

## Original article



# The effect of eight weeks of high-intensity interval training and moderate-intensity continuous training on some factors causing oxidative stress in the cardiomyocytes of mice with type II diabetes

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## Abstract

**Background and aims:** Diabetic cardiomyopathy (DCM) is a major cause of morbidity and mortality among diabetic patients. This study aimed to compare and investigate the effects of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on oxidative stress as one of the key links in the development of DCM in mice with type 2 diabetes mellitus (T2DM).

**Methods:** Forty male C57BL/6J mice were randomly assigned to four groups of equal number (Control, T2DM, T2DM+HIIT, and T2DM+MICT). After the induction of T2DM, HIIT and MICT programs were conducted 5 days a week for 8 weeks. At the end of the experimental period, blood and heart samples were collected for subsequent measurements.

**Results:** T2DM significantly up-regulated the levels of advanced glycation end products (AGEs,  $P=0.001$ ) and the expression of 15-lipoxygenase (15-LOX,  $P=0.001$ ) and 4-hydroxy-2-nonenal (4-HNE,  $P=0.001$ ) compared to controlled mice. After 8 weeks of training, HIIT and MICT programs increased the levels of insulin ( $P<0.001$ ) while reducing the levels of fasting blood glucose ( $P<0.001$ ,  $P=0.024$ , respectively). On the other hand, it was found that HIIT and MICT programs significantly decreased the levels of AGEs ( $P<0.001$ ), expression of 15-LOX ( $P=0.006$ ,  $P=0.019$ , respectively), and 4-HNE ( $P=0.008$  and  $P=0.035$ , respectively).

**Conclusion:** Our findings revealed that exercise training, particularly MICT, will be highly helpful in the prevention of DCM through the improvement of glucose metabolism and reduction of 15-LOX expression.

**Keywords:** Type 2 diabetes mellitus, Diabetic cardiomyopathy, Exercise, Oxidative stress

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## Introduction

Diabetes mellitus (DM) is defined by the World Health Organization as a group of metabolic disorders characterized by insulin resistance and hyperglycemia together with disturbances of carbohydrate, protein, and lipid metabolism. Type 2 diabetes mellitus (T2DM) is the most common type of DM and accounts for 90%-95% of the population with DM (1). In addition, it has been recently reported that nearly 300 million people worldwide live with T2DM, and the prevalence of this condition will rise to 590 million by 2035 (2). T2DM, caused by insulin resistance and a progressive loss of  $\beta$ -cell insulin secretion (3), leads to cardiovascular disease, chronic heart disease, and microvascular complications (4). In the absence of other cardiac risk factors described in the animal and human models of DM, a heart muscle-specific disease termed diabetic cardiomyopathy (DCM) (5) imposes a huge burden on society as one of the major complications of DM (6).

Increasing the levels of metabolites and glucose residues in the setting of T1DM and T2DM up-regulates the production of advanced glycation end-products (AGEs), which can affect endothelial cells and cardiomyocytes (7). AGEs are a general term for a class of heterogeneous groups of oxidative molecules with pathogenic capability derived from nonenzymatic reactions (Maillard reactions) between carbohydrate residues and lipids, proteins, and nucleic acids (8). As a result of the interaction between the AGEs and the receptor for AGEs (RAGE), the cytoplasmic domain of RAGE activates different signaling pathways (9,10). The systemic glucotoxicity (through the accumulation of AGEs) associated with T2DM can activate lipoxygenase (LOX) enzymes, which promote mitochondrial dysfunction and oxidative stress (OS), which can mediate metabolic derangements, hypertrophy, loss of contractility, and cardiomyocyte death (11).

