Original Article

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Swimming training by affecting the pancreatic Sirtuin1 (*SIRT1*) and oxidative stress, improves insulin sensitivity in diabetic male rats

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Abstract:
Background: Sirtuin1 is a regulator of oxidative stress involved in the management of diabetes complications. Due to the beneficial effects of swimming training in diabetes, this study aimed to investigate the effects of swimming training on pancreatic Sirtuin1, oxidative stress and metabolic parameters in type 2 diabetic male rats.
Materials and methods: Twenty-eight male Wistar rats (200–250 g) were randomly divided into four groups: control, diabetic, swim trained and swim trained diabetic rats (n = 7). Diabetes was induced by a high-fat diet and streptozotocin injection [35/kg intraperitoneally]. After 72 hours, animals with blood glucose levels ≥300 mg/dL were considered diabetic. Seven days after the induction of diabetes, animals in the exercise groups were subjected to swimming training (60 min/daily, 5 days/week) for 12 weeks. At the end of the intervention, the animals were anesthetized, and tissue/blood samples were prepared for measurements of metabolic parameters, albumin, the Sitruin1 gene and its protein expression levels, oxidative stress and histological study.
Results: This study indicated that the diabetic rats had a significant decrease ($p < 0.01$, $p < 0.05$) in pancreatic Sitruin1 gene and its protein expression levels, antioxidant enzymes, serum albumin, and the quantitative insulin sensitivity check index, but a significant increase ($p < 0.01$) in malondialdehyde level. Swimming training resulted in a considerable improvement ($p < 0.01$, $p < 0.05$) in pancreatic Sitruin1 gene and its protein expression levels, antioxidant enzymes, serum levels of albumin and metabolic parameters. In addition, histological findings indicated the beta-cells conservation.
Conclusions: This study suggested that pancreatic Sitruin1 may be a promising therapeutic target for diabetic complications.

Keywords: diabetes, insulin sensitivity, oxidative stress, sirtuin1, swimming training
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Introduction

Type 2 diabetes is a metabolic disorder characterized by insulin resistance, relative insulin deficiency and a disturbance in the metabolism of lipids and carbohydrates [1]. High levels of blood glucose, called "hyperglycemia", have been shown through several mechanisms such as the induction of inflammatory cytokines, p38 mitogen-activated protein kinase (*MAPK*), chemokines, reactive oxygen species (ROS), protein kinase C (*PKC*) and nuclear factor (*NF*)-*κB* activity, which can lead to diabetes complications [2], [3], [4], [5], [6].

Oxidative stress is believed to have a key role in the onset and development of complications resulting from diabetes [2]. Several general risk factors, as well as obesity, increased age and unhealthy diets cause oxidative reactions, decreased insulin sensitivity and damaged glucose tolerance, leading to insulin resistance. Hyperglycemia is the common consequence of both types of diabetes, which, in turn, participates in the development

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and maintenance of a general oxidative condition [7]. Many studies have demonstrated a fundamental role for oxidative stress in the induction of diabetes complications [7], [8]. Excessive production of ROS and defects in their scavenging can result in oxidative stress [6]. Previously, studies have shown that nicotinamide adenine dinucleotide phosphate (*NADPH*) oxidase had an important role in ROS generation [9].

Recent studies have recognized Sirtuin1 (*SIRT1*), an NAD-dependent class III histone deacetylase, as a member of the mammalian sirtuins family and a fundamental enzyme that mediates calorie restriction and lifespan in mammals [10], [11]. Moreover, *SIRT1* functions as a regulator of cell death/survival and stress response in mammals. *SIRT1* prevents apoptosis and repairs oxidative cellular damage and damaged DNA, thereby promoting cell survival [12], [13]. It is a well-known fact that inappropriate regulation of sirtuins proteins leads to a number of diseases, such as Bowen's disease [14], type I diabetic nephropathy, *Alzheimer's* disease, amyotrophic lateral sclerosis and nonalcoholic fatty liver disease [11]. In addition, defects and reduction in *SIRT1* activity may be related to metabolic diseases such as atherosclerosis and type 2 diabetes [15], [16]. With exercise, an *SIRT1* regulator, in rats fed high-fat diets (HFDs) has an effective impact on prolonged cell survival by improving insulin resistance and enhancing mitochondrial content [16], [17].

SIRT1 prompts insulin secretion from pancreatic beta cells (β-cells) through a glucose-dependent manner and directly activates insulin signaling pathways in insulin-sensitive organs [18]. Likewise, *SIRT1* can contribute to the improvement of insulin resistance by controlling adiponectin secretion, inflammatory parameters, gluconeogenesis and ROS levels. Moreover, overexpression of *SIRT1* and its activators has beneficial effects on glucose homeostasis and insulin sensitivity in obese mice models. These results suggest that *SIRT1* might be a new therapeutic target for the prevention of diseases related to insulin resistance, such as metabolic syndrome and diabetes mellitus [18], [19]. Physical activity and exercise are known to normalize hyperglycemia and greatly improve hyperinsulinemia in the diet-induced obese and diabetic conditions [20], [21].

