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The effect of swimming training on oxidative stress, SIRT1 gene expression, and histopathology of hepatic tissue in type 2 diabetic rats

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Original Article

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Introduction: Elevated oxidative stress in type 2 diabetes mellitus (T2DM) has been proposed as one of the major risk factors in pathophysiology of several organ damages including liver tissue. *Materials and methods:* In this study, we evaluated the effect of swimming training on hepatic oxidative markers, SIRT1 gene expression, and histological alterations in T2DM. Twenty-eight male Wistar rats were randomly assigned into four groups ($N = 7$): control, exercise, diabetic, and diabetic + exercise. One week after the induction of T2DM, rats were subjected to swimming (60 min/5 days a week) for 12 weeks. At the end of the experiment, oxidative markers (SOD, GPx, CAT activities, and MDA level) and SIRT1 gene expression were measured in the liver by special kits and RT-PCR, respectively. Hematoxylin–eosin statins were used for histological alterations. *Results:* Swimming training attenuated MDA levels and enhanced SOD, GPx, and CAT activities in the liver of diabetic animals. Furthermore, swimming training restored the expression of SIRT1 in T2DM. Histopathological finding of the hepatic tissue confirmed a protective role for swimming training in diabetic rats. *Conclusion:* Our findings indicate that swimming training attenuates oxidative stress probably by upregulation of SIRT1 in the liver of type 2 diabetic rats.

INTRODUCTION

Due to urbanization and prosperous life, the prevalence of type 2 diabetes mellitus (T2DM) is increasing worldwide (Franz et al., 2015). It is triggered by insulin resistance, and 80% of people in the community suffer from T2DM. It is known that diabetes is greatly accompanied by several disorders, such as chronic kidney disease, cardiovascular disease, myocardial infarction, and NAFLD as well as both non-fatal and fatal outcomes (Dart et al., 2014).

Previously, it was demonstrated that lipid metabolism disorders and insulin resistance considered as pathophysiological outcomes in diabetic liver (Smith & Adams, 2011). These abnormalities lead to accumulation of triglycerides in the liver, one of the key factors in the progression of T2DM, which is called NAFLD and ultimately lead to cirrhosis and liver fibrosis (Smith & Adams, 2011). It has been shown that fat accumulation as the first sign of liver damage can make the liver susceptible to oxidative stress (Zheng et al., 2018). Oxidative stress, due to increased production of reactive oxygen species (ROS) and decreased hepatic antioxidant defense system, plays a major role in the pathophysiological mechanisms of diabetes linked to impairment in glucose and lipid metabolism (Tucker et al., 2008). Therefore, reducing oxidative stress could limit the toxic effects of peroxides and free radicals in several organ damages including liver tissue (Zheng et al., 2018). In this regard, there are some studies on the potential of antioxidant therapy in liver including vitamin E, N-acetylcysteine, betaine,

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and probucol supplementation (Al-Busafi et al., 2012). Instead, chronic physical exercise is an important non-drug therapy to prevent and improve diabetes, because of its useful effects on antioxidant enzyme expression and activity in several tissues in healthy and diabetic conditions (Chiş et al., 2016; Mohammadi et al., 2013; Naderi et al., 2015b). However, there are a little data on the impact of exercise training on T2DM-induced oxidative stress in the liver; even so, chronic exercise seems to improve insulin sensitivity, glucose, and lipid metabolism of the liver and prevent high fat diet (HFD)-induced NAFLD via the regulation of fatty acid transport, lipogenesis, and β-oxidation-associated genes (Wu et al., 2015; Yi et al., 2013).

SIRT1 is a class III protein deacetylase, emerging as an important signaling molecule in the prevention of oxidative damage (Yamazaki et al., 2009). It has previously been demonstrated that SIRT1 is an important agent in regulating metabolic and endocrine signals and also has extensively a key role in regulating the mammalian cell life span, insulin secretion, and glucose/lipid metabolism (Song et al., 2011). It was reported that in the liver, SIRT1-knockout was associated with insulin resistance, impairment in plasma glucose, free fatty acid, cholesterol levels and may explain the increased risk of T2DM in the Chinese Han population (Han et al., 2015; Lovis et al., 2008). Therefore, SIRT1 may have a therapeutic effect following disturbances in metabolic pathways in T2DM and inhibit the development of T2DM (Szkudelski & Szkudelska, 2015).

However, in contrast to the attention given to improvement of antioxidant defense following exercise in many organs, the consequences of swimming training on oxidative stress in type 2 diabetes in the liver remain poorly understood. Even so, there is no reported study in the literature examining whether the beneficial effect of swimming training on oxidative stress can be mediated via the activation of SIRT1 in diabetic liver. Considering the above, this study was conducted with the following aims: (a) to investigate whether swimming training could attenuate oxidative stress in the liver with T2DM and (2) to evaluate the potential role of SIRT1 in mitigating oxidative stress following swimming training.

MATERIALS AND METHODS

Animals and experimental design

Twenty-eight male Wistar rats (200–250 g) aged 90 days were housed four per cage and kept in a 12-h light–dark cycle in an air-conditioned constant temperature (22 ± 1 °C) room. Food and water were made available ad libitum. Animal care was in accordance with the National Institute of Health Guide for the Care of the Animal and Human Ethical Committee of Tabriz Medical Sciences University. Animals were randomly divided into four experimental groups ($N = 7$):

Control.

