Exercise Improves Cardiac Function in the Aged Rats With Myocardial Infarction

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Summary

Exercise can improve the cardiovascular health. However, the mechanism contributing to its beneficial effect on elderly patients with myocardial infarction is obscure. 20-month-old male Sprague-Dawley rats were used to establish myocardial infarction (MI) model by permanent ligation of the left anterior descending coronary artery (LAD) of the heart, followed by 4-week interval exercise training on a motor-driven rodent treadmill. The cardiac function, myocardial fibrosis, apoptosis, oxidative stress, and inflammatory responses were determined by using pressure transducer catheter, polygraph physiological data acquisition system, Masson's trichrome staining, and ELISA to evaluate the impact of post-MI exercise training on MI. Western blot were performed to detect the activation of AMPK/SIRT1/PGC-1α signaling in the hearts of aged rats. Exercise training significantly improved cardiac function and reduced the cardiac fibrosis. In infarcted heart, the apoptosis, oxidative stress, and inflammation were significantly reduced after 4-week exercise training. Mechanistically, AMPK/SIRT1/PGC-1α pathway was activated in the myocardial infarction area after exercise training, which might participate in the protection of cardiac function. Exercise training improves cardiac function in MI rats through reduction of apoptosis, oxidative stress, and inflammation, which may mediate by the activation of AMPK/SIRT1/PGC-1α signaling pathway.

Key words

Myocardial infarction • Exercise • Apoptosis • Oxidative stress • Inflammation

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Introduction

Myocardial infarction (MI) is one of the highest morbidity and mortality cardiovascular diseases, and its incidence increases sharply with age in the world [1]. In the past decades, MI cases have increased dramatically, for example, there were 16 million new cases occurred in 2015 alone [2,3]. With the growth of aged population globally, the importance of prevention and treatment of MI will further increase. The prevalence of myocardial infarction in the elderly is much higher than that in the young, and effective interventions are urgently needed for adjuvant therapy [4]. There is a growing clinical consensus that exercise training may help to change myocardial remodeling and improve cardiac function after myocardial infarction [5-7], but the related mechanism is still controversial, and there are gaps in research on the elderly.

Myocardial ischemia is the major cause of MI, which promotes the overexpression of reactive oxygen species (ROS) in the injured myocardium [8]. ROS overproduction after MI increase the oxidative stress responses and the production of inflammatory cytokines, and then disrupt the membranes of myocardium, which cause serious necrosis and apoptosis of infarcted heart [9]. Both myocardium injury and cell death lead to the inflammatory responses and cardiac remodeling, such as hypertrophy of cardiomyocyte of the

surrounding myocardium and cardiac fibrosis [10,11]. These remodeling of cardiomyocytes result in deterioration of cardiac function and eventually lead to cardiac dysfunction and heart failure [12]. Large number of signal pathways altered during the above pathological processes, including PTEN/PI3K/Akt, Akt3/mTOR, NF-κB, and AMPK/SIRT1/PGC-1α signaling pathways [13-16]. Zeng *et al.* reported that enhancing AMPK-dependent autophagy can improve MI injury in rat model [16]. Quercetin administrations improve I/R-induced cardiomyocyte apoptosis through activating the SIRT1/PGC-1α signaling pathway *in vitro* and *in vivo* [17].

As a non-pharmacological intervention, appropriate exercise training has been reported to have benefits for improving cardiovascular functions in healthy population and cardiovascular patients [18,19]. Post-MI exercise training can change the MI-induced myocardial remodeling and improve the cardiac function [20]. Moreover, exercise training also promotes the production of antioxidant and improve the cardiorespiratory capacity in heart failure patients [21]. Although studies have shown that exercise can improve the cardiovascular health, the impact of exercise on elderly patients with myocardial infarction is currently unclear. In current study, the elderly MI rats were used as the research object to explore the impact of exercise on MI. We found that the heart function, myocardial fibrosis, apoptosis, inflammation, oxidative stress of the elderly MI rats in the exercise group were improved. AMPK/SIRT1/PGC-1α pathway is activated in the myocardial infarction area, which participates in the protection of cardiac function. Therefore, moderate exercise may become a new intervention method for the elderly patients with myocardial infarction.