Due to the useful effect of *SIRT1* on diabetes, the current study was designed to investigate the effect of swimming training on *SIRT1* expression, oxidative stress and metabolic parameters of swimming training in type 2 diabetic male rats.

Materials and methods

Twenty-eight Wistar male rats (200–250 g) were obtained from the Animal Conservation Center of Tabriz University of Medical Sciences. The rats were randomly divided into four groups control (Con), diabetic (Dia), swim trained control (Exe) and swim trained diabetic rats (Dia+Exe) (n = 7). The animals had completely free access to food and water, and at the end of the project, they experienced 12 h of light and 12 h of darkness at a temperature of 22–25 °C. This study was designed based on the protocol in accordance with the National Institutes of Health (NIH) Guide, for laboratory animals' care (Figure 1).

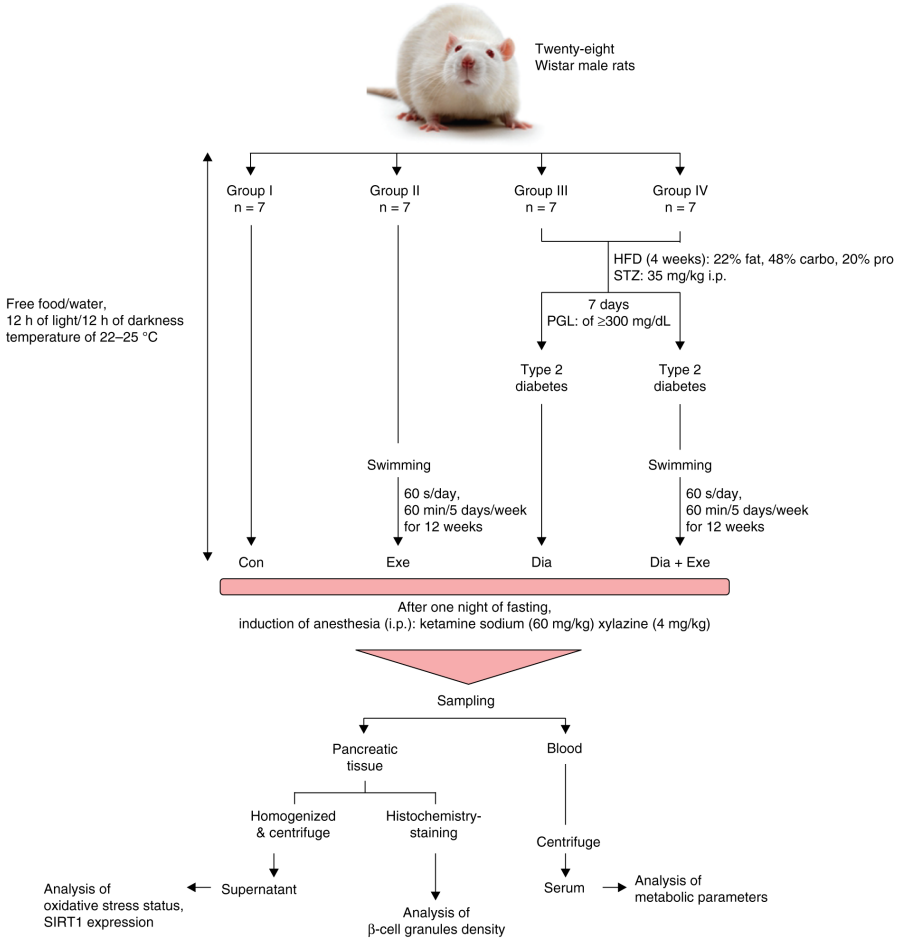

Figure 1: Schematic view of experimental procedure.
HFD, high-fat diet; STZ, streptozotocin; Carbo, carbohydrate; Pro, protein; i.p., intraperitoneal; PLG, plasma glucose; Con, control; Dia, diabetic; Exe, exercise; Dia-Exe, diabetic+exercise; *SIRT1*: Sirtuin 1.

Induction of type 2 diabetes

For induction of type 2 diabetes, a combination of both a HFD and a low dose streptozotocin (STZ) were used [22], [23]. According to this method, the animals received a HFD for 4 weeks (including 22% fat, 48% carbohydrate, 20% protein). At the end of the period, the rats received 35 mg/kg intraperitoneal (i.p.) injection of STZ. After 7 days of injection, animals with the non-fasting basal plasma glucose (PGL) ≥300 mg/dL were considered diabetic and selected for further studies.

Swimming training

The swimming training was performed in a cylindrical tank (60×100 cm) filled with water (height of 35–45 cm, 35 °C). During the training period, the training groups performed to swim for 60 min/5 days/weekly for 12 weeks, and at the end, the rats were dried with a clean towel and kept for an hour in a hot environment [24]. It should be noted that the weight of the animals was measured at the beginning and end of the intervention.