LOXs are a family of iron-containing enzymes that catalyze arachidonic acid to form biologically

active products such as leukotrienes, prostaglandins, thromboxanes, epoxyeicosatrienoic acids, and hydroperoxy eicosatetraenoic acids. Moreover, 15-LOX is a member of the LOX family that has gained particulate attention because of its increased expression in inflammatory diseases such as DM, DCM, and atherosclerosis (12). Suzuki et al reported that the expression of 15-LOX and 4-HNE as a by-product of lipid peroxidation associated with persistent hyperglycemia is significantly upregulated in the heart of diabetic mice, developing DCM via cardiac OS and inflammation (13).

In recent years, DCM treatments included lipid and glucose control, cardiovascular disease intervention, and hypertension treatment. Recently, pharmacological treatments such as  $Ca^{2+}$  antagonists, renin-angiotensin-aldosterone system inhibitors, and  $\beta$ -blockers are accepted as the common strategy for cardiovascular disease in diabetic patients (14). Furthermore, exercise has been considered an important non-pharmacological treatment for the treatment and prevention of DM and its complications. The American Diabetes Association proposed that patients with T2DM should undertake at least 150 minutes of moderate to vigorous aerobic exercise per week to benefit from the protective effects of exercise against diabetic complications (15).

Considering that AGEs, LOXs, OS, and their interactions are highly related to the progression of DCM, therapies that involve AGEs and 15-LOX may help reduce OS and cardiovascular complications in diabetic patients. Additionally, Novoa et al found that 4 weeks of high-intensity exercise could not restore the nitroso-redox imbalance in the diabetic heart (16). Therefore, this study aimed to investigate the effect of 8 weeks of two exercise protocols, including high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), on the levels of fasting blood glucose (FBG), insulin, AGEs, and expression of 15-LOXs and 4-HNE in the heart of mice with T2DM. Given that OS is associated with DCM and can be influenced by exercise, it was hypothesized that HIIT and MICT would improve glycemic control and attenuate OS in mice with T2DM.

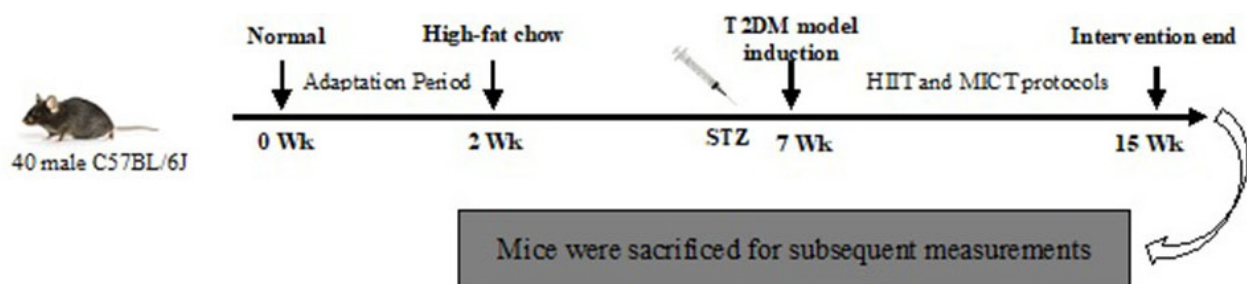
## Materials and Methods

Forty male C57BL/6J mice with a weight of 23-24 g were

procured from Royan Biotechnology Research Institute at the age of eight weeks. The animals were housed in a temperature-controlled room ( $25 \pm 2^\circ\text{C}$ ) with a relative humidity of 50-70% and a 12-hour light/dark cycle (lighting from 6:00 hours to 18:00 hours) and had free access to standard water and chow. Before the beginning of T2DM induction, all animals underwent a 2-week acclimatization period (Figure 1).

