doi: 10.4149/gpb_2022010

Aerobic exercise ameliorates myocardial ischemia/reperfusion injury and thrombosis of diabetic rats *via* activation of AMPK/Sirt1/PGC-1α pathway

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Abstract. This current work is aimed to make investigations for the action mechanism of aerobic exercise in rats with type 2 diabetes mellitus (T2DM) after myocardial ischemia/reperfusion injury (MI/RI). The high-fat diet was used to induce T2DM in male Wistar rats. After treatments, the rats in the exercise groups were underwent swimming training for 8 weeks. Two days later, all the rats were subjected to perform MI/RI experiments *via* left anterior descending artery ligation and reperfusion. The blood samples and myocardial tissues were collected for biochemistry analysis and histology assessment. The results demonstrated that aerobic exercise reduced the levels of serum glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and thrombosis in T2DM rats. In addition, aerobic exercise further decreased the levels of myocardial injury markers and also repressed inflammation responses. Furthermore, the AMP-activated protein kinase (AMPK)/silent information regulator factor 2-related enzyme 1 (Sirt1)/peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) pathway could be activated by aerobic exercise. In a word, aerobic exercise may attenuate myocardial ischemia/reperfusion injury and repress thrombosis *via* activation of the AMPK/Sirt1/PGC-1α pathway in DM rats.

Key words: Aerobic exercise — Diabetes mellitus — Myocardial ischemia/reperfusion injury — Thrombosis — AMPK/Sirt1/PGC-1α

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease, which is characterized by chronic hyperglycemia accompanied by impaired metabolism of carbohydrates, lipids and proteins, and lack of insulin secretion or decreased sensitivity to insulin metabolism (Motahari-Tabari et al. 2014). It is statistically that there are approximately 330 million adults suffering from DM worldwide and this number will reach 700 million in 2045 (Saeedi et al. 2019). Among different subtypes of DM, type 2 diabetes mellitus (T2DM) is the most prevalent and accounts for more than 90% of all cases (Shaw et al. 2010). Shockingly, there are more than 100 complications of T2DM, which is the disease with the most known complications (Zheng et al, 2018). Among these complications, cardiovascular diseases and thrombosis are the leading causes of mortality for T2DM (Chung et al. 2015; Saeedi et al. 2019). Therefore, exploring effective therapies to decrease the morbidity of cardiovascular diseases is imperative for T2DM treatments.

Of all the treatment options available, taking medications and diet control, in combination with aerobic exercise are considered to effectively delay the occurrence and development of DM complications (Hussey et al. 2012; Mullugeta

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et al. 2012; Karstoft and Pedersen 2016), pointing out the important role of aerobic exercise in DM treatments. For instance, Hussey et al. (2012) demonstrated that for T2DM patients, aerobic exercise can increase the absorption of glucose in skeletal muscle. Mullugeta and colleagues indicated that aerobic exercise can elevate the activity of hormone-sensitive triglyceride lipase, and thus promote the hydrolysis of triglyceride (Mullugeta et al. 2012). Karstoft and Pedersen (2016) found that regular aerobic exercise not only reduces the secretion of inflammatory cytokines tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) and represses the infiltration of inflammatory cells, but also promotes some anti-inflammatory factors such as IL-10 to be released into the blood). More importantly, clinical research conducted by Wang et al. (2015) believed that regular exercise can effectively enhance the tolerance of myocardial cells against ischemia/reperfusion injury. However, the possible action mechanism of exercise on myocardial ischemia/reperfusion injury (MI/RI) remains unclear.

The AMP-activated protein kinase (AMPK)/silent information regulator factor 2-related enzyme 1 (Sirt1)/peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) is a classical pathway that has been reported to be deeply involved in energy metabolism (Chau et al. 2010; Yang et a. 2020). Yang et al. (2020) have revealed that AMPK/Sirt1/PGC-1α signaling pathway is associated with mitochondrial biogenesis and function. Chau et al. (2010) have found that fibroblast growth factor 21 (FGF21) can activate the AMPK/Sirt1/PGC-1α pathway, enhance the oxidative capacity of mitochondrial, and promote oxygen consumption in the human body). In addition, AMPK/Sirt1/PGC-1α seems to be also correlated with the progression of DM (Jiang et al. 2015; Hou et al. 2017; Huang et al. 2019; Liao et al. 2019; Yap et al. 2020). More importantly, numerous studies have demonstrated that the activation of the AMPK/Sirt1/PGC-1α pathway attenuates MI/RI in a rat model (Meng et al. 2019; Tian et al. 2019). Interestingly, some pharmacological agents such as metformin and catalpol can enhance insulin sensitivity *via* activation of the AMPK/Sirt1/PGC-1α pathway (Banerjee et al. 2016; Yap et al. 2020). In addition, Cho et al. (2015) have indicated that exercise training can improve insulin resistance *via* adiponectin receptors. Nevertheless, the accurate function of the AMPK/Sirt1/PGC-1α pathway on DM-induced MI/RI complication and the interaction with aerobic exercise is still unknown.