Materials and Methods

Animals

Aged Sprague-Dawley male rats (20-month-old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats were housed under the 12/12 light/dark cycles and 22-24 ℃ conditions with free access for food and water. All animal experiments were performed according to the protocol established by the University Committee on Use and Care of Animals at the Affiliated Wuxi No. 2 People's Hospital of Nanjing Medical University.

Myocardial infarction model

The rat MI surgery was carried out by permanent ligation of the left anterior descending coronary artery, flowing the protocols published previously [22,23]. During the acute phase of MI, 4 rats died and 2 rats without ST segment elevation were excluded in this study. A successful operation of surgery was confirmed by sign of MI, and electrocardiogram was used to confirm the regional myocardial ischemia. The Sham and MI rats were divided into four groups: Sham, Sham+exercise (Sham+Ex), MI, MI+Ex, with 10 rats for each group.

Post-MI exercise training

Both Sham+Ex and MI+Ex groups were subjected to interval exercise training on the motor-driven rodent treadmill (Harvard Apparatus, Holliston, USA). The first week after MI, rats were allowed to recover. One week after MI, the rats received adaptive exercise training for one week (10 m/min for 30 min), and then daily exercise. From the second week, rats performed daily interval exercise training at treadmill speeds of 10 m/min (10 min), then 25 m/min (7 min), and 15 m/min (3 min). This daily exercise training was carried out 5 days/week for four weeks.

Cardiac function analysis

At the end of the experiment, rats were anesthetized using 30 mg/kg pentobarbital. The pressure transducer catheter (Utah Medical Products, Inc., Midvale, USA) and Powerlab ML 870 polygraph physiological data acquisition system (AD Instruments, Sydney, Australia) were used to quantify the left ventricle end diastolic pressure (LVEDP), left ventricle systolic pressure (LVSP), and maximal positive and minimal negative first derivative of LV pressure (±dp/dt maximum). Rats were euthanized after the measurement and left ventricle of peri-infarcted zone was used for molecular biological and histological analyses.

Masson's trichrome staining

Collagen volume fraction (CVF) was quantified with the infarct ratio after Masson's trichrome staining by using the ImageJ software (NIH, Bethesda, USA). Briefly, heart tissues were fixed by 4 % PFA for 2 days and embedded into paraffin blocks, and then cut into sections with 7 μm thickness. Sections were performed Masson's trichrome staining, and cardiac fibrosis status of myocardium infarction in aged rat was evaluated by the collagen volume fraction, which defines as the sum fall the connective tissue areas of the entire section, following the previous protocol [24].

Western blot

Left ventricle samples of peri-infarcted zone were homogenized and lysed by using the Cole-Parmer LabGEN 125 Homogenizer (Cole-Parmer, Vernon Hills, USA) and Beyotime RIPA lysis buffer (Shanghai, China). Total protein concentration was determined by BCA protein assay (Thermo Fisher Scientific, Waltham, USA). Target protein levels were analyzed by Western blot as described previously [25]. The primary antibodies of Bax (#14796, 1:1000 dilution), Bcl-2 (#2764, 1:1000 dilution), SirT1 (#8469, 1:1500 dilution), and PGC-1α (#2178, 1:1000 dilution) were ordered from Cell Signaling Technology, Inc. (Danvers, USA); primary antibodies of p-AMPK (T172, ab133448, 1:1000 dilution), AMPK (ab110036, 1:1500 dilution), and GAPDH (ab9485, 1:2000 dilution) were purchased from Abcam plc. (Cambridge, United Kingdom). Each group had 3 samples, and the protein levels were quantified by using Image J software (NIH, Bethesda, USA).