All mice were randomly distributed into two control ( $n=10$ ) and T2DM ( $n=30$ ) groups. The control group was fed with standard laboratory chow, and the T2DM group was fed a high-fat diet for four consecutive weeks. After four weeks, the T2DM group was injected intraperitoneally with 25 mg/kg of streptozocin (STZ, CAS 18883-66-4- Calbiochem) for 5 consecutive days. Three days after the final injection, the FBG of mice in the T2DM group was measured, and mice having  $\text{FBG} > 16.7$  mmol/L were considered a successful model of T2DM (15). Thirty diabetic mice were randomly divided into three groups of equal numbers ( $n=10$ ), including T2DM, T2DM+HIIT, and T2DM+MICT. Then, T2DM and T2DM+exercise groups were fed with standard laboratory chow, and mice in the T2DM+exercise groups started their training programs.

All mice at the age of 3 months in T2DM+HIIT and T2DM+MICT groups underwent one week (3 days, 15 min/d) of treadmill acclimation on a 6 lanes motorized mice treadmill (MazeRouter, Tabriz, Iran) at a speed of 10-15 m/min, followed by performing a maximal running capacity test (MRCT). Briefly, the animals started to run at 8 m/min for 2 minutes, and progressively the speed was creased to 1 m/min every 2 minutes until exhaustion (mice were unable to maintain the treadmill speed exceeding 10 consecutive seconds). The highest running speed and duration were recorded to determine the  $S_{\text{max}}$  (average maximum speed). Both HIIT and MICT protocols were performed 5 days/week for 8 weeks in T2DM+HIIT and T2DM+MICT groups. The training sessions were monitored by the same person, and mice were placed on the treadmill every day at the same hour (10.00-12.00 am). The training protocols started with a 5-minute warm-up and ended with a 5-minute cool-down period at 40%  $S_{\text{max}}$ . The HIIT protocol consisted of 1.5 minutes of high-intensity running at 85%  $S_{\text{max}}$ ,



**Figure 1.** Schematic representation of the experimental design. Note: T2DM: Type 2 diabetes mellitus; MICT: Moderate-intensity continuous training; HIIT: High-intensity interval training; STZ: Streptozocin. After 2 weeks of the adaptation period and 4 weeks of feeding with a high-fat diet, animals were treated with STZ (25 mg/kg) for five consecutive days, followed by 8 weeks of MICT and HIIT protocols.

followed by 2 minutes of active recovery at 45% Smax (9 bouts), whereas the MICT protocol consisted of distance-matched continuous running at 60% Smax. To adjust treadmill speed, the MRCT was conducted at the beginning, 2 weeks, 4 weeks, 6 weeks, 8 weeks, and the end of the exercise period (Table 1) according to previous research (17).

The tail vein blood was collected every 2 weeks, and FBG concentration was directly assessed using glucometers (Infopia EasyGluko blood glucose monitor, South Korea). After 8 weeks of exercise training and after an overnight fast, the final tail vein blood was collected, and body weight (BW) underwent measurement. Then, all mice were sacrificed under anesthesia attained with an intraperitoneal injection of 2% sodium pentobarbital at a dose of 50 mg/kg of BW. A blood sample was collected via cardiac puncture to determine the levels of serum insulin and AGEs. After collecting the blood samples, mice hearts were dissected and submerged into liquid nitrogen and stored at -70 °C for further use. The serum was separated by centrifugation at 3000 rpm for 15 minutes and kept at -70 °C until later assay. The levels of serum insulin were measured using the Insulin Mouse ELISA kit (Cat. No: MBS038565). ZellBio GmbH assay kit was used to quantitative assay Mouse AGEs (Cat. No.: ZB-10589C-M9648).