In the current study, the effects of aerobic exercise (swimming) on DM rats underwent MI/RI surgery and the relationships with the AMPK/Sirt1/PGC-1α pathway were investigated. The findings uncover an underlying downstream regulatory pathway of aerobic exercise on DM-induced MI/RI complication.

Methods

Animals

Forty male Wistar rats (150–200 g, 10 weeks) were purchased from EseBio, Co., Ltd (Shanghai, China). The rats were allowed to adapt to the laboratory environment with a normal diet for 1 week before testing. All animal experiments in this study were in strict accordance with the protocols stated in the Guide for the Care and Use of Laboratory Animals and approved by the ethical committee of Henan University.

DM rat model and swimming exercise

After habituation, the rats were assigned into four groups ($n = 10$ in each) *ad libitum*: the control (Con) group, the control + exercise (Con+Ex) group, the diabetes mellitus (DM) group, and the diabetes mellitus + exercise (DM+Ex) group. To induce a T2DM rat model, the rats in the DM groups were fed with a high-fat diet (formula: 3% cholesterol, 0.3% sodium cholate, 5% yolk powder, 0.2% propylthiouracil, 5% sugar, 10% lard, 15% whole milk powder, and 61.5% basic diet) for 4 weeks. Meanwhile, streptozotocin (20 mg/kg/day; Sigma Aldrich, San Luis, MO, USA) was intraperitoneally (i.p.) injected into the rats on day 1, 3, 5, 21, 23, and 25, respectively. The rats in the Con and DM+Ex groups were still fed with a normal diet and i.p. injected with citrate buffer at the same time points. Before aerobic exercise, the rats with blood glucose levels over 300 mg/dl were considered as diabetic, while the blood glucose levels of control rats were in the range of 97.32 to 98.87 mg/dl. After treatments, the rats in the Con+Ex and DM+Ex groups were underwent swimming training for 8 weeks (90 min/day). The swimming exercises were lasting for 8 weeks.

Insulin resistance sensitivity assay by short insulin tolerance test (ITT) using capillary blood glucose

Rats were weighed and placed into mouse cages after fasting overnight. Blood sugar in rats was detected six times after i.p. insulin (0.05 U/kg) using a blood sugar detector. Abscissa indicates time and ordinate expresses nature logarithm of blood sugar. Regression coefficient (r) or slope was determined by linear regression and glucose disappearance constant (K_{ITT}) was calculated by multiplying r by 100. K value indicates insulin sensibility with smaller K values for lower sensibilities.

Hyperinsulinemic-euglycemic clamp experiment

Food was withdrawn 12 h before the experiment. The rats were then anesthetized by amobarbital sodium (i.p.,

50 mg/kg) after they had been weighed. Rats were cannulated in the jugular vein for infusion of glucose and insulin (dual cannula) and in the carotid artery for sampling. All catheters were tunnelled subcutaneously, and encased in silastic tubing (0.08 cm) sutured to the skin. After infusion of glucose (10%) and insulin (1 IU/ml) from dual cannula (constant velocity), blood sugar was measured. To keep the blood sugar in a relatively steady state, the rate of glucose infusion was continuously adjusted. Glucose injection rate (GIR) was measured under homeostasis six times during the experiment.

Experiment protocol for MI/RI

Approximately 48 h after the last swimming exercise, MI/RI was performed *via* ligation of the left anterior descending (LAD) coronary artery as previously described (Niu et al. 2020). In brief, rats in the above four groups were anticoagulanted by i.p. injection of heparin sodium (1000 IU/kg). Afterwards, rats were received endotracheal intubation, and the trachea was cannulated by a polyethylene tube connected to a rodent respirator with a tidal volume of 60 breaths/min. A 6-0 prolene suture was used for ligating LAD to induce ischemia. Half an hour later, the ligature was loosened to reperfuse for 3 h.

Platelet aggregation analysis

Blood samples (3 ml) were collected from carotid artery, added sodium citrate for anticoagulation, and centrifuged (25°C, 500 × *g* for 8 min) for the preparation of platelet-rich plasma (PRP). Meanwhile, the platelet-poor plasma (PPP) was obtained with centrifugation (25°C, 1900 × *g* for 10 min). PRP was induced using adenosine diphosphate (ADP, 4 μM) or thrombin (5 μM). Approximately 10 min later, the platelet aggregation (%) was calculated using PACKS-4 aggregometer (Helena Laboratories). Additionally, activated partial thromboplastin time (APTT) was measured by an ELISA kit (Jiancheng Chemical, Nanjing, China).