ELISA

The protein levels of oxidative stress makers and inflammatory cytokines released by heart tissues were determined by using the commercial ELISA kits. Superoxide dismutases (SOD) and Glutathione (GSH) were determined by Beyotime Superoxide Assay Kit (S0060) and Total Glutathione Assay Kit (S0052), respectively. Thiobarbituric Acid Reactive Substances (TBARS) was measured by the TBARS Parameter™ Kit (R&D Systems (Montgomery, USA). The concentration of IL-1β, IL-6, and TNF-α were determined by commercial kits ordered from Thermo Fisher Scientific (Waltham, USA).

Statistical analysis

All statistical analyses in this study were performed by using the Prism 8.0 software. One-way ANOVA analysis with a *post hoc* test was used to analyze the differences between groups. All data were represented as mean ± standard deviation (SD). It was considered statistically significant when p value was less than 0.05.

Results

Exercise alleviates MI-induced cardiac dysfunction

Twenty-month-old Sprague-Dawley rats were used to establish MI model; male rats were used in this study to avoid that hormone level changes in elderly female rats influence the results. To investigate the benefits of post-exercise training on MI-induced heart damage, we performed cardiac function analysis at the end of training. As shown in Figure 1a, MI significantly reduced the LVSP of rats compared to that in Sham group. Post-exercise training improved LVSP of Sham rats, interestingly, exercise also significantly restored the MI-induced LVSP reduction. Similar ameliorative result of post-exercise training on MI was observed on LVEDP (Fig. 1b). Moreover, the maximum time derivatives of the pressure change during left ventricular contraction (+dp/dt) and relaxation (-dp/dt) were significantly improved after 4 weeks of exercise training in both Sham and MI rats (Fig. 1c, 1d). We also recorded the body weight and heart rate of treated rats at the beginning and end of the experiment, interestingly, exercise could significantly increase body weight (Fig. S1a) and reduce heart rate (Fig. S1b) of Sham and MI tarts. All these data suggested that exercise alleviates MI-induced cardiac dysfunction.

Exercise reduces MI-induced cardiac fibrosis

Cardiac fibrosis is one of the important indicators of heart function [26,27], hence, we measured the cardiac fibrosis analysis of myocardium infarction in treated rats. Although there was no change of CVF between exercise and non-exercise rats in Sham groups, post-exercise training decreased the CVF in MI rats significantly (Fig. 2).

Exercise improves apoptosis in myocardium of MI rats

To explore the underlying mechanism of exercise on MI amelioration, we performed Western blot to detect the apoptotic markers in myocardium. In comparison with non-exercise Sham rats, apoptosis regulator Bax protein level was decreased after exercise and increased in MI heart; however, post-exercise training significantly reduced the MI-induced Bax elevation (Fig. 3a, 3b). In contract, Bcl-2 protein level was decreased in the myocardium of MI rats, which was restored by post-exercise training (Fig. 3a, 3c). The above data indicated that post-exercise training suppresses MI-induced apoptosis in myocardium of rats.

Exercise suppresses oxidative stress responses and inflammatory cytokines release in heart tissues of MI rats

Next, oxidative stress and inflammatory responses, two major risk factors of myocardium [28,29], were detected by ELISA. The concentration of

antioxidants, SOD and GSH, was decreased in MI rats and restored in MI+Ex rats significantly (Fig. 4a, 4b). In contract, oxidative stress marker TBARS increased in MI heart and reduced after exercise training (Fig. 4c). The release of inflammatory cytokines, IL-1β, IL-6, and TNF-α, increased in MI heart significantly; upon 4 weeks of exercise training, their releases had dropped significantly (Fig. 5a-c).