The expressions of 15-LOX and 4-HNE were quantitatively detected using a western blot. The heart tissue was lysed in the radioimmunoprecipitation assay buffer (50 mM Tris-HCl, 1% Triton X-100, 1 mM ethylenediaminetetraacetic acid, 150 mM sodium chloride, 0.1% sodium dodecyl sulfate [SDS], and 0.5% sodium deoxycholate) supplemented with phosphatase and protease inhibitors (Sigma, St Louis, MO). After the sonication of samples over ice, the concentration of protein was determined the by Pierce BCA protein assay kit (Pierce Biotechnology) (18). The SDS-polyacrylamide gel electrophoresis was employed for protein separation. Then, separated proteins were transferred to an activated *hydrophobic poly (vinylidene fluoride)* membrane

(FFP26, Beyotime, Shanghai, China), blocked with 5% skim milk powder (P0216, Beyotime, Shanghai, China) for 2 hours at room temperature. The membrane was subsequently incubated with mouse monoclonal 4-HNE antibody (LS-C777291, Santa Cruz Biotechnology, Dallas, TX, USA), mouse monoclonal 15-LOX antibody (SC-133085, Santa Cruz Biotechnology, Dallas, TX, USA), and glyceraldehyde-3-phosphate dehydrogenase (Sigma-Aldrich) overnight at 4°C with mild agitation. After three times washing, the membrane was incubated with peroxidase-conjugated secondary antibody (Thermo Fisher Scientific) for 1 hour at room temperature. The protein was detected by SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). The specific protein bands were quantified using ImageJ software (ImageJ U.S. National Institutes of Health, Bethesda, Maryland, USA).

The collected data were statistically analyzed with SPSS (version 20.0) software (SPSS Inc. USA) using an independent sample t-test and one-way analysis of variance (ANOVA). Data were expressed as the mean ± standard deviation (SD), and  $P < 0.05$  was statistically significant.

## Results

The BW changes at baseline, 5th, 7th, 9th, 11th, and 13th weeks were investigated in all groups. The result showed no difference in the BW of mice between the groups at baseline. The control animals progressively gained weight from the beginning to the end of the experiment. As illustrated in Figure 2, the BW in the T2DM group began to decrease slowly after T2DM induction ( $P < 0.001$ ). In addition, there was a slow reduction in BW after the beginning of the exercise intervention in the T2DM + MICT ( $P < 0.001$ ) and T2DM + HIIT ( $P < 0.001$ ) groups. Furthermore, diabetic animals in T2DM + MICT and T2DM + HIIT groups represented lower weight compared to the T2DM group ( $P < 0.001$  and  $P = 0.001$ , respectively).

The biochemical analysis demonstrated no significant difference in baseline FBG among groups. However,

**Table 1.** Exercise training protocols

	Average Maximum Speed	HIIT	Low intensity	MICT	Total running distance
		High intensity (85% Smax) session (9 intervals, 1.5 min)	(45% Smax) session (9 intervals, 2 min)	Moderate Intensity (60% Smax) Session	
Pre	22.35 m/min				
Week 1		18 m/min	10 m/min	12 m/min, 35 min	423 m
Week 2		18 m/min	10 m/min	12 m/min, 35 min	423 m
Week 3	23.52 m/min	20 m/min	11 m/min	14 m/min, 34 min	468 m
Week 4		20 m/min	11 m/min	14 m/min, 34 min	468 m
Week 5	25.88 m/min	22 m/min	12 m/min	16 m/min, 32 min	513 m
Week 6		22 m/min	12 m/min	16 m/min, 32 min	513 m
Week 7	28.23 m/min	24 m/min	13 m/min	17 m/min, 33 min	558 m
Week 8		24 m/min	13 m/min	17 m/min, 33 min	558 m
Post	29.41 m/min				

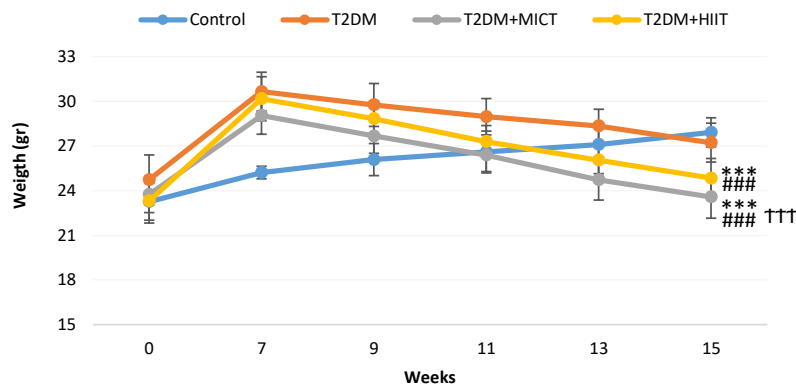
Note: MICT: Moderate-intensity continuous training; HIIT: High-intensity interval training.