Assessment of MI/RI and biochemical indexes

After reperfusion, blood samples (5 ml) from each rat were collected and centrifuged. The biomarkers of myocardial injury such as creatine kinase-MB (CK-MB) (Logotech India Pvt, Delhi, India), lactic dehydrogenase (LDH), cardiac troponin I (cTnl), and myoglobin (Mb) (Abcam, Cambridge, UK), the levels of biochemical indexes such as glucose (Sigma Aldrich, San Luis, MO, USA), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) (SPINREACT, Girona, Spain), and the levels of inflammatory markers such as TNF-α, IL-6, and IL-10 (Abcam) in serum of rats with MI/RI were measured by the corresponding commercial kits.

Measurement of heart weight index

The body weight (BW) of rats was measured. After sacrifice of rats, the hearts were rapidly removed, washed in cold saline and weighed. Subsequently, the heart weight index (HWI) was calculated (HWI = weight of the heart/BW).

Hematoxylin-eosin (HE) staining assay

The myocardial tissues of rats were fixed in 4% paraformaldehyde for one day, followed by embedding in paraffin sectioned at 5 μm thickness. All the sections were stained with HE staining immediately and then were observed by light microscopy.

Western blot assay

The levels of AMPK/Sirt1/PGC-1α pathway-related proteins (p-AMPK, AMPK, Sirt1, and PGC-1α) in cardiac tissues of MI/RI rats were determined by Western blotting. The proteins were lysed with RIPA buffer, and then determined the concentrations using a BCA Protein Assay Kit (Thermo Fisher Scientific). After that, the proteins were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto polyvinyldifluoride (PVDF) membranes. The relevant primary antibodies (1:1,000) were incubated at 4°C for overnight and then the secondary antibody (1: 5,000) for 1 h at room temperature. GAPDH was used as the internal control. Immunoblottings were visualized using an ECL detection kit (Amersham Biosciences, Sweden).

Statistical analysis

SPSS 20.0 software (Chicago, USA) was used for statistical analysis. One-way ANOVA followed by Tukey's multiple comparisons test was used to assess the experimental data. Data were shown as means ± SD. *p*-value less than 0.05 indicated a statistically significant difference.

Results

Swimming exercise ameliorates the hyperglycemia and hyperlipidemia of DM rats

To assess the possible function of swimming exercise on hyperglycemia and hyperlipidemia in DM rat models, the levels of serum glucose, TC, TG, HDL-C, and LDL-C were