Preparation of tissue and blood samples

At the end of intervention, after one night of fasting, deep anesthesia was induced by injection of ketamine sodium (60 mg/kg) and xylazine (4 mg/kg) i.p. Pancreatic tissues were immediately removed, washed with cold saline normal serum, and then homogenized as described by Carrillo et al. [25]. After centrifuge, the obtained supernatant was stored at –70 °C until the measurement of oxidative stress status. The blood samples were collected from the inferior vena cava vein. All samples were considered for molecular analysis.

Measurement of metabolic parameters

Serum glucose and insulin levels were measured using a digital glucometer (Gluco Sure, Star, Taiwan) and rat insulin ELISA kit (Chongqing Biospes Co., Ltd, Chongqing, China), respectively. The insulin sensitivity index carried was out using the quantitative insulin sensitivity check index (QUICKI) method, $(= 1/[\log (I (0)) + \log (G (0))])$, that has a substantially stronger correlation with SI_{Clamp} ($r = 0.78$) [26]. Also, serum samples were used for evaluating levels of insulin through related enzyme-linked immunosorbent assay (ELISA) kit, total cholesterol, triglycerides (TGs), high-density lipoprotein (HDL), and albumin by using an auto blood analyzer (Bayer Corp., USA). The TGs, total cholesterol, HDL and albumin kits were obtained from Pars Azmoon Co. (Iran).

Oxidative stress status assay

The supernatant superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) activities and malondialdehyde (MDA) contents were measured by commercial kits (Randox, Italy), Cayman kit (Cayman, USA) and the barbituric acid method, respectively. The automated Abbott Biochemistry Analyzer (Alcyon 300, USA), after standardizing and validating the biochemical tests, was used for performing the tests. All the supernatants' results were divided and normalized with the supernatants' protein contents, which were assessed by a commercial kit (Parsazmun, Iran) and expressed as per mg tissue protein [24].

Measurement of the pancreatic *SIRT1* gene and protein expression levels

Real-time polymerase chain reaction (PCR) was used for measurement of the *SIRT1* gene expression in the pancreatic tissue. Isolation of total RNA and synthesis of cDNA in pancreas samples were performed as previously described [27]. Real-time PCR was performed with 2 μL cDNA and PCR master mix (2 μL forward and reverse primers, 12.5 μL SYBR Green PCR Master Mix, and 8.5 μL water) in a final reaction volume of 23 μL. After normalization to the housekeeping gene, beta-actin (β-actin), relative quantification expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method. Also, the *SIRT1* protein expression levels was measured using related ELISA kit (Chongqing Biospes Co, L td, Chongqing, China).

Evaluating the β-cells granules density in the pancreatic islets

For the demonstration of the β-cells granules in the pancreatic islets, the aldehyde-fuchsine and counterstaining with fast green were used [28]. In summary, after preparing the sections (thickness of 4 μm), deparaffinization and hydration steps were done. After embedding with alcohol, they were entered in the aldehyde-fuchsine solution. Then, the sections were distilled into water and after several washings, they were immersed into a fast-green solution. Subsequently, they were covered with lamella and prepared for microscopic examination. For assessment of histochemical-staining, the intensity of the staining was scored as 0 (<10%), 1 (10–25%), 2 (25–50%), 3 (50–75%) and 4 (75–100%) [29]. Based on the intensity of staining, the density of pancreatic β-cells granules were determine.

Data analysis and statistics

Software SPSS version 16 was used for data analysis. After confirming the normal distribution of data, the one-way analysis of variance (ANOVA) followed by Tukey's test for assessment of data were used. The significant level was considered at $p < 0.05$. Results are expressed as means ± SEM.

Results

Metabolic parameters and body weight (BW)

The fast blood glucose (FBG), fast serum insulin lipid profile and QUICK levels in groups are shown in Table 1. The FBG, TGs and cholesterol were significantly increased in the diabetic groups compared to non-diabetic

group ($p < 0.05$; $p < 0.01$), and swimming training induced a significant decrease in these parameters in diabetic treated rats ($p < 0.05$; $p < 0.01$). Also, the QUICKI rate and serum levels of HDL decreased in diabetic rats compared to the corresponding control group ($p < 0.05$). While, these parameters were significantly improved in the diabetic training group compared to the diabetic group ($p < 0.01$). In addition, the results of this study showed that after diabetes induction (immediately before intervention), the weights of the diabetic group were significantly lower than the control group ($p < 0.01$). While, swimming training significantly prevented weight loss in diabetic rats at the end of intervention ($p < 0.05$).

Table 1: The effect of swimming training on metabolic parameters: FBG, fast serum insulin, QUICKI rate, lipid profile, TG, cholesterol, HDL and BW in the studied groups.