Exercise: Rats were subjected to swimming (60 min/5 days a week) for 12 weeks.

Diabetic: T2DM was induced by HFD and a low dose of streptozotocin [STZ; 35 mg/kg, intraperitoneally (i.p)].

Diabetic + exercise: Animals that received HFD and a low dose of STZ subjected to swimming (60 min/5 days a week) for 12 weeks.

Induction of T2DM

In order to induce T2DM, rats received HFD regimens consisting 22% fat, 48% carbohydrates, and 20% protein for 4 weeks. The composition and preparation of HFD was as reported previously (Ghiasi et al., 2015).

After 4 weeks of dietary usage, the rats were injected i.p with a low dose of STZ (35 mg/kg). After 3 days of injection, animals with the non-fasting plasma glucose of >300 mg/dl (16.67 mmol/L) were assumed to be diabetic and were included in the experiments.

Swim training protocol

To allow adaptation, swimming training was confined to 5 min on the first day (3 days after injection of STZ) and enhanced by 5 min each day. Rats were subjected to 60 min/day of swimming, 5 days/week for 12 weeks (Ghiasi et al., 2015). Swimming exercise was performed in a rectangular tank (100 × 60 × 80 cm) with water maintained at 34–36 °C. After swimming, rats were dried and maintained in a warm place for 1 h, and then returned back to their houses.

Fasting blood glucose and serum insulin levels

At the end of the training protocol, fasting plasma glucose and serum insulin levels were measured. Blood samples were collected from the tip of tail and plasma glucose levels were measured by a digital glucometer (Gluco Sure, Star, Taiwan). Quantitative estimation of serum insulin was determined by rat insulin ELISA kit (Bioseps, Co. Ltd., China).

Tissue processing and homogenate preparation

After 12 weeks of exercise, the deep anesthesia was induced by injection of ketamine sodium (60 mg/kg) and xylazine (4 mg/kg) i.p. Livers were taken out, frozen in liquid nitrogen, and kept at deep freeze (−70 °C) for later measurements. For antioxidant activities measurement, the liver samples were homogenized in 1.15% KCl solution. The homogenates were centrifuged at 1,000 rpm for 1 min at 4 °C. The tissue homogenate was then stored at −20 °C for determination of lipid peroxides and catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPx) levels (Paglia & Valentine, 1967).

Determination of antioxidant enzymes

SOD activity was determined using a commercial kit (RANSOD, Randox co., Antrim, UK) according to Delmas-Beauvieux et al. (1996). Spectrophotometer was used for determination SOD activity at 505 nm (Pharmacia Biotech, Cambridge, England). In this method, xanthine and xanthine oxidase were used to produce superoxide radicals that react with 2-(4-iodophenyl)-3 (4-nitrophenol)-5-phenyl

tetrazolium chloride (ITN) to generate a red formazan dye. Concentrations of substrates were 0.05 mmol/L for xanthine and 0.025 mmol/L for ITN. SOD activity was measured by the degree of the hindrance of this reaction. After computing the percent of inhibition using the related formula, SOD activity value was computed by comparing with the standard curve and was exhibited as U/mg protein in the liver. GPx activity was determined using the commercial kit (RANSEL, Randox co.) according to the method of Paglia and Valentine. GPx catalyzes the oxidation of glutathione (at a concentration of 4 mmol/L) by cumene hydroperoxide. In the presence of glutathione reductase (at a concentration = 0.5 units/L) and 0.28 mmol/L of nicotinamide adenine dinucleotide phosphate (NADPH; reduced form of $NADP^+$), oxidized glutathione is immediately converted to the reduced form with participant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm (37 °C) was measured using a spectrophotometer (Pharmacia Biotech), and then GPx concentration was calculated by the correlated formula (Mohammadi et al., 2013; Naderi et al., 2015a).

MDA and CAT assessments

Thiobarbituric acid reactive substances (TBARS) method was used for determining MDA levels (Naderi et al., 2015a). CAT activity was measured using the method of Aebi (1984). Measurement was based on the dissociation rate of H2O2 in 240 nm at 20 °C. Liver homogenate aliquots were centrifuged at 1,000 × *g* for 10 min at 4 °C. The adequate amount of supernatant (60 µl equivalent to 1.5 mg tissue wet weight) was added to a reaction mixture that contained 0.002% Triton X-100, 0.1 mM EDTA, 0.5 M potassium phosphate buffer, and 15 mM H_2O_2 in 1 ml final volume at pH 7.0. Activity was calculated within the initial 15 s decomposition rate. The initial absorbance was recorded (A240 at $t = 0$). Then, it was mixed well with a plastic paddle and a decrease in absorbance was recorded again for about 15 s (A240 at $t = 15$), and CAT activity (K) was calculated by the related formula and was expressed as U/mg protein: $K = 0.153$ (log A240 at $t = 0$/A240 at $t = 15$).

