали та методи. Самці щурів лінії Wistar (віком 10 тижнів, вага 200–250 г) загальною кількістю 40 рандомізовано розподілили на групи здорового контролю (ЗК), здорових тренованих (ЗТ), з діабетом (контроль, ДК) та з діабетом із фізичним навантаженням (ДН). Діабет був індукований одноразовою інтратеперитонеальною ін’єкцією стрептозоцину (45 мг/кг). Тренувальні групи виконували вправу на біговій доріжці п’ять днів подряд в течінні шести тижнів. У експериментальних групах значно підвищили рівень білка Bcl-2 і знизили рівень білка Bax у тканині підшлункової залози (p < 0,05). Окрім того, результати показали, що шість тижнів аеробних тренувань значно підвищили рівень білка Bcl-2 та знизили рівень білка Bax у тканині підшлункової залози. Антиапоптична роль аеробних вправ у бік зумовлена ускладненням апоптозу через зниження виходу і зменшення передачі фактора, що індукує апоптоз, який спостерігається в тканинах тих, хто відбував аеробні тренування. Висновки. Згідно з результатами цього дослідження, фізичні вправи можна розглядати як ефективну стратегію зменшення частоти спричиненого діабетом апоптозу та контролю його ускладнень.

Ключові слова: фізичні вправи; Вах; Bcl-2; апоптоз; цукровий діабет

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Физическая активность как нефармакологическая и эффективная стратегия в контроле симптомов апоптоза у крыс с экспериментальным диабетом

Резюме. Актуальность. За последние годы сахарный диабет (СД) стал глобальной проблемой здравоохранения. Апоптоз бета-клеток поджелудочной железы играет важную роль в патогенезе СД 1-го типа. Физическая активность как нефармакологическая стратегия для уменьшения частоты осложнений, вызываемых диабетом, всегда интересовала исследователей. Поэтому целью данного исследования было установление влияния аэробных упражнений на уровень апоптоза в белях Вах и Bcl-2 в ткани поджелудочной железы. Материалы и методы. Сами хомяков линии Wistar (в возрасте 10 недель, вес 200–250 г) общим количеством 40 были разделены на группы здорового контроля (ЗК), здоровых тренированных (ЗТ), с диабетом (контроль, ДК) и с диабетом и физической нагрузкой (ДН). Диабет был индуцирован одиннадцатое интратеперитонеальной инъекцией стрептозоцина (45 мг/кг). Тренеровочные группы выполняли упражнения на беговой дорожке пять дней подряд в течение шести недель. Уровни белька Bax у групах ДН (p < 0,05). Усиление выделения и передачи фактора, индуцирующего апоптоз, который наблюдается в состоянии окислительного стресса, уменьшается в тканях тренированных, что указывает на гальмувание в сигнализации апоптозу. Выводы. Согласно результатам этого исследования, физические упражнения можно рассматривать как эффективную стратегию уменьшения частоты апоптоза, вызванного диабетом, и контроля его осложнений.

Ключевые слова: физические упражнения; Вах; Bcl-2; апоптоз; сахарный диабет