Groups	Con	Dia	Exe	Dia-Exe
FBG, mg/dL	105.43 ± 1.67	421.14 ± 23.40[a]	106 ± 23.40	348.71 ± 28.83[b,c]
Insulin, μU/mL	2.95 ± 0.37	9.8 ± 0.65[a]	2.75 ± 0.39	6.77 ± 0.52[a,c]
QUIKI	0.40 ± 0.009	0.27 ± 0.02[a]	0.40 ± 0.01	0.31 ± 0.01[a,c]
Cho, mg/dL	60.42.00 ± 10.04	89.28 ± 5.67[a]	56.28 ± 4.71	69.14 ± 3.32[c]
TG, mg/dL	48.42 ± 5.44	90.42 ± 4.64[b]	43 ± 6.35	67.28 ± 5.86[b,c]
HDL, mg/dL	46.28 ± 3.40	19.58 ± 3.06[a]	51.14 ± 3.40	33.00 ± 2.53[b,c]
BW (g, immediately before intervention)	246 ± 2.9	270 ± 4.9[a]	239 ± 4.18	275 ± 5.6[b,c]
BW (g, at the end of intervention)	311 ± 3.9	191 ± 4.2[b]	307 ± 5.1	215 ± 6.3[b,c]

Data are shown as mean ± SEM.
[a]$p < 0.01$ vs. Cont group; [b]$p < 0.05$ vs. Con group; [c]$p < 0.05$, vs. Dia group.
FBG, fast blood glucose; QUICKI rate, quantitative insulin sensitivity check index, lipid profile; TG, triglyceride; cholesterol; HDL, High-density lipoprotein; BW, body weight; Con, control; Dia, diabetic; Exe, swim trained control; Dia+Exe, swim trained diabetic group.

Oxidative stress injury and antioxidant enzymes

The MDA levels in pancreatic tissues were elevated in the diabetic groups compared to the control group ($p < 0.01$). The swimming training significantly reduced the MDA levels in the pancreas of the diabetic exercised group ($p < 0.01$). Table 2 shows that SOD activity was significantly decreased by diabetes ($p < 0.01$). Also, non-diabetic exercised rats have greater SOD activity in the pancreatic tissues than the control group (not significant). In addition, the activity of SOD was significantly increased in the diabetic training group compared to the diabetic group ($p < 0.01$).

Table 2: The effects of swimming training on oxidative stress status: MDA, GPX, SOD, CAT and serum levels of albumin in the studied groups.

Groups	Con	Dia	Exe	Dia-Exe
MDA, nmol/g protein	24.98 ± 2.04	67.23 ± 5.78[a]	27.28 ± 5.78	46.22 ± 2.60[a,b]
GPX, U/g protein	1.25 ± 0.06	0.50 ± 0.06[a]	1.12 ± 0.06	0.78 ± 0.03[a,b]
SOD, U/mg protein	0.36 ± 0.02	0.14 ± 0.01[a]	0.39 ± 0.04	0.31 ± 0.01[a,b]
CAT, nmol/min/mg protein	0.72 ± 0.03	0.33 ± 0.04[a]	0.89 ± 0.03	0.46 ± 0.04[a]
Serum albumin, ng/dL	2.22 ± 0.13	1.39 ± 0.48[a]	2.18 ± 57	2.18 ± 57[b]

Data are shown as mean ± SEM, [a]$p < 0.01$ vs. Con group; [b]$p < 0.01$, vs. Dia group.
MDA, malondialdehyde; GPX, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; Con, control; Dia, diabetic; Exe, swim trained control; Dia+Exe, swim trained diabetic rats.

As it is shown in Table 2, the rate of GPX activity in the diabetes and diabetes training groups significantly decreased ($p < 0.01$) in the pancreatic tissue compared to the control group ($p < 0.01$). Also, diabetes significantly reduced ($p < 0.01$) the CAT activity. However, CAT activity in the pancreatic tissues in the exercise group was higher in comparison with the control group although this difference was not significant. Also, swimming training had no significant effect on CAT activity in the diabetes condition. In addition, the serum levels of albumin significantly reduced in the diabetic group compared to the control group ($p < 0.01$). While, swimming training significantly reversed this parameter ($p < 0.01$).

Expression levels of the *SIRT1* gene and protein in the pancreatic tissue

The *SIRT1* gene and protein expression levels in the pancreatic tissue are shown in Figure 2 A, B. Comparison of the four studied groups indicates a considerable decrease in the *SIRT1* gene and protein expression levels through diabetes ($p < 0.05$, $p < 0.01$). Swimming training caused a significant increase in the expression of the *SIRT1* gene and protein in the training and diabetic training groups compared with the comparable groups ($p < 0.05$, $p < 0.01$).