Real-time PCR

Total RNA was extracted from the liver tissue using miRCURYTMRNA isolation kit (Exiqon, Vedbaek, Denmark) in accordance with manufacturer's instructions (Biyashev et al., 2012; Lässer et al., 2012). RNA concentration and purity were estimated by Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington, DE 19810, USA) and characterized by TAE-agarose gel electrophoresis. Complementary DNA (cDNA) synthesis from total RNA extracts was performed using universal cDNA synthesis kit. In brief, total RNA was reversed to cDNA using a poly(T) primer with a three degenerate anchor and a five universal tag (Exiqon, Vedbaek, Denmark). Each cDNA was used as a template for RNA quantitative real-time PCR by using SYBR Green master mix (Exiqon).

The following primers were used: SIRT1 forward, 5′-GTG AGA AAA TGC TGG CCT AA-3′; SIRT1 reverse, 5′-CTG CCA CAG GAA CTA GAG GA-3′; beta Gusb sense: 5′-GGCTCGGGGCAAATT-3′; and beta Gusb antisense: 3′-GGGGCAGCACGAT-5′. Sequences were derived from GenBank. Furthermore, the primers were checked using a Gene Runner software (Syngene, Cambridge, UK). We chose beta Gusb as an internal control for quantitative real-time PCR. Relative quantitative expressions of these genes were assessed through the $2^{-(\Delta\Delta Ct)}$ method. The results were expressed as the fold-difference to the relevant controls.

Histological evaluation

A part of liver tissue was isolated and then fixed in 10% buffered-formalin solution, dehydrated in ascending grades of alcohol, and embedded in paraffin. We prepared sections of 5 µm and stained with hematoxylin–eosin (H&E) for the assessment of histopathological changes under a light microscope (Olympus BH-2, Tokyo, Japan) in a blinded manner. Liver tissues were evaluated in terms of hepatic sinus structure, congestion, vacuolization, degeneration, pyknosis of the nuclei, fragmentation of plasma membrane, and inflammatory cell infiltration. Histological alterations were scored on a 4-point scale: (–) none, (+) mild, (++) moderate, and (+++) severe damage.

Statistical analysis

After ensuring a normal distribution of data, they were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The significant level was set at $p < .05$. The results are expressed as means ± *SD*.

RESULTS

Plasma glucose and serum insulin levels

Following 12 weeks of training, blood glucose and serum insulin levels were measured in overnight-fasted rats. There are significant differences among the groups for blood glucose using ANOVA test ($f = 139.861$, $df = 3$, $p < .001$). As shown in Fig. 1a, fasting blood glucose in diabetic and diabetic + exercise groups were significantly ($p < .001$) increased compared with the control group. However, swimming training decreased blood glucose in diabetic animals in comparison to the diabetic group ($p < .05$). Similarly, the insulin levels among the groups were significantly different using ANOVA test ($f = 44.734$, $df = 3$, $p < .001$). In Fig. 1b, the serum concentration of insulin was elevated ($p < .001$) in the diabetic group compared to that in the control group, whereas it was lowered in diabetic + exercise group ($p < .001$) relative to the diabetic group. Furthermore, insulin level in diabetic + exercise group was significantly higher than control group ($p < .001$).

Lipid peroxidation

There are significant differences among the groups for MDA levels using ANOVA test ($f = 95.024$, $df = 3$, $p < .001$). According to Table 1, diabetic rats underwent a significant increase in the level of MDA ($p < .001$) in liver homogenized in comparison with the control group. However, swimming training in diabetic animals significantly

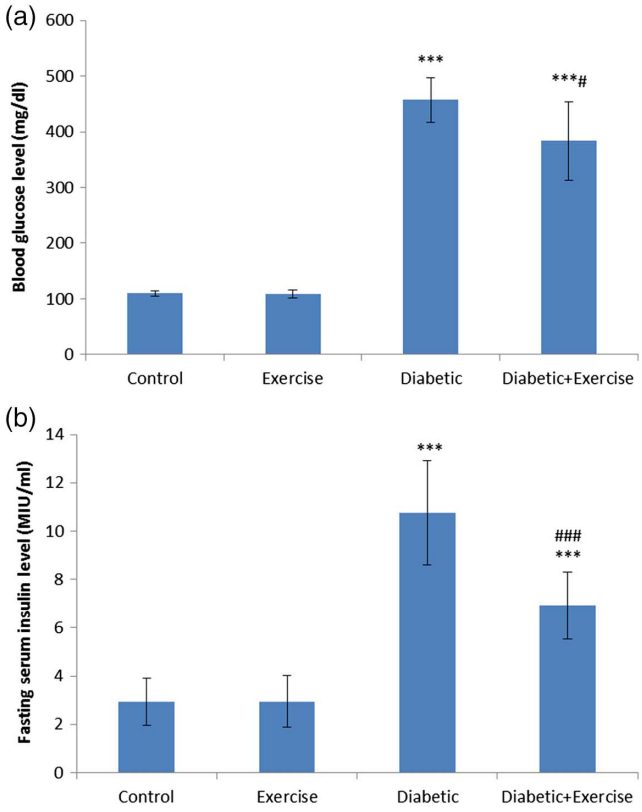

Fig. 1. (a) Effect of swimming training on blood glucose at the end of experiment. (b) Effect of swimming training on insulin levels at the end of experiment. Data are represented as the mean ± *SD* ($n = 7$). ***$p < .001$ vs. control group. $^{\#}p < .05$. $^{\#\#\#}p < .001$ vs. diabetic group

($p < .001$) decreased MDA level in the liver tissue compared to the diabetic group. Furthermore, MDA level in diabetic + exercise group was significantly higher than control group ($p < .001$).