Exercise activates AMPK/SIRT1/PGC-1α signaling pathway in MI heart

To further reveal the molecular regulation of exercise on MI damage, we detected the activation of AMPK/SIRT1/PGC-1α signaling in control and MI heart. MI surgery significantly decreased the phosphorylation level of AMPK in myocardium, 4 weeks of exercise training was able to restore the activation of AMPK signaling (Fig. 6a and 6b). Similar rescue effect of post-exercise training on MI damage was observed on the expression of SIRT1 and PGC-1α (Fig. 6a, 6c, and 6d). Interestingly, the activation of AMPK/SIRT1/PGC-1α signaling only happened in MI heart instead of Sham heart. These results suggested that post-exercise training improves cardiac function might through activation of AMPK/SIRT1/PGC-1α signaling pathway.

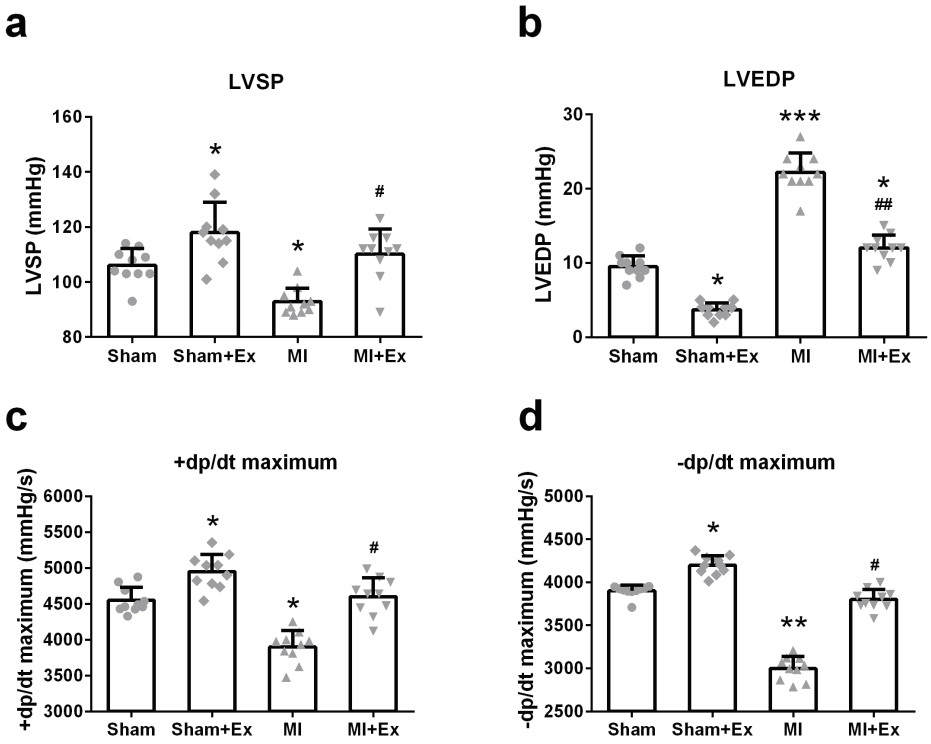

Fig. 1. The effects of exercise training on the cardiac functions. LVSP (**a**), LVEDP (**b**), +dp/dt maximum (**c**) and -dp/dt maximum (**d**) were measured. Data were expressed as mean ± SD. n=10 for each group. * p<0.05, ** p<0.01, *** p<0.001 compared with sham group; # p<0.05, ## p<0.01 compared with MI group. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; ±dp/dt maximum, maximal rate of rise or decline of left ventricular pressure.