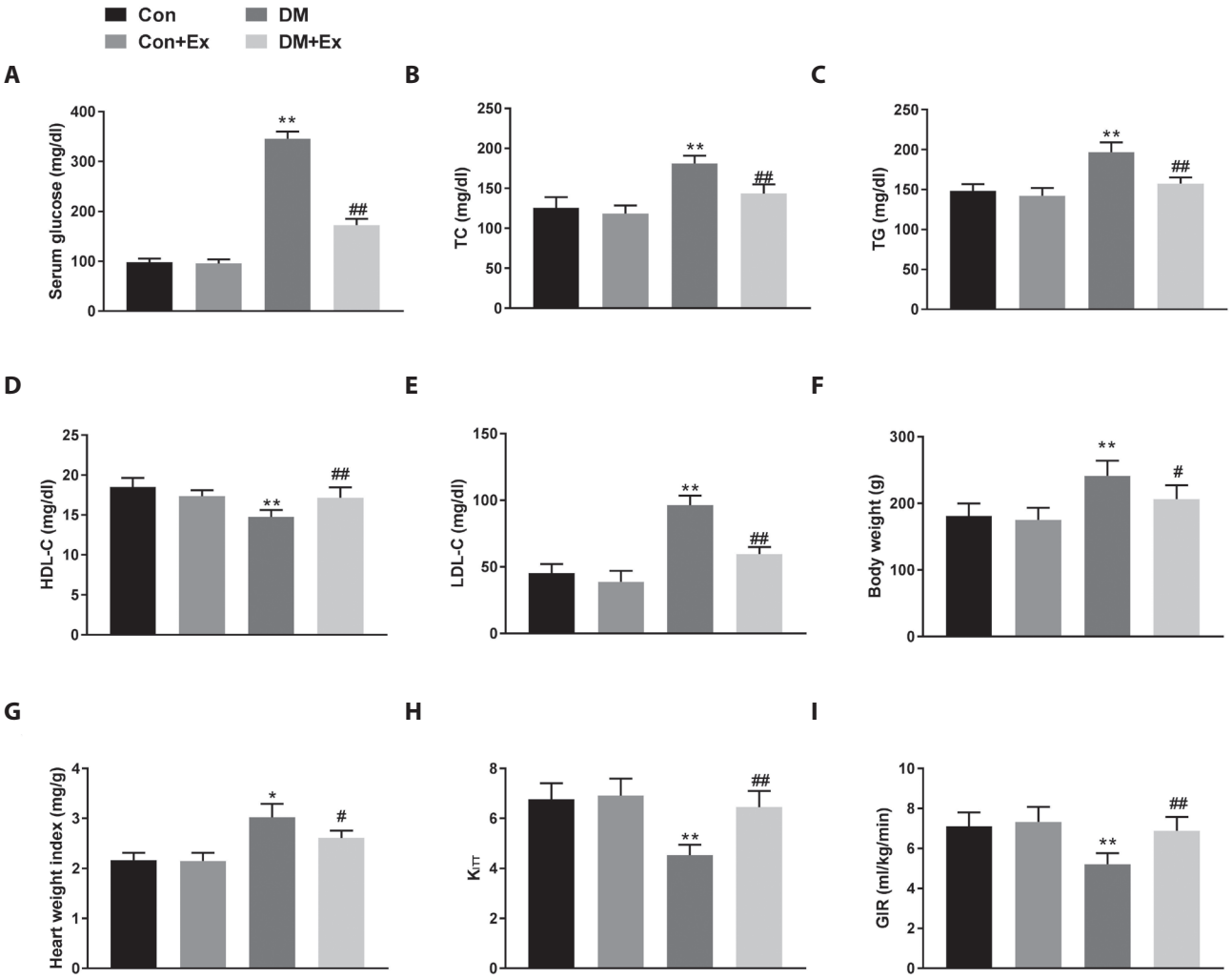

Figure 1. Swimming exercise ameliorates the hyperglycemia and hyperlipidemia of diabetes mellitus rats. The level of serum glucose (**A**), total cholesterol (TC; **B**), triglyceride (TG; **C**), high-density lipoprotein cholesterol (HDL-C; **D**), and low-density lipoprotein cholesterol (LDL-C; **E**) of rats in Con, Con+Ex, DM and DM+Ex groups. Body weight (**F**) and heart weigh index (**G**) of rats were calculated. Results of insulin resistance sensitivity assay in changes of glucose induced by insulin injection (**H**) and hyperinsulinemic-euglycemic clamp test (**I**). * $p < 0.05$, ** $p < 0.01$ *vs*. Con group; # $p < 0.05$, ## $p < 0.01$ *vs*. DM group. Con, control; DM, diabetes mellitus; Ex, exercise; GIR, glucose injection rate; K_{ITT}, glucose disappearance constant.

measured. As illustrated in Figure 1A–E, the levels of serum glucose, TC, TG, and LDL-C in DM rats were significantly increased, whereas HDL-C level was decreased ($p < 0.01$) when compared to the rats of the control group. Interestingly, exercise effectively reduced the hyperglycemia and hyperlipidemia caused by DM ($p < 0.01$). Additionally, the BW and HW index in the DM group was higher than in the control group, while it was partially decreased in the DM+Ex group (Fig. 1F and G, $p < 0.05$). The results of short insulin tolerance test using capillary blood glucose revealed that K_{ITT} was decreased in DM rats, and was increased after exercise (Fig. 1H, $p < 0.01$). The hyperinsulinemic-euglycemic clamp test showed similar results in GIR of different groups (Fig. 1I, $p < 0.01$).

Swimming exercise inhibits the thrombosis of DM rats

As presented in Figure 2A, the DM group showed significantly increased platelet aggregation induced by ADP when compared to the control ($p < 0.05$). As expected, swimming exercise repressed the excessive aggregation of platelets ($p < 0.05$). Meanwhile, I also found that there were no significant differences in thrombin-induced platelet aggregation between the DM and DM+Ex groups (Fig. 2B). Additionally,