following T2DM induction, diabetic animals displayed a higher FBG level than the control group. After 8 weeks of training, the analysis of blood samples indicated that FBG was significantly decreased in HIIT ( $P < 0.001$ ) and MICT ( $P = 0.024$ ) groups. Based on the results, the levels of FBG in the T2DM+HIIT group were markedly decreased compared with the T2DM group ( $P = 0.014$ ). Although MICT slightly decreased FBG, there was no significant difference between T2DM+MICT and T2DM groups (Figure 3).

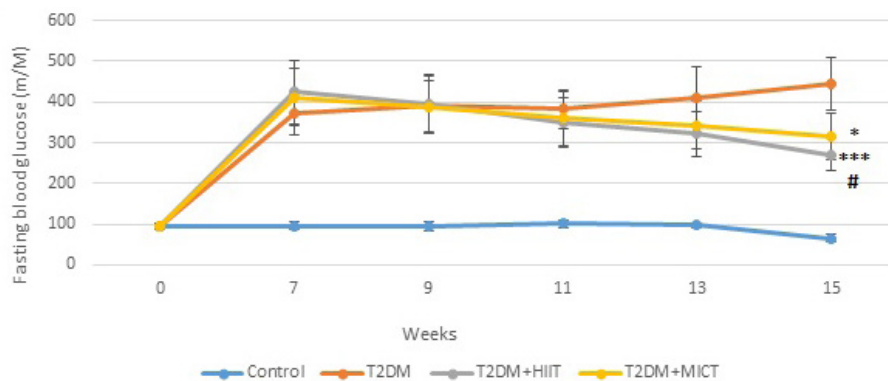
The results of one-way ANOVA showed a significant

reduction in the levels of fasting serum insulin levels in the T2DM group compared with the control group ( $P < 0.001$ ). However, the levels of fasting serum insulin in the T2DM+HIIT ( $P = 0.004$ ) and T2DM+MICT ( $P = 0.003$ ) groups were higher in comparison with the T2DM group (Figure 4).

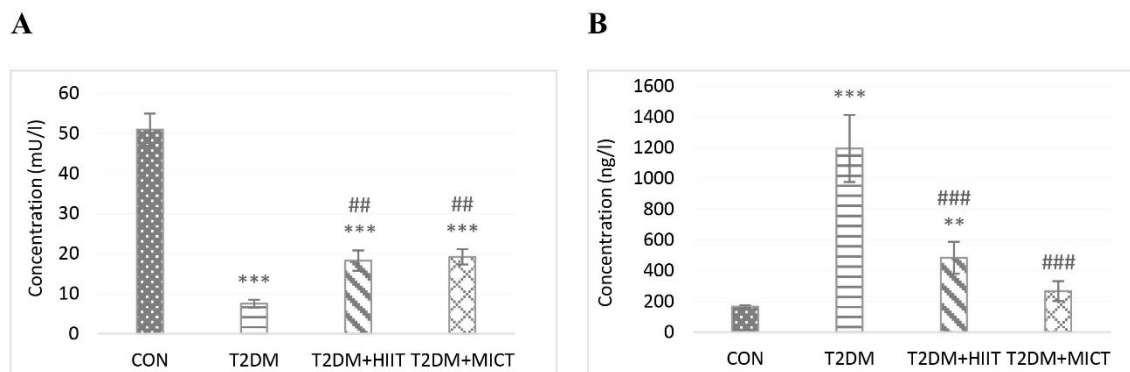
The evaluation of the effects of T2DM and exercise on the AGE level represented that both effects are significant. There were higher levels of AGEs in the T2DM group compared with control groups ( $P < 0.001$ ). Moreover, the treatment of HIIT and MICT produced significantly