Antioxidant enzymes

There are significant differences among the groups for SOD, CAT, and GPx using ANOVA test ($f = 48.890$, $f = 19.43$, $f = 21.74$ respectively, $df = 3$, $p < .001$). Table 1 also demonstrates the values determined for the antioxidant enzyme levels (SOD, CAT, and GPx) in the hepatic homogenates of all groups of rats. The findings indicated that, compared to the control group, the diabetic rats underwent significant decreases in the SOD, CAT, and GPx ($p < .001$) levels in the hepatic homogenized. Twelve-week swimming training reversed these values, whereas only post-training SOD ($p < 0.05$) was significant. Furthermore, SOD, CAT ($p < .01$), and GPx ($p < .001$) levels in diabetic + exercise group were significantly lower than control group.

SIRT1 gene expression

There are significant differences among the groups for SIRT1 gene expression using ANOVA test ($f = 35.271$, $df = 3$, $p < .001$). As shown in Fig. 2, hepatic SIRT1 mRNA level, which measured by RT-PCR, was significantly ($p < .001$) decreased in diabetic group compared with the control group. However, this suppression was ameliorated by 12 weeks of swimming training ($p < 0.001$). Neverthless, SIRT1 expression in diabetic + exercise group was significantly lower than control group ($p < .05$).

Histopathological changes in liver tissue

Liver histology studies reveal that control and exercise groups show the normal architecture of tissue insections stained with H&E. All diabetic animals exhibited

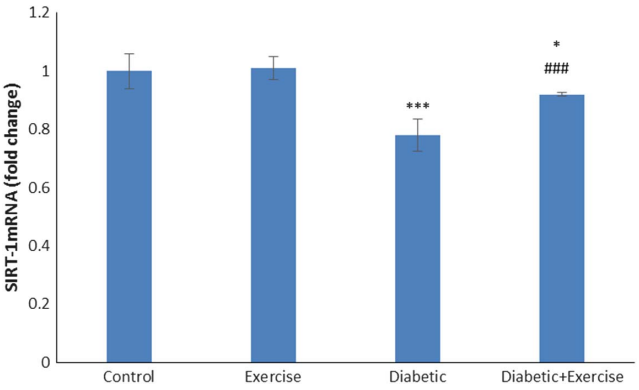

Fig. 2. Real-time quantitative PCR analysis of hepatic SIRT1 gene expression in the liver tissue of experimental groups. The expression level of SIRT1 was normalized to that of the gene encoding beta Gusb. The values represent means ± *SD* for seven animals. *$p < .05$. ***$p < .001$ vs. control group. $^{\#\#\#}p < .001$ vs. diabetic group

Table 1. The effect of swimming training on MDA (nmol/g protein), GPx (U/g protein), SOD (U/g protein), and CAT (nmol/min/mg protein) levels in the liver tissue of different groups of rats

	Groups			
Variants	Control	Exercise	Diabetic	Diabetic + exercise
MDA	0.43 ± 0.11	0.44 ± 0.09	2.36 ± 0.38***	1.23 ± 0.26***,###
SOD	30.16 ± 4.6	41.3 ± 7.2**	13.1 ± 2.3***	20.02 ± 2.6**,#
GPx	43.9 ± 5.4	34.02 ± 11.8*	15.65 ± 2.8***	23.47 ± 4.01***
CAT	63.3 ± 3.8	57.1 ± 23.02	19.7 ± 3.91***	33.1 ± 6.4**

Note. The values represent means ± *SD* ($n = 7$). MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase.
*$p < .05$ vs. control group. **$p < .01$ vs. control group. ***$p < .001$ vs. control group.
$^{\#}p < .05$ vs. diabetic group. $^{\#\#\#}p < .001$ vs. diabetic group.

histo-architectural distortion or disarrangement such as hepatic sinusoidal dilation with signs of congestion, inflammatory cell infiltration, hepatocellular destruction with acidophilic cytoplasm, and obvious degeneration of nuclei and signs of apoptotic cell death (nuclear pyknosis). Some of the hepatic cells did not have regular borders and were fragmented, when compared with control rats. In addition, liver sections of diabetic rats revealed cytoplasmic vacuolization. However, training mitigates these pathological changes in diabetic animals. It suggests that swim training can repair damaged liver in this rat model (Fig. 3; Table 2).

DISCUSSION

In this study, we evaluated the effect of swimming training on oxidative stress, SIRT1 gene expression, and histopathology of hepatic tissue in experimentally type 2 diabetic rats. Diabetic rats exhibited significantly higher glucose levels, higher insulin levels, and severe liver impairment compared to the control rats. These results indicated that the model for type 2 diabetes was constructed successfully. These observations are in agreement with previous studies (Ghiasi et al., 2015; Oliveira et al., 2012). Interestingly, these effects were reversed or significantly improved by swimming training, suggesting that swimming training mitigates T2DM.