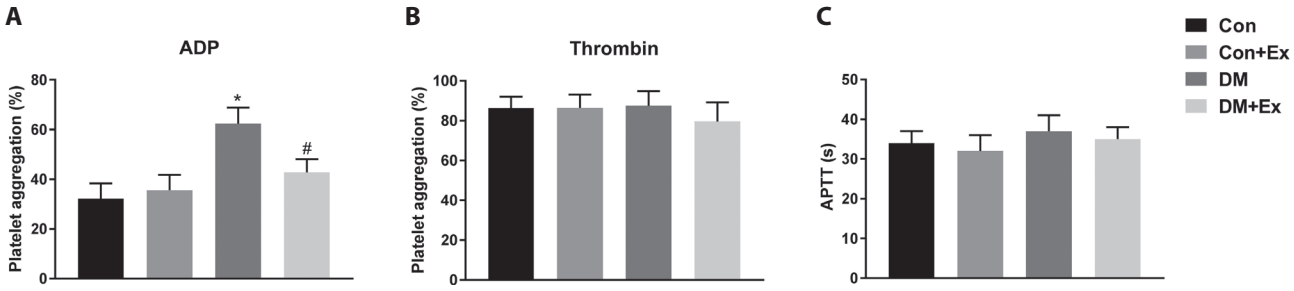

Figure 2. Swimming exercise inhibits the thrombosis of DM rats. The platelet aggregation in rats of different groups response to adenosine diphosphate (ADP; **A**) and thrombin (**B**). **C.** The activated partial thromboplastin time (APTT) in rats of different groups was measured by the ELISA kit. * $p < 0.05$ *vs*. Con group; # $p < 0.05$ *vs.* DM group. For abbreviations, see Fig. 1.

also no significant changes were found in APTT of the four groups (Fig. 2C).

Swimming exercise improves MI/RI in DM rats

Emerging evidence has demonstrated that aerobic exercise promotes myocardial tolerance to MI/RI injury in animal models (Powers et al. 2008). Therefore, the effect of swimming exercise on cardioprotection against MI/RI was explored. As shown in Figure 3A–D, the levels of cardiac biomarkers (CK-MB, LDH, cTnl, and Mb) were all significantly elevated in DM rats underwent MI/RI experiments ($p < 0.05$). As expected, exercise training reversed the adverse effects of MI/RI on DM rats ($p < 0.05$). In addition, HE staining assay further indicated that the ratio of inflammatory cells in MI/RI tissues of DM rats was remarkably increased, whereas the infiltration of inflammatory cells was significantly suppressed after exercise (Fig. 3E).

Swimming exercise exhibits an anti-inflammation role in DM rats with MI/RI

TNF-α and IL-6 are two common inflammatory cytokines existed in MI/RI (Zhang et al. 2020), and IL-10 has been confirmed to play a protective role in MI/RI rats (Hou et al. 2020). Based on these previous results, the levels of TNF-α,

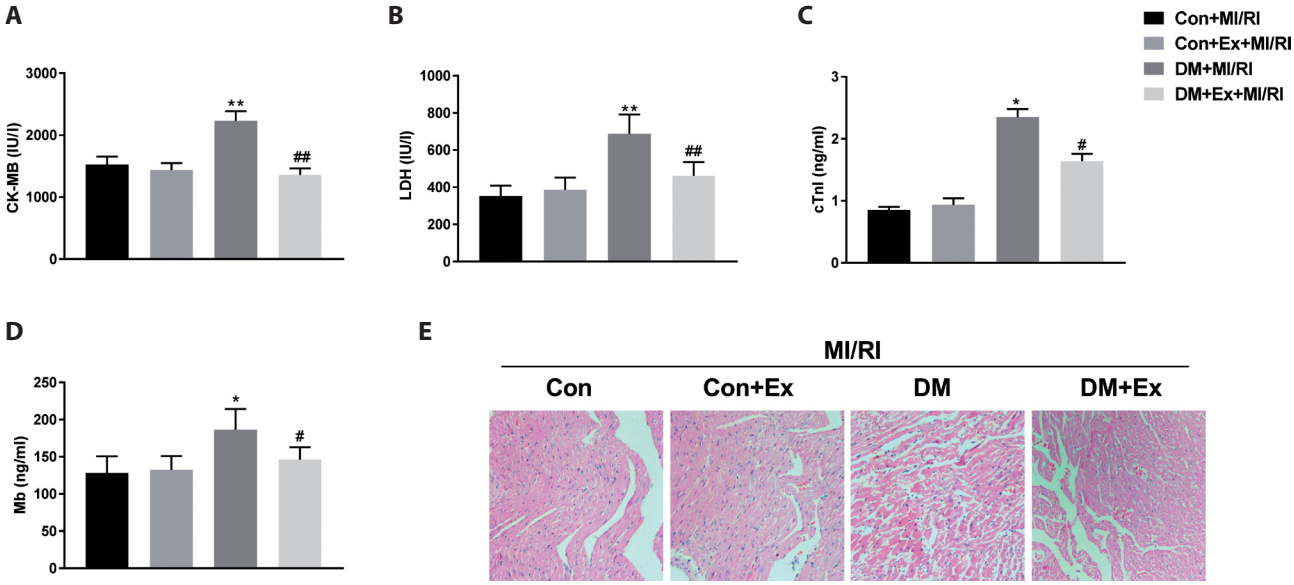

Figure 3. Swimming exercise improves myocardial ischemia/reperfusion injury (MI/RI) in DM rats. The level of creatine kinase-MB (CK-MB; **A**), lactic dehydrogenase (LDH; **B**), cardiac troponin I (cTn1; **C**) and myoglobin (Mb; **D**) in rats of different groups after MI/RI. **E.** The histology of myocardial tissues in rats of different groups after MI/RI was assessed by hematoxylin-eosin (HE) staining assay. * $p < 0.05$, ** $p < 0.01$ *vs*. Con group; # $p < 0.05$, ## $p < 0.01$ *vs*. DM group. For abbreviations, see Fig. 1.