**Figure 2.** The BW of four groups during 15 weeks. Note. BW: Body weight; T2DM: Type 2 diabetes mellitus; MICT: Moderate-intensity continuous training. \*\*\*  $P < 0.001$  the inter group, \*\*\*\*  $P < 0.001$  VS T2DM group, \*\*\*  $P < 0.001$  vs. the MICT group



**Figure 3.** The fasting blood glucose of four groups during 15 weeks. Note. T2DM: Type 2 diabetes mellitus; HIIT: High-intensity interval training; MICT: Moderate-intensity continuous training. \*  $P < 0.05$ , \*\*\*  $P < 0.001$  before and after 8 weeks, #  $P < 0.05$  vs. T2DM



**Figure 4.** Exercise intervention reduced the levels of AGEs while increasing the levels of insulin: insulin (A) and AGE (B) evolution from mice of all groups. Note. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  vs. CON, ##  $P < 0.01$ , ###  $P < 0.001$  T2DM+HIIT and T2DM+MICT vs. T2DM, CON: Control; T2DM: Type 2 diabetes mellitus; MICT: Moderate-intensity continuous training; HIIT: High-intensity interval training; AGE: Advanced glycation end product; SEM: Standard error of the mean. All Data are presented as the mean  $\pm$  SEM



lower AGEs in the blood ( $P < 0.001$ ) in comparison with the T2DM group (Figure 4).

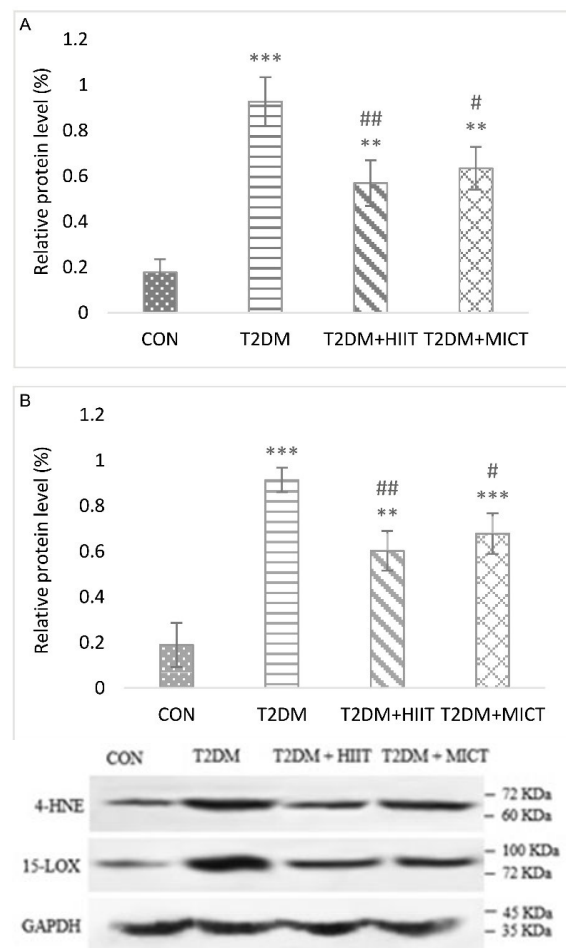
Western blot analysis revealed that the protein expression of cardiac 15-LOX was upregulated in the cardiomyocytes of diabetic mice compared with that in the control group ( $P < 0.001$ ), and this enhancement was significantly abolished by exercise in T2DM+HIIT ( $P = 0.006$ ) and T2DM+MICT ( $P = 0.019$ ) groups. Similarly, the protein levels of 4-HNE were significantly higher in the T2DM group ( $P < 0.001$ ). After completing the study intervention, the HIIT and MICT programs induced a significant decrease in the protein levels of 4-HNE compared with the T2DM group ( $P = 0.008$  and  $P = 0.035$ , respectively). Under these conditions, there was no significant difference in the protein levels of 15-LOX and 4-HNE between exercise groups ( $P = 0.697$ , Figure 5).