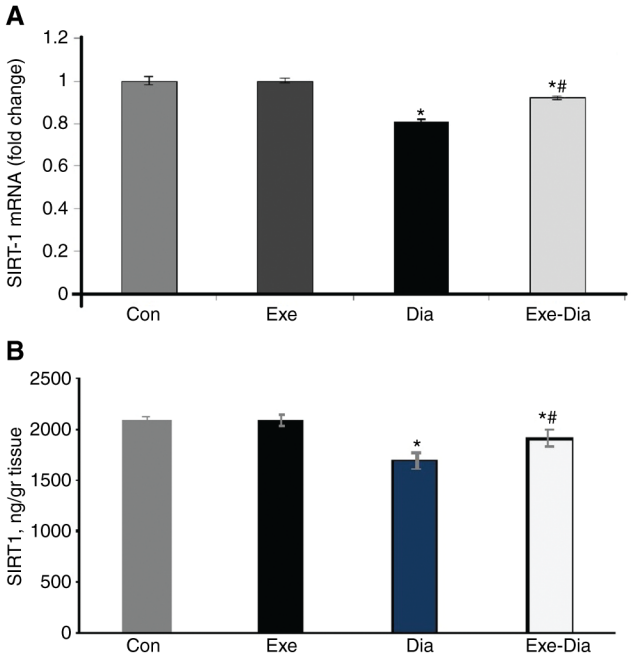

Figure 2: The effect of swimming training on (A) *SIRT1* gene and (B) *SIRT1* protein expression in the studied groups: control (Con), diabetic (Dia), swim trained control (Exe) and swim trained diabetic rats (Dia+Exe).
Results indicated a significant decrease in the *SIRT1* gene and *SIRT1* protein expression through Dia rats ($p < 0.05$, $p < 0.01$), and swimming mediated a considerable improvement ($p < 0.05$, $p < 0.01$) in the expression of *SIRT1* in the Exe and Dia-Exe rats compared with the two other groups (data are shown as mean ± SEM, *$p < 0.05$ vs. Con group, #$p < 0.05$ vs. Dia group, **$p < 0.01$ vs. Con group, ##$p < 0.01$ vs. Dia group).

β-cell granules' density in the pancreatic islets in the diabetic condition

Investigation of pancreatic tissue by the aldehyde-fuchsine and counterstaining with fast green showed that the β-cell granules were stained deep purple and showed clearly against the colorless cytoplasmic background. Diabetes significantly reduced the pancreatic β-cells granules density (too pale), as well as the extent of the islands of Langerhans in the diabetic group reduced compared to the control group ($p < 0.01$). According to the results, β-cells granules density for the diabetic group and the control group were 1.5 ± 0.22 and 3.83 ± 0.4, respectively. Also swimming training, significantly increased pancreatic β-cells granules density in the islands of Langerhans in the diabetic training group compared to the diabetic group ($p < 0.01$). According to the results, β-cells granules density for training control group and diabetic training were 4 ± 0.00 and 3.33 ± 0.21, respectively (Figure 3 and Figure 4).