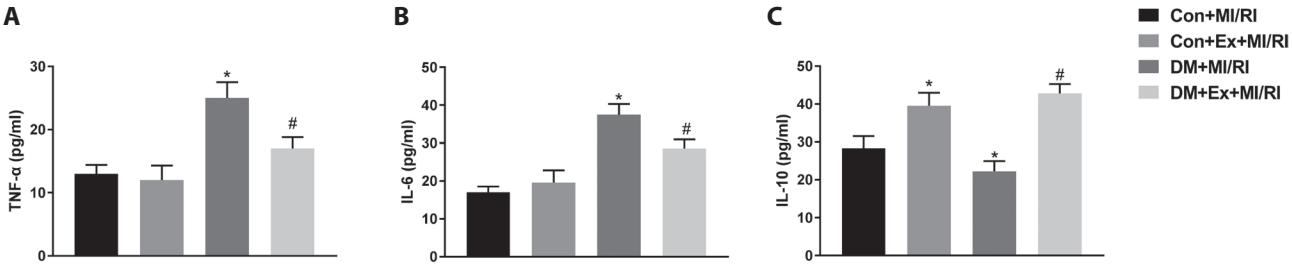

Figure 4. Swimming exercise exhibits an anti-inflammation role in DM rats with myocardial ischemia/reperfusion injury (MI/RI). The level of tumour necrosis factor-alpha (TNF-α; **A**), interleukin 6 (IL-6; **B**) and interleukin 10 (IL-10; **C**) in rats of different groups after MI/RI. * $p < 0.05$ *vs*. Con group; # $p < 0.05$ *vs*. DM group. For abbreviations, see Fig. 1.

IL-6, and IL-10 in the serum of rats with MI/RI were assessed. I found that both the secretion of TNF-α and IL-6 were remarkably enhanced in DM rats with MI/RI compared to controls with MI/RI ($p < 0.05$, Fig. 4A and B). Unsurprisingly, swimming exercise is helpful for the decrease of TNF-α and IL-6 ($p < 0.05$). Interestingly, compared to the control group, IL-10 level was increased in the Con+Ex group and was reduced in the DM group ($p < 0.05$, Fig. 4C). Meanwhile, the level of IL-10 was also elevated in the DM+Ex group relative to the DM group ($p < 0.05$).

The AMPK/Sirt1/PGC-1α pathway is activated by swimming exercise

It is noticed that the activation of the AMPK/Sirt1/PGC-1α pathway protects against MI/RI in murine models (Tian et al. 2019; Wu et al. 2020). Therefore, the interactions between exercise training and AMPK/Sirt1/PGC-1α pathway-related proteins were investigated. As shown in Figure 5A–D, the protein levels of p-AMPK/AMPK and Sirt1 in DM rats with MI/RI were significantly inhibited ($p < 0.05$). In addition, there seemed no significant difference between the Con and DM groups at PGC-1α protein level. However, swimming exercise effectively increased the levels of those proteins ($p < 0.05$), implying that the AMPK/Sirt1/PGC-1α pathway may be activated by exercise training.

Discussion

DM is a common metabolic disorder with high mortality and morbidity all over the world and is generally accompanied