Furthermore, according to histopathological findings, distortion of liver architecture, cytoplasmic vacuolization, hepatic sinusoidal dilation, inflammatory cell infiltration, unclear cell boundaries with acidophilic cytoplasm, and obvious degeneration of nuclei and signs of apoptotic cell death (nuclear pyknosis) were observed in diabetic animals. However, swimming training improved these histopathological alterations in the liver of T2DM. These data are in accordance with previous results (Ghiasi et al., 2015;

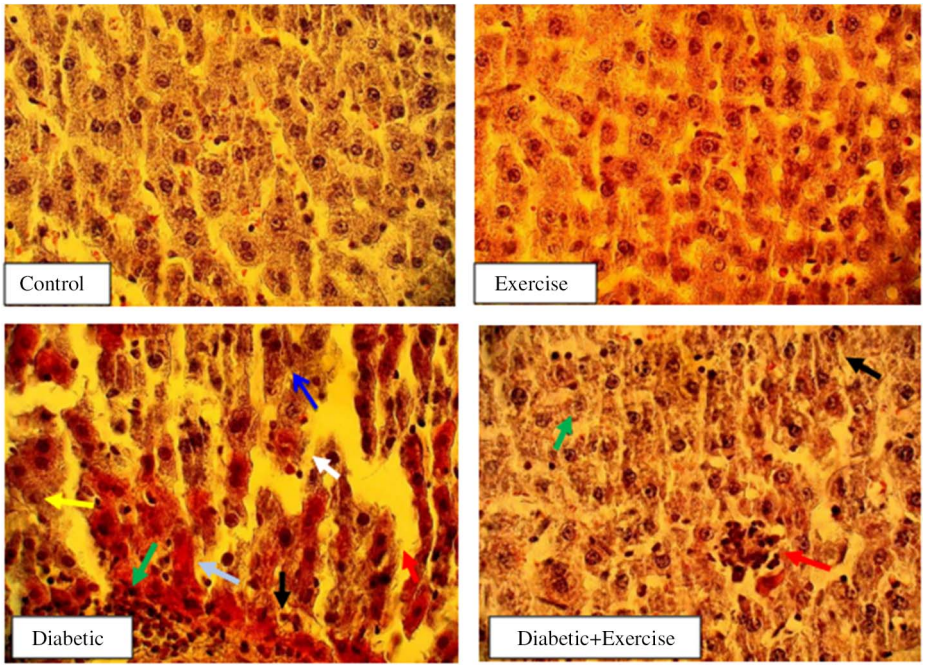

Fig. 3. Histological analysis of liver tissues sections in each group. Conventional H&E staining was performed (magnification: 400×). In diabetic group, distortion of liver architecture (blue arrow), cytoplasmic vacuolization (black arrow), hepatic sinusoidal dilation (red arrow), inflammatory cell infiltration (green arrow), unclear cell boundaries (white arrow) with acidophilic cytoplasm (indigo arrow), and obvious degeneration of nuclei and signs of apoptotic cell death (nuclear pyknosis; yellow arrow) were observed. Swimming training improved histopathological alterations. In diabetic + exercise group, mild injury such as cytoplasmic vacuolization, inflammatory cell infiltration, and hepatic sinusoidal dilation was observed in hepatocytes

Table 2. Comparison of histological alterations of hepatocytes in different groups after exercise training (hematoxylin and eosin staining)

Groups	Distortion of liver architecture	Leukocyte infiltration	Cytoplasmic vacuolization	Sinusoidal dilation	Nuclear pyknosis
Control	–	–	–	–	–
Exercise	–	–	–	–	–
Diabetic	+++	+++	+++	+++	+++
Diabetic + exercise	++	+	+	+	+

Note. A minimum of 10 fields for each hepatocyte slide were examined and assigned for severity of changes using scores on a scale of: (–) none, (+) mild, (++) moderate, and (+++) severe damage ($n = 7$).

Yang et al., 2017) and suggest that exercise training is very effective for the management of diabetes mellitus. However, its mechanism is not fully elucidated.

It was known that oxidative stress plays a key role in the pathogenesis of diabetes and its complications especially in hepatic abnormalities are called NAFLD (Klisic et al., 2018). In this study, we found that diabetes reduced SOD, GPx, and CAT in the liver tissue. The opposite trend was observed in MDA level, which is a marker for lipid peroxidation, with respect to the control group. Similar results are available in the literature (de Bem et al., 2018; Oliveira et al., 2012). Our investigation in T2DM is novel and suggests that swimming training elevated SOD, GPx, and CAT and reduced MDA levels in the liver of diabetic animals. However, significant alterations in GPx and CAT levels were not observed in the liver of diabetic rats. These findings indicate that swimming training may be beneficial to type 2 diabetic rats via attenuating oxidative stress in the liver.

High level of MDA leads to diabetic liver damage and other complications (Klisic et al., 2018). According to the literature, glucose is oxidized to produce reactive ketoaldehyde and superoxide radicals prone to complications in diabetes. Therefore, excessive production of free radicals causes the demolition of macromolecules including carbohydrates, proteins, lipids, and DNA (Padiya et al., 2011). In addition, ROS production further leads to hepatic structural and functional disorders (Iskender et al., 2017). Klisik et al. (2018) reported that MDA significantly correlated with hepatic histo-architectural distortion and making a virtuous circle between oxidative stress and NAFLD. It was indicated that neutralization of reactive species has significantly been able to inhibit the development of several organ damages in diabetes. For this purpose, antioxidant defense mechanisms consisting of SOD and CAT are available in cells (Naderi et al., 2015b). However, high levels of free radicals production and the simultaneous decline of endogenous antioxidant reserves may result in cell injury and in the development of insulin resistance (Padiya et al., 2011). In light of recent investigations, as well as our own results, overproduction of ROS due to hyperglycemia leads to oxidative stress, which plays a substantial role in the pathogenesis of liver damage due to diabetes (Naderi et al., 2015b).