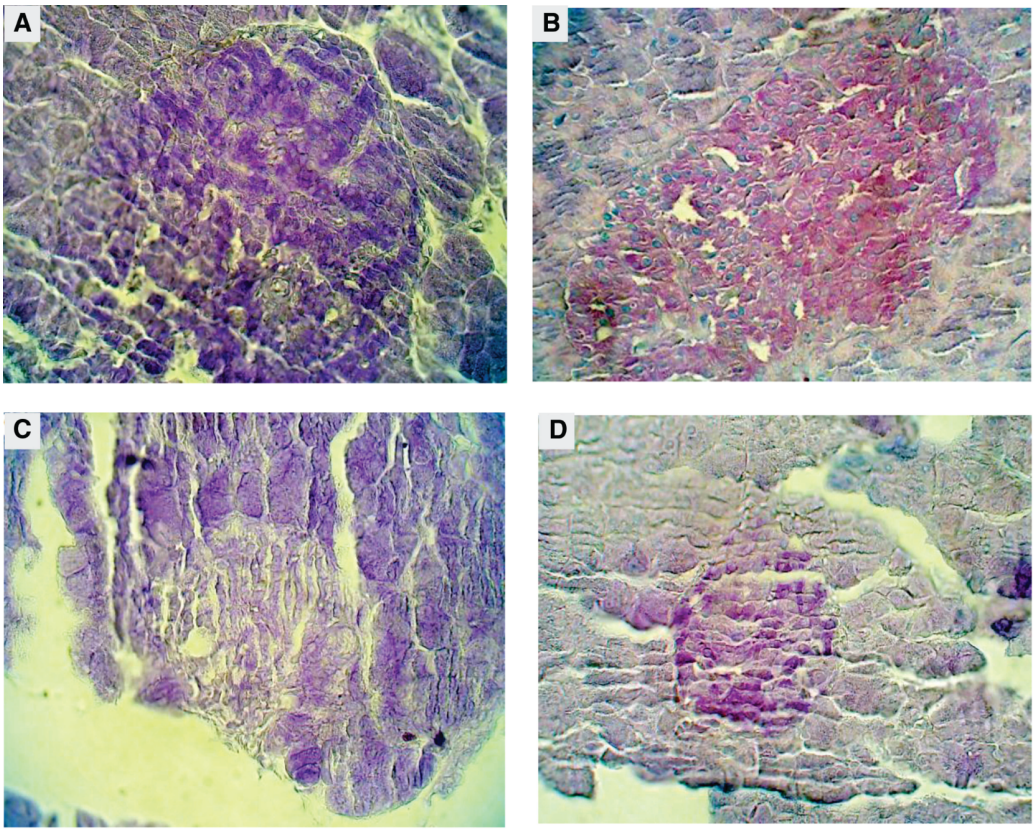

Figure 3: Histochemical detection of pancreatic β-cells granules (staining by aldehyde-fuchsine and counterstaining with fast green) in the studied groups: control (Con), diabetic (Dia), swim trained control (Exe) and swim trained diabetic rats (Dia+Exe).
(Data are shown as Mean ± SEM, **P< 0.01vs Con group, ##P< 0.01 vs Dia group).
Purple stained tissues show histochemistry-stained β-cell granules.

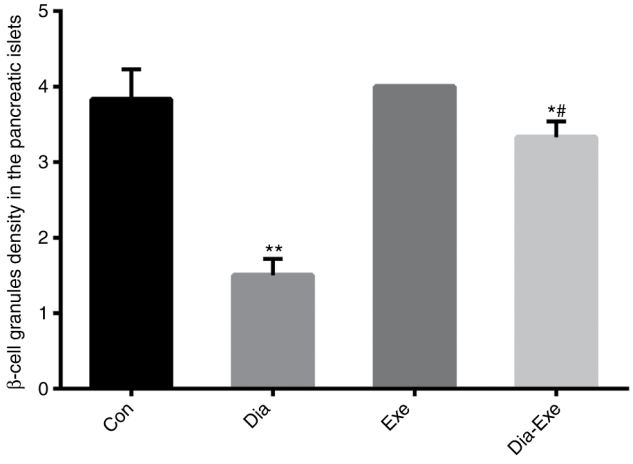

Figure 4: Effect of swimming training on the pancreatic β-cells granules density in the studied groups: control (Con), diabetic (Dia), swim trained control (Exe) and swim trained diabetic rats (Dia+Exe).
Diabetes reduced the pancreatic β-cells granules density ($p < 0.01$) and the extent of the islands of Langerhans compared to the Con group. Swimming training significantly elevated ($p < 0.01$) pancreatic β-cells granules density in the Dia-Exe group compared to the Dia rats. (Data are shown as mean ± SEM, **$p < 0.01$ vs. Con group, ##$p < 0.01$ vs. Dia group).

Discussion

Findings of the study revealed that swimming training could increase the pancreatic expression of the *SIRT1* gene and the *SIRT1* protein, and beyond that, improve pancreatic oxidative stress injuries in the type 2 diabetes condition. One reason is that the *SIRT1* gene and protein expression were reduced in diabetic pancreas in type

2 diabetes induced by a HFD and low dose STZ. Another reason is that swimming training by overexpression of *SIRT1* relieved pancreatic oxidative stress injuries, improved the conservation of β-cells, and consequently ameliorated metabolic function and insulin sensitivity in the diabetic rats.

This study showed that mRNA and protein expression of pancreatic *SIRT1* were significantly reduced in the HFD-STZ-induced type 2 diabetic rats. Previous studies revealed that the *SIRT1* gene and protein expression decreased in STZ-induced type 1 diabetic animals [30]. Accordingly, it is believed that *SIRT1* might relatively decrease by hyperglycemia, which is a common feature between type 1 and type 2 diabetes [30], [31]. Moreover, consistent with our results, previous studies showed that *SIRT1* decreased in the liver, muscle and adipose tissue of HFD-fed rats. From their findings, it appears that the use of HFDs can affect these variables. In this regard, Lee et al. showed that *SIRT1* protected cells against cytokines and increased NO production in pancreatic β-cells [32]. It can be also mentioned that *SIRT1* can also regulate inflammatory responses. It appears that in inflammatory conditions such as diabetes, the increased expression of inflammatory cytokines can reduce the expression of *SIRT1* [33]. Moreover, our findings demonstrated that swimming training significantly increased the expression of pancreatic *SIRT1* in the exercise-diabetic group compared with the diabetic group. Meanwhile, inconsistent with this study, Ferrara et al. showed that long-term exercise through induction of *SIRT1* expression could have an antioxidant effect in heart and adipose tissues of rats [34].