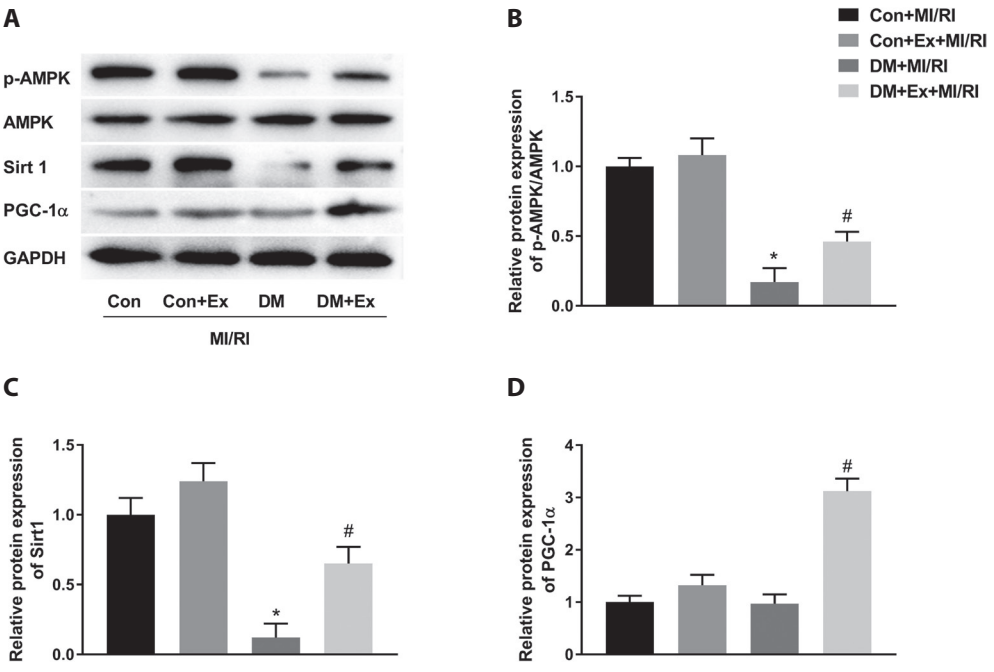

Figure 5. The AMP-activated protein kinase (AMPK)/silent information regulator factor 2-related enzyme 1 (Sirt1)/peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) pathway is activated by swimming exercise. **A.** The Western blotting of AMPK/Sirt1/PGC-1α pathway-related proteins. The level of p-AMPK/AMPK (**B**), Sirt1 (**C**) and PGC-1α (**D**) in rats of different groups after myocardial ischemia/reperfusion injury (MI/RI). * $p < 0.05$ *vs*. Con group; # $p < 0.05$ *vs*. DM group. For abbreviations, see Fig. 1.

by multiple complications including cardiovascular diseases, the leading causes of mortality for DM patients (Whiting et al. 2011; Saeedi et al. 2019). Therefore, exploring the effective methods for DM and cardiovascular complication therapy is a main social problem to be solved worldwide. Aerobic exercise is a conventional and integrated therapeutic management with a long history to regulate metabolism (Tipton 2014). This study focused on the detailed effects of aerobic exercise on MI/RI in DM rats, and revealed the AMPK/Sirt1/PGC-1α pathway may be activated by aerobic exercise to attenuate MI/RI in DM rats.

The features of hyperglycemia and hyperlipidemia existed in most DM patients, which are associated with metabolic disorder and also an increased risk of cardiovascular complication (Eckel et al. 2005). Hyperglycemia means high concentration of blood glucose, and high levels of TC, TG, and LDL-C are closely correlated with hyperlipidemia (Kannel 1985; Eckel et al. 2005). In this study, it was found that rats in the DM group had significantly high levels of serum glucose, TC, TG, and LDL-C, and relatively low HDL-C concentration, suggesting that DM rat model was established successfully. Meanwhile, the level of serum glucose was reduced in DM rats with swimming exercise. Similarly, Sigal and colleagues believed that aerobic exercise enhances the ability of muscles to uptake dissociative glucose and therefore glucose level in blood is decreased (Sigal et al. 2007). Additionally, the dyslipidemia of DM rats was also improved after swimming training. I speculated aerobic exercise such as swimming is also helpful for the decrease of blood lipids. It is reported that DM can induce vascular injury, alter platelet function, increase the synthesis of thromboxane, and eventually accelerate thrombus formation (Chung et al. 2015). Interestingly, Lundberg Slingsby and colleagues conducted research on the association between exercise and platelet aggregation in pre- and postmenopausal women; they indicated that platelet aggregation was significantly repressed by aerobic exercise (Lundberg Slingsby et al. 2017). Based on these previous results, I speculated aerobic exercise may be also effective for the decrease of platelet aggregation in DM. In my current work it was found that in rat models, DM indeed increased the platelet aggregation response to ADP but not to thrombin, and more importantly, aerobic exercise could remarkably inhibit thrombus formation. It is well known that platelet aggregation is mediated by the activated glycoprotein IIb/IIIa (GP IIb/IIIa), which binds fibrinogen and von Willebrand factor at high affinity (Lefkovits et al. 1995). I speculated that there were no differences in the percentage of platelet aggregation among the four groups because thrombin may act directly on the GP IIb/IIIa receptor and activate it, and it seems to be not affected by diabetes or aerobic exercise. Interestingly, Smyth et al. (2009) have indicated that thrombin can bind to protease-activated receptors (PARs) and further activate GP IIb/IIIa receptors *via* phospholipase C (PLC) and protein kinase C (PKC) pathways, which may validate my hypothesis to some extent. In addition, ADP and its analogues bind to P2 receptors, which are expressed ubiquitously throughout the human body including on platelets (Di Virgilio and Solini 2002). Stable and firm platelet aggregation requires ADP binding to P2Y12 receptor and activation of GP IIb/IIIa receptors in the platelet membrane (Nguyen et al. 2005). These data suggested that ADP may be an important factor in activator GPIIb/IIIa receptors. Additionally, previous clinical reports have indicated that suppression of central adrenergic activity or catecholamine release by clonidine and reserpine reduced the diabetes-enhanced ADP-induced platelet aggregation in diabetic animals (Dunbar et al. 1990). I further speculated that the increased responsiveness of platelets to ADP-induced aggregation may be coupled to an adrenergic-mediated mechanism. But the detailed mechanisms need to be explored in the future.