Moreover, another finding is increasing SOD enzyme in sedentary animals. It was known that SOD level is associated with the level of oxidative stress (Liu et al., 2015) and exercise training vigorously affect the liver SOD enzyme, that is because of temporary production of ROS following aerobic exercise in the liver (Lima et al., 2013). Instead, antioxidant enzymes are activated due to free radicals productions including SOD in healthy and diabetic rats indicating that exercise has an important role in detoxification superoxide by increasing SOD enzyme. This is in line with the previous findings that demonstrated liver SOD activity is upregulated following exercise training in rats (Kakarla et al., 2005; Wilson & Johnson, 2000). There are seven mammalian sirtuins (SIRT1–SIRT7) with different activities and functions. It was known that SIRT1 is the most widely studied (Blander & Guarente, 2004). SIRT1 has been identified in various metabolic tissues, such as liver that regulates hepatic glucose and lipid metabolism (Nassir & Ibdah, 2016), insulin secretion, and oxidative stress. Taken together, these events may cause insulin resistance suggesting a new therapeutic target for the prevention of disorders in association with insulin resistance and T2DM (Kitada et al., 2013). In line with our study, it was shown that the expression of SIRT1 is low in rat liver of T2DM with NAFLD (Xu et al., 2012, 2013). Thereby, SIRT1activators could be a potential therapeutic target in controlling insulin resistance and T2DM (Liu et al., 2018). Cho et al. (2014) demonstrated that hepatic SIRT1 protein level was decreased following HFD-induced hyperglycemia and insulin resistance. Nevertheless, aerobic exercise training normalized SIRT1 in these animals and it claims that exercise mitigates the development of metabolic disorders via SIRT1 (Cho et al., 2014). However, to date, there have been no data elucidating the roles of SIRT1 in hepatic tissue with T2DM in response to swimming training. This study has clarified that swimming training is able to mitigate diabetes-induced inhibition of SIRT1 gene expression. Previously, it was reported that insulin resistance is related to mitochondrial dysfunction and oxidative stress has an important role in this event (Cao et al., 2018). Meanwhile, SIRT1 incidentally increases cellular responses to oxidative stress through deacetylation of FOXO3 (Colak et al., 2011) and modulation of p53 and NF-κB (Zhang et al., 2015). It was mentioned that FOXO3 upregulates CAT and MnSOD to detoxify ROS (Colak et al., 2011). Thereby, antioxidant defense was enabled to counteract free radicals in response to SIRT1 upregulation (Iskender et al., 2017). Therefore, it seems that swimming training promotes liver oxidative stress in part by increasing the expression of this gene, which is confirmed by improvement of histological alterations.

Further research is required in comprehensive details to evaluate other mediators involved in protecting effect of swimming training in the diabetic liver. Consequently, this study relied on the histological findings in the liver sections of male rats to detect any direct toxicity evidence in T2DM. In fact, the histological sections of the liver showed necrotic lesions, inflammatory signs, and congestion status, whereas swimming training attenuated these abnormalities as compared to diabetic animals.

CONCLUSION FOR FUTURE BIOLOGY

In conclusion, the results of this study revealed that swimming training could protect against liver injury induced by T2DM via oxidative stress attenuation mediated by SIRT1 gene expression. The current findings implied that swimming training might be an effective and non-pharmacological strategy for liver injury in T2DM. However, further studies should be carried out to assess whether the present results can be generalized to human. In addition, molecule inhibitors suggested to be used for accrediting the signaling pathways.

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Competing Interests: The authors declare no conflict of interest.

Authors' Contributions: AM contributed in practical stages. AA contributed in hystological analysis. RG contributed in analysis, interpretation of data, and writing of the manuscript. RN contributed in all steps of the study.

REFERENCES

Aebi, H. (1984) Catalase *in vitro*. *Methods Enzymol. 105*, 121–126.

Al-Busafi, S. A., Bhat, M., Wong, P., Ghali, P., Deschenes, M. (2012) Antioxidant therapy in nonalcoholic steatohepatitis. *Hepat. Res. Treat. 2012*, 947575.

Biyashev, D., Veliceasa, D., Topczewski, J., Topczewska, J. M., Mizgirev, I., Vinokour, E., Reddi, A. L., Licht, J. D., Revskoy, S. Y., Volpert, O. V. (2012) miR-27b controls venous specification and tip cell fate. *Blood 119*, 2679–2687.

Blander, G., Guarente, L. (2004) The Sir2 family of protein deacetylases. *Annu. Rev. Biochem. 73*, 417–435.

Cao, M. M., Lu, X., Liu, G. D., Su, Y., Li, Y. B., Zhou, J. (2018) Resveratrol attenuates type 2 diabetes mellitus by mediating mitochondrial biogenesis and lipid metabolism via Sirtuin type 1. *Exp. Ther. Med. 15*, 576–584.