### Discussion

The current study evaluated and compared the effects of HIIT and MICT on factors causing OS in the cardiomyocytes of mice with T2DM. Based on the findings, the 8 weeks of HIIT and MICT programs improved BW, physical capacity, and glycemic control while decreasing OS in the mice with T2DM. Therefore, HIIT and MICT are effective interventions to prevent the complications of T2DM, especially DCM.

Recent studies have focused on a therapeutic strategy for the management of the cardiovascular complications of DM. It was reported that the crosstalk between OS and the AGE-RAGE axis plays an important role in the context of DM-associated cardiovascular diseases (19). It was found that the levels of AGEs in the blood of mice with T2DM were increased in comparison with nondiabetic mice. Moreover, the results showed that the increased production of AGEs in diabetic mice was significantly reversed in exercised mice with T2DM. Few studies demonstrated the effects of exercise training on the AGE/RAGE axis. Similar to our study outcomes, Wright et al reported that treadmill exercise had reduced AGE levels in cardiac tissues in the late middle-to-old age rats compared to age-matched control animals (20). Santilli et al found that exercise training reduces advanced glycation, lipid peroxidation, and reactive oxidation species (ROS) in the aortas of aged rats (21). Additionally, it was shown that regular moderate exercise causes a significant reduction in serum AGEs, insulin levels, and FBG in diabetic rats (22). These findings highlight the importance of AGEs in the prevention of DCM and exercise as a non-pharmacological intervention.

It is well established that sustained hyperglycemia causes more rapid Maillard reactions and results in an increase in AGEs (23). Regular exercise improves insulin and glucose metabolism and efficiently contribute to the prevention of cardiovascular complication in a patient with DM (24). Our study represented significant increases in insulin levels following HIIT and MICT for 8 weeks, which was associated with FBG reduction in the exercise



**Figure 5.** Exercise decreased the cardiac oxidative stress induced by 15-LOX in the diabetic heart of mice: western blot analysis of 15-LOX (A) and 4-HNE expression (B) in the hearts of CON, T2DM, T2DM+HIIT, and T2DM+MICT using antibody, and analysis of 15-LOX and 4-HNE western blot bands (C) in all groups. Note. Data are presented as the mean  $\pm$  SEM. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  vs. CON, #  $P < 0.05$ , ##  $P < 0.01$  HIIT and MICT vs. CON. CON: Control; T2DM; Type 2 diabetes mellitus; MICT: Moderate-intensity continuous training; HIIT: High-intensity interval training; AGE: Advanced glycation end product; SEM: Standard error of the mean; LOX: Lipoxygenase.

groups and approved the beneficial clinical practice of exercise in diabetic mice. In line with our study findings, Seo et al reported that exercise could activate the insulin signaling pathway through increased insulin secretion from pancreatic beta cells and upregulation of glucose transporter 4 expression (25). It has been also indicated that regular exercise could improve glycemic control, while it reduces the availability of reactive precursors for glycation reactions by increasing energy requirements, reducing the accumulation of AGEs in DM (26). It seems that exercise through improving glucose and insulin metabolism could decrease AGE levels. In our study, there were no significant differences in the levels of insulin and FBG between the MICT and HIIT groups. Therefore, the positive effects of MICT and HIIT on glycemic control and glucotoxicity may be similar.