MI/RI experimental scheme in DM rats is generally used for the research of cardiovascular complication (Ranjbar et al. 2018); Sharma et al. 2019). CK-MB, LDH, cTnl, and Mb mainly expressed in heart are the most sensitive and specific biomarkers for the assessment of acute myocardial damage (Ranjbar et al. 2018; Xie et al. 2020). Herein, I demonstrated that the levels of CK-MB, LDH, cTnl, and Mb were all increased in DM rats but were repressed after aerobic exercise, which was consistent with the previously described (Sharma et al. 2019). It is noticed that both inflammatory cytokines and anti-inflammation factors are deeply involved in not only the progression of DM but also cardiovascular complication (Sharma et al. 2019; Dallak et al. 2020; Xie et al. 2020). Similarly, HE staining assay indicated that inflammatory cells were remarkably infiltrated into myocardial tissues of DM rats underwent MI/RI surgery. Interestingly, inflammatory infiltration was significantly suppressed in the above rats after swimming exercise. The ELISA for the measurement of TNF-α, IL-6, and IL-10 further validated these results. Therefore, I believed that aerobic exercise could attenuate the inflammatory reactions existing in DM and cardiovascular complication.

It is acknowledged that the activated AMPK/Sirt1/PGC-1α pathway can facilitate mitochondrial metabolism and improve muscular dysfunction Chau et al. 2010; Yu et al. 2018; Yang et al. 2020), and is also associated with DM and cardiovascular complication (Jiang et al. 2015; Hou et al. 2017; Huang et al. 2019; Liao et al. 2019; Meng et al. 2019; Tian et al. 2019; Yap et al. 2020). Given the important role of aerobic exercise such as swimming in the reduction of blood lipids, blood glucose, thrombus formation, and inflammation in DM and MI/RI, I speculated AMPK/Sirt1/PGC-1α may be activated by aerobic exercise. Therefore, AMPK/Sirt1/PGC-1α pathway-related proteins were deter-

mined. As illustrated in Figure 5, I indicated that the levels of p-AMPK/AMPK, Sirt1, and PGC-1α were inhibited in DM rats but were significantly elevated after swimming training, which confirmed my assumption. Therefore, it was believed that aerobic exercise may activate the AMPK/Sirt1/PGC-1α pathway to affect DM and cardiovascular complication. Additionally, the interaction of the AMPK/Sirt1/PGC-1α pathway with carbohydrate-lipid metabolism has been confirmed in numerous previous researches. AMPK is considered a key regulator of fat and carbohydrate metabolism (Canto and Auwerx 2009), and its activation enhances pathways that generate ATP, such as phosphorylation and activation of PGC-1α, glucose transport, and fatty acid oxidation, and inhibits ATP-consuming pathways including triglycerides and cholesterol synthesis (Winder 2000; Musi et al. 2001; Witczak et al. 2008). Furthermore, SIRT1 increases lipolytic rates in white adipose tissue (Picard et al. 2004) and insulin secretion in beta cells of the pancreas (Bordone et al. 2006). In addition, AMPK/Sirt1/PGC-1α pathway is also reported to be closely related to the weakening of MI/RI and thrombosis. For example, Liu et al. (2020) found that the AMPK/Sirt1/PGC-1α pathway can inhibit oxidative stress and inflammation in the progression of MI/RI. Kim et al. (2013) demonstrated that resveratrol can interact with AMPK/Sirt1/PGC-1α pathway to protect against thrombosis and renal lipotoxicity in *db/db* mice. I speculated that aerobic exercise may also directly target the AMPK/Sirt1/PGC-1α pathway to affect these pathological processes.

Conclusion

Taken together, aerobic exercise ameliorates MI/RI and thrombosis in diabetic rats *via* activation of the AMPK/Sirt1/PGC-1α pathway. These findings clarify the action mechanism of aerobic exercise on the progression of DM and related cardiovascular complication. Support for the use of aerobic exercise may be strengthened by understanding the action mechanism and establishing clinical indications and appropriate recommendations.

Statement of ethics. This study was conducted after obtaining Biomedical Research Ethics Committee of Henan University's approval (No. HUSOM2021-192).

Conflict of interest. The authors have no conflicts of interest to declare.

Funding. Not applicable.

Data availability statement. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Received: October 25, 2021
Final version accepted: February 16, 2022