Moreover, our finding showed that 12 weeks of regular swimming training improved lipid profile and glucose intolerance in the type 2 diabetes condition. These effects could be attributed to *SIRT1*. In addition to other effects of *SIRT1*, although inconsistent with our results, it has been shown that *SIRT1* is also involved in protection against metabolic syndrome. However, the suggested involvement of *SIRT1* in protection against metabolic syndrome is commonly based on the use of chemical activators of *SIRT1*. In particular, two studies revealed useful effects of resveratrol in mice on a HFD, such as the improved resistance of insulin, alleviation of steatosis and survival enhancement of β-cells [32]. Lately, *SIRT1* activators distinct to resveratrol have been displayed to have useful effects on mitochondrial function and metabolic conditions in overweight rodents [35]. Swimming training, that was used as an intervention in the present study, resulted in an improvement in insulin sensitivity that occurred in concert with an increase in the expression of *SIRT1* [36]. Regarding the results of this study, regular long-term swimming training improved the insulin sensitivity and metabolism of lipids and glucose by increasing the expression of *SIRT1* and reducing oxidative stress. *SIRT1* through several mechanisms can contribute to regulating glucose homeostasis, controlling insulin excretion, protecting pancreatic β-cells, improving insulin resistance through modulation of post-insulin receptor signaling diminishing inflammation, lipid recruiting and secreting adiponectin, controlling fatty acid oxidation and mitochondrial biogenesis [37], and regulating hepatic glucose production and circadian rhythms in pancreatic-cells, skeletal muscles, adipose tissues, monocytes/macrophages and the liver [38]. Bordone et al. proposed that *SIRT1* regulated insulin secretion from pancreatic β-cells. Overexpression of *SIRT1* in β-cells by repressing uCP2 increases ATP production through uncoupling of ATP synthesis from glucose, and a raised ATP level causes cell membrane depolarization and Ca2+-dependent exocytosis. However, β-cells produce less ATP in response to glucose in *SIRT1* deficient mice than in normal mice. Moreover, *SIRT1* by deacetylating FoxO1 preserves insulin secretion and reduces the death of β-cells in vivo [38]. By SIRT1 overexpressing in β-cell-specific mice, the enhanced level of *SIRT1* in pancreatic β-cells rallies glucose tolerance and increases insulin secretion in response to glucose [19], [37]. Furthermore, *SIRT1* activity diminishes with aging to reduced systemic NAD biosynthesis, which leads to the failure of insulin secretion in β-cells in response to glucose [39]. These results demonstrated that *SIRT1* regulated glucose-ATP signaling and insulin secretion from pancreatic β-cells. Thus, swimming training by increasing the expression and activity of *SIRT1* can be considered as a potential therapeutic target for improvement of insulin-sensitivity and following type 2 diabetes [40].

This study revealed that 12 weeks of regular swimming training not only reduced the production of MDA, but also augmented the activity of antioxidant enzymes in the diabetic pancreatic tissue and serum levels of albumin. As estimated, diabetes enhances the production of lipid peroxidation as an index of oxidative stress and reduces antioxidant protection in pancreas as proved by reduced SOD, GPX and CAT activity. Overproduction of ROS induced by hyperglycemia has a vital role in the development and worsening of diabetic complications [8], [9], [11]. The exact mechanism for regulation of oxidative stress in diabetes is yet to be understood. It has been suggested that sirtuins are involved in stress resistance and metabolic processes. Researchers have proposed an important role for the mammalian *SIRT1* in the suitable cellular response to metabolic stress processes such as excessive nutrition or nutrient deprivation [41]. In fact, impacts of SIRTs are believed to be useful. *SIRT1* limited or excessive expression in mouse models has revealed metabolic benefits of SIRT1 activation. Recently, it has been proposed that *SIRT1* is a main regulator of many factors prompting obesity and type 2 diabetes [42] and also a potential target for the management and treatment of metabolic syndrome [42], [43]. *SIRT1*, as a member of a class defined as histone deacetylases (class III HDAC), is the most widely studied type of sirtuins. *SIRT1*, due to deacetylation activity, can be considered as an essential regulator of cell protection and survival under different stress situations by deacetylating the p53 and forkhead transcription factors (a critical