Chiş, I., Mureşan, A., Oros, A., Nagy, A., Clichici, S. (2016) Protective effects of quercetin and chronic moderate exercise (training) against oxidative stress in the liver tissue of streptozotocin-induced diabetic rats. *Acta Physiol. Hung. 103*, 49–64.

Cho, J., Lee, I., Kim, D., Koh, Y., Kong, J., Lee, S., Kang, H. (2014) Effect of aerobic exercise training on non-alcoholic fatty liver disease induced by a high fat diet in C57BL/6 mice. *J. Exerc. Nutr. Biochem. 18*, 339.

Colak, Y., Ozturk, O., Senates, E., Tuncer, I., Yorulmaz, E., Adali, G., Doganay, L., Enc, F. Y. (2011) SIRT1 as a potential therapeutic target for treatment of nonalcoholic fatty liver disease. *Med. Sci Monit. 17*, HY5.

Dart, A. B., Martens, P. J., Rigatto, C., Brownell, M. D., Dean, H. J., Sellers, E. A. (2014) Earlier onset of complications in youth with type 2 diabetes. *Diabetes Care 37*, 436–443.

de Bem, G. F., da Costa, C. A., Cordeiro, V. D. S. C., Santos, I. B., de Carvalho, L. C. R. M., de Andrade Soares, R., Ribeiro, J. H., de Souza, M. A. V., da Cunha Sousa, P. J., Ognibene, D. T. (2018) Euterpe oleracea Mart. (açaí) seed extract associated with exercise training reduces hepatic steatosis in type 2 diabetic male rats. *J. Nutr. Biochem. 52*, 70–81.

Delmas-Beauvieux, M. C., Peuchant, E., Couchouron, A., Constans, J., Sergeant, C., Simonoff, M., Pellegrin, J. L., Leng, B., Conri, C., Clerc, M. (1996) The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or beta-carotene. *Am. J. Clin. Nutr. 64*, 101–107.

Franz, M. J., Boucher, J. L., Rutten-Ramos, S., VanWormer, J. J. (2015) Lifestyle weight-loss intervention outcomes in overweight and obese adults with type 2 diabetes: a systematic review and meta-analysis of randomized clinical trials. *J. Acad. Nutr. Diet. 115*, 1447–1463.

Ghiasi, R., Soufi, F. G., Hossein Somi, M., Mohaddes, G., Bavil, F. M., Naderi, R., Alipour, M. R. (2015) Swim training improves HOMA-IR in type 2 diabetes induced by high fat diet and low dose of streptozotocin in male rats. *Adv. Pharm. Bull. 5*, 379.

Han, J., Wei, M., Wang, Q., Li, X., Zhu, C., Mao, Y., Wei, L., Sun, Y., Jia, W. (2015) Association of genetic variants of SIRT1 with type 2 diabetes mellitus. *Gene Expr. 16*, 177–185.

Iskender, H., Dokumacioglu, E., Sen, T. M., Ince, I., Kanbay, Y., Saral, S. (2017) The effect of hesperidin and quercetin on oxidative stress, NF-κB and SIRT1 levels in a STZ-induced experimental diabetes model. *Biomed. Pharmacother. 90*, 500–508.

Kakarla, P., Vadluri, G., Reddy Kesireddy, S. (2005) Response of hepatic antioxidant system to exercise training in aging female rat. *J. Exp. Zoolog. A Comp. Exp. Biol. 303*, 203–208.

Kitada, M., Kume, S., Kanasaki, K., Takeda-Watanabe, A., Koya, D. (2013) Sirtuins as possible drug targets in type 2 diabetes. *Curr. Drug Targets 14*, 622–636.

Klisic, A., Isakovic, A., Kocic, G., Kavaric, N., Jovanovic, M., Zvrko, E., Skerovic, V., Ninic, A. (2018) Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes 126*, 371–378.

Lässer, C., Eldh, M., Lötvall, J. (2012) Isolation and characterization of RNA-containing exosomes. *J. Vis. Exp. 59*, e3037.

Lima, F. D., Stamm, D. N., Della-Pace, I. D., Dobrachinski, F., de Carvalho, N. R., Royes, L. F. F., Soares, F. A., Rocha, J. B., González-Gallego, J., Bresciani, G. (2013) Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. *PLoS One 8*, e55668.

Liu, H., Sheng, M., Liu, Y., Wang, P., Chen, Y., Chen, L., Wang, W., Li, B. (2015) Expression of SIRT1 and oxidative stress in diabetic dry eye. *Int. J. Clin. Exp. Pathol. 8*, 7644.

Liu, P., Feng, T., Zuo, X., Wang, X., Luo, J., Li, N., Han, X., Zhu, N., Xu, S., Xu, Y. (2018) A novel SIRT1 activator E6155 improves insulin sensitivity in type 2 diabetic KKAy mice. *Biochem. Biophys. Res. Commun. 498*, 633–639.

Lovis, P., Gattesco, S., Regazzi, R. (2008) Regulation of the expression of components of the exocytotic machinery of insulin-secreting cells by microRNAs. *Biol. Chem. 389*, 305–312.

Mohammadi, M., Ghaznavi, R., Keyhanmanesh, R., Sadeghipour, H. R., Naderi, R., Mohammadi, H. (2013) Voluntary exercise prevents lead-induced elevation of oxidative stress and inflammation markers in male rat blood. *Sci. World J. 2013*.