There are several possible mechanisms by which exercise through the reduction of AGEs can improve OS. First, the positive effects of MICT and HIIT on

the AGEs under diabetic conditions can improve OS through the Sirt1/Nrf2 axis (27). Second, the reduction of AGE levels can decrease the activity and expression of NADPH oxidase, which is an important source of ROS in cardiovascular complications of DM (23,28). Furthermore, it has been found that arachidonic acid is a major source of OS in the diabetic heart. The activation of 15-LOX, which catalyzes the step from arachidonic acid, induced by hyperglycemia is associated with DCM and cardiac OS (29). Cardiac OS is associated with the elevation of 4-HNE levels. The levels of 4-HNE, as a by-product of lipid peroxidation, were significantly elevated in the myocardium of C57BL/6 mice after STZ treatment but not in diabetic Alox15-deficient mice (ALOX15: Arachidonate 15-Lipoxygenase is a Protein Coding gene) (13). It has been reported that exercise positively influences the cardiorespiratory capacity and contributes to the attenuation of OS (30). To our knowledge, no study has so far investigated the effect of exercise on 15-LOX in the cardiomyocyte of diabetic hearts. In our study, increased 15-LOX and 4-HNE expressions were observed in diabetic groups compared with the control group. Furthermore, our results showed the effectiveness of HIIT and MICT in decreasing 15-LOX and 4-HNE expression. According to a recent study, the secretion of 15-HETE as a metabolite of arachidonic acid tended to increase 2 hours after exercise (31). Moreover, it has been demonstrated that 4 weeks of moderate-intensity exercise training did not modify the plasma 15-LOX content (32). This is probably because of the short duration of exercise training in the included studies, leading to a lack of significant changes. As mentioned earlier, AGEs can activate 15-LOX enzymes and induce cardiovascular injury. Likewise, Golbidi et al concluded that AGEs can damage cells by the modification of intracellular proteins which are involved in gene-transcription regulation. Further, the diffusion of AGEs in extracellular space leads to the modification of extracellular proteins, disturbing signaling between the matrix and the cells (33). It seems that HIIT and MICT, through the reduction of AGEs associated with T2DM, can attenuate the overexpression of 15-LOX. Further research is warranted to elucidate the mechanisms involved in the exercise-induced decrease in the production of 15-LOX and myocardial OS during DM.

We acknowledge several limitations of our study. First, we only examined the expression of cardiac 15-LOX and 4-HNE. Western blots would provide further results on the signaling pathways involved in the improvement of cardiac OS in T2DM. Second, we focused on the changes in the protein expression of 15-LOX in the heart muscle but did not measure the possible changes in its activity. However, we believe that we have provided important new insights into DCM.

## Conclusion

The findings of this study demonstrated that eight weeks

of regular exercise, including HIIT and MICT, exerted beneficial effects on T2DM by decreasing the risk factors of DCM. Exercise training as a non-pharmacological treatment can improve glycemic control and attenuate OS. These results suggested that HIIT and MICT have a potential role in the prevention of DCM. However, more studies are needed to provide clinical and molecular evidence about how exercise training can be affected DCM in patients with T2DM.

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## Authors' Contribution

**Conceptualization:** Ali Khosravizadeh, Farhad Rahmani Nia, Arsalan Damirchi.

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**Funding acquisition:** Ali Khosravizadeh.

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**Project administration:** Ali Khosravizadeh.

**Resources:** Ali Khosravizadeh.

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**Supervision:** Farhad Rahmani Nia, Arsalan Damirchi.

**Validation:** Farhad Rahmani Nia, Arsalan Damirchi.

**Visualization:** Farhad Rahmani Nia, Arsalan Damirchi.

**Writing—original draft:** Ali Khosravizadeh.

**Writing—review & editing:** Ali Khosravizadeh.

## Competing Interests

The authors declare no conflict of interests.

## Ethical Approval

All animal experiments were approved by the Ethics Committee of Sport Sciences Research Institute (IR.SSRC.REC.1401.039).

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