component of NADPH oxidase), NADPH oxidase is a central source of superoxide anion production [44]. A pervious study demonstrated that overexpression of *SIRT1* decreased myocardial gp91phox expression and MDA content in diabetic I/R rats. The MDA content in tissue usually reflects the level of oxidative stress [45]. Moreover, *SIRT1* administration reduced MDA formation in the diabetic I/R heart tissue. Previous researches have described that *SIRT1* enhanced the expression of Mn-SOD (a mitochondria-specific isoform of SOD) and relived mitochondrial oxidative injuries [46]. These findings proposed that overexpression of *SIRT1* may relive both mitochondria and NADPH oxidase-derived ROS production. Moreover, oxidative stress disturbs the insulin signaling pathway and results in the development of insulin resistance in type 2 diabetes [11]. In the hyperglycemia condition, other metabolites comprising free fatty acid and various cytokines such as tumor necrosis factor-alpha (*TNF-α*) lead to the excess production of ROS by mitochondria. ROS initiates the activation of serine/threonine kinases, for example, apoptosis signal-regulating kinase (ASK) 1, Jun N-terminal kinase (JNK) and IκB kinase (IKK), which consecutively enhance the serine phosphorylation of insulin receptor substrate 1 (IRS-1) and reduce the tyrosine phosphorylation of IRS-1, leading to insulin resistance and inflammation (oxidative stress linked to inflammation) [47]. Therefore, decreased mitochondrial oxidative capacity and oxidative stress can lead to insulin resistance. *SIRT1* mediates mitochondrial biogenesis by PGC-1 deacetylation, in addition to the excess expression of anti-oxidative enzymes such as Mn-SO [48], thereby reducing oxidative stress from the damaged mitochondria. Furthermore, FOXO3a is deacetylated by *SIRT1* and translocated to the nucleus, which results in the excess production of CAT and protection against oxidative stress [48], [49]. In pancreatic β-cells, *SIRT1* affects insulin secretion positively and protects them from oxidative stress and inflammation. In addition, *SIRT1* regulates the metabolic pathway and oxidative stress through modulation in insulin signaling [42]. Another study on skeletal muscles suggested an improvement in the SIRT1 protein content through endurance exercise [50]. Yet another research team showed that exercise training through significantly elevated the *SIRT1* activity could counteract impairment of age-related systems [34]. Throughout the body, *SIRT1* is expressed and regulates both cellular survival and longevity during acute and long-term injuries, which involve both oxidative stress and cell metabolism [51]. In agreement with our findings, other studies showed that long-term exercise through induction of *SIRT1* expression could have oxidative effects on the heart and fat tissues of rats [34], [52].

As mentioned, serum levels of albumin significantly decreased in the diabetic group compared to the other groups. While swimming training significantly increased serum albumin levels in the diabetic training group compared to the diabetic group. It has been indicated that albumin has many bioactive functions; for example, it can regulate the plasma osmotic pressure, bind and transport several endogenous or exogenous compounds, and extracellular antioxidant defenses. Albumin has a unique biochemical structure, which provides most of the antioxidant properties of albumin. The protein has antioxidant properties such as binding copper tightly and iron weakly, scavenging free radicals, for example, hypochlorous acid (HOCl) and peroxynitrite (ONOOH) and providing a thiol group (-SH) [53]. Moreover, this study showed that swimming training had no significant effect on pancreatic MDA levels in control rats after exercise. It is specified that the amount of lipid peroxidation depends on the intensity, duration and mode of exercise. For example, a short-term high-intensity exercise can increase the speed of lipid peroxidation induced by oxidative stress. While the chronic repetition of exercise can diminish the lipid peroxidation process and prevent tissue from being damaged [54]. Furthermore, at the end of the intervention the non-diabetic exercised rats had greater SOD, and CAT activity (was non-significant) in the pancreatic tissues as compared to the control group. While swimming training had no significant effect on pancreatic GPX levels in comparison to the control group. The association between exercise and oxidative stress is not always negative. Long-term exercise, i.e. exercise training, may compensate for the oxidative stress through an adaptation of the antioxidant and repair systems. This might lead to a diminished level of an oxidative injury and an augmented resistance to oxidative stress. In fact, the impact of training on oxidative stress depends on training characteristics (i.e. intensity, type, volume, duration [55]).

In the present study, histopathological findings showed that pancreatic β-cell granules were unchanged in the control and swim-trained control groups. Diabetes had extensive alterations in the islands, reduced β-cells granules. Swimming training, due to antioxidant properties increased the number of β-cells and granules of β-cells compared to the diabetic group. Previous studies showed that the use of antioxidants, such as herbal antioxidants or some drugs, led to β-cell regeneration and β-cell survival, improvement of the diameter and the number of the islands of Langerhans, and finally improved side effects of diabetes in the pancreatic tissue [56]. Protective effects of swimming training on β-cells granules and the number of the islands of Langerhans can be attributed to the overexpression of *SIRT1*. Thus, *SIRT1*, by preventing apoptosis caused by stresses, containing DNA damage and oxidative stress led to β-cell survival [47]. Moreover, the results of this study showed that after diabetic induction (immediately before intervention); the weight of the diabetic group was significantly lower than that of the control group. While swimming training significantly prevented weight loss in diabetic rats at the end of the intervention. However, the limitation of this study is that BW gain, food/water intake, body composition or energy consumption during experiments were not measured. In future studies, these variables

are required to be included. Based on previous studies and the findings of this study, *SIRT1* is one the main factors of controlling oxidative stress, which functions based on the mentioned mechanisms or other unknown mechanisms. Thus, more studies are needed to be conducted in this field.

Conclusion

The current study revealed that swimming training by overexpression of *SIRT1* could protect β-cells from death, and improve diabetes-exacerbated oxidative stress injury, insulin sensitivity, and metabolic parameters. These findings suggested that the content of expression of *SIRT1* in a pancreatic tissue may be a promising novel therapeutic target for diabetic complications.

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Author Statement

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