Naderi, R., Mohaddes, G., Mohammadi, M., Alihemmati, A., Badalzadeh, R., Ghaznavi, R., Ghyasi, R., Mohammadi, S. (2015a) Preventive effects of garlic (*Allium sativum*) on oxidative stress and histopathology of cardiac tissue in streptozotocin-induced diabetic rats. *Acta Physiol. Hung. 102*, 380–390.

Naderi, R., Mohaddes, G., Mohammadi, M., Ghaznavi, R., Ghyasi, R., Vatankhah, A. M. (2015b) Voluntary exercise

protects heart from oxidative stress in diabetic rats. *Adv. Pharm. Bull. 5*, 231.

Nassir, F., Ibdah, J. A. (2016) Sirtuins and nonalcoholic fatty liver disease. *World J. Gastroenterol. 22*, 10084.

Oliveira, V. N. D., Bessa, A., Jorge, M. L. M. P., Oliveira, R. J. D. S., de Mello, M. T., De Agostini, G. G., Jorge, P. T., Espindola, F. S. (2012) The effect of different training programs on antioxidant status, oxidative stress, and metabolic control in type 2 diabetes. *Appl. Physiol. Nutr. Metab. 37*, 334–344.

Padiya, R., Khatua, T. N., Bagul, P. K., Kuncha, M., Banerjee, S. K. (2011) Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats. *Nutr. Metab. 8*, 53.

Paglia, D. E., Valentine, W. N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med. 70*, 158–169.

Smith, B. W., Adams, L. A. (2011) Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. *Nat. Rev. Endocrinol. 7*, 456.

Song, R., Xu, W., Chen, Y., Li, Z., Zeng, Y., Fu, Y. (2011) The expression of Sirtuins 1 and 4 in peripheral blood leukocytes from patients with type 2 diabetes. *Eur. J. Histochem. 55*, e10.

Szkudelski, T., Szkudelska, K. (2015) Resveratrol and diabetes: from animal to human studies. *Biochim. Biophys. Acta 1852*, 1145–1154.

Tucker, P. S., Fisher-Wellman, K., Bloomer, R. J. (2008) Can exercise minimize postprandial oxidative stress in patients with type 2 diabetes? *Curr. Diabetes Rev. 4*, 309–319.

Wilson, D., Johnson, P. (2000) Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver. *J. Appl. Physiol. 88*, 1791–1796.

Wu, H., Jin, M., Han, D., Zhou, M., Mei, X., Guan, Y., Liu, C. (2015) Protective effects of aerobic swimming training on high-fat diet induced nonalcoholic fatty liver disease: regulation of lipid metabolism via PANDER-AKT pathway. *Biochem. Biophys. Res. Commun. 458*, 862–868.

Xu, J., Li, N., Wang, J., Zhang, C., Ding, S., Jiao, Y. (2012) Hepatic SIRT1 and UCP2 expressions in rats with type 2 diabetes mellitus and nonalcoholic fatty liver. *Nan fang yi ke da xue xue bao [J. South. Med. Univ.] 32*, 726–729.

Xu, J., Li, N., Wang, J., Zhang, C., Ding, S., Jiao, Y., Zhang, J. (2013) Effect of metformin on the expression of SIRT1 and UCP2 in rat liver of type 2 diabetes mellitus and nonalcoholic fatty liver. *Zhong Nan Da Xue Xue Bao Yi Xue Ban 38*, 882–887.

Yamazaki, Y., Usui, I., Kanatani, Y., Matsuya, Y., Tsuneyama, K., Fujisaka, S., Bukhari, A., Suzuki, H., Senda, S., Imanishi, S. (2009) Treatment with SRT1720, a SIRT1 activator, ameliorates fatty liver with reduced expression of lipogenic enzymes in MSG mice. *Am. J. Physiol. Endocrinol. Metab. 297*, E1179–E1186.

Yang, Q., Wang, W-W., Ma, P., Ma, Z-X., Hao, M., Adelusi, T. I. (2017) Swimming training alleviated insulin resistance through Wnt3a/β-catenin signaling in type 2 diabetic rats. *Iranian J. Basic Med. Sci. 20*, 1220.

Yi, X., Cao, S., Chang, B., Zhao, D., Gao, H., Wan, Y., Shi, J., Wei, W., Guan, Y. (2013) Effects of acute exercise and chronic exercise on the liver leptin-AMPK-ACC signaling pathway in rats with type 2 diabetes. *J. Diabetes Res. 2013*, 946432.

Zhang, F., Li, Z. L., Xu, X. M., Hu, Y., Yao, J. H., Xu, W., Jing, H. R., Wang, S., Ning, S. L., Tian, X. F. (2015) Protective effects of icariin-mediated SIRT1/FOXO3 signaling pathway on intestinal ischemia/reperfusion-induced acute lung injury. *Mol. Med. Rep. 11*, 269–276.

Zheng, Y., Liu, T., Wang, Z., Xu, Y., Zhang, Q., Luo, D. (2018) Low molecular weight fucoidan attenuates liver injury via SIRT1/AMPK/PGC1α axis in db/db mice. *Int. J. Biol. Macromol. 112*, 929–936.