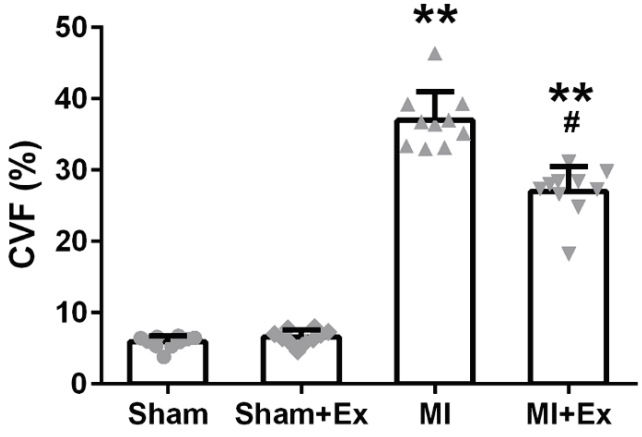

Fig. 2. Cardiac fibrosis analysis of heart from indicated groups. Cardiac fibrosis analysis of myocardium infarction in aged rat by evaluating the collagen volume fraction (CVF). Data were expressed as mean ± SD. n=10 for each group. ** p<0.01 compared with sham group; # p<0.05 compared with MI group.

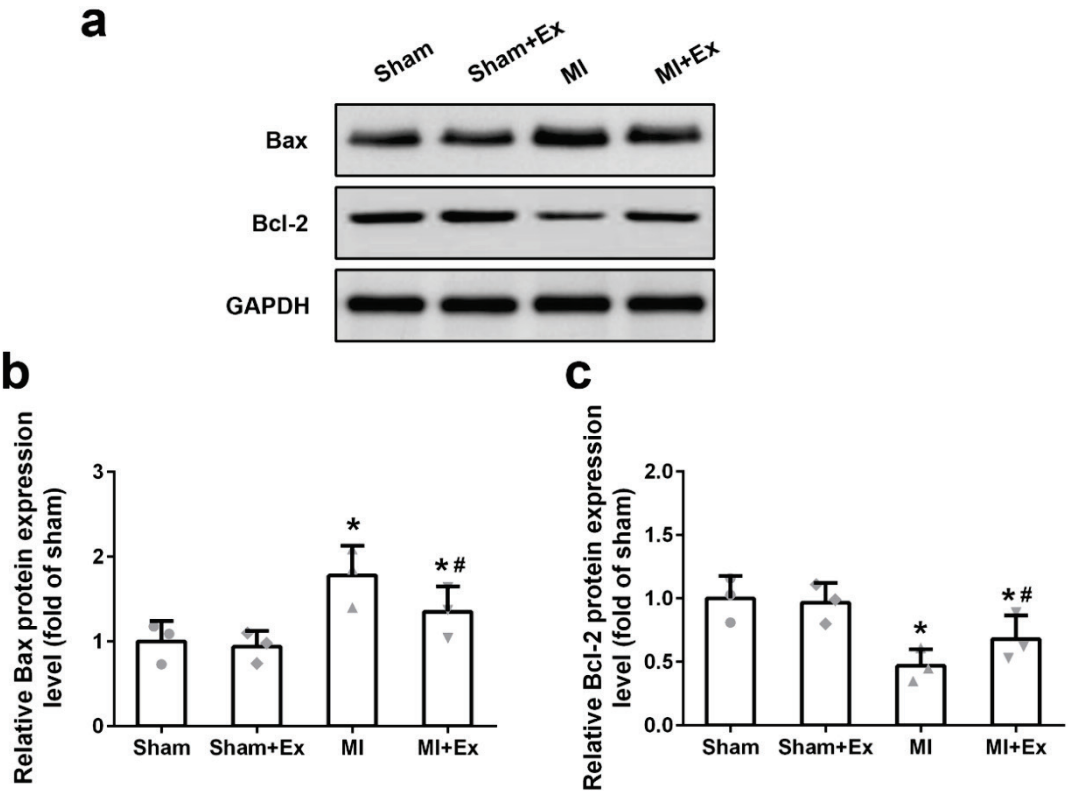

Fig. 3. The effects of exercise on Bax and Bcl-2 in myocardium of aged rat. Protein expression of Bax and Bcl-2 were analyzed by western blot (**a**) and relative expression levels of Bax (**b**) and Bcl-2 (**c**) were quantified. Data were expressed as mean ± SD. n=3 for each group. * p<0.05 compared with sham group; # p<0.05 compared with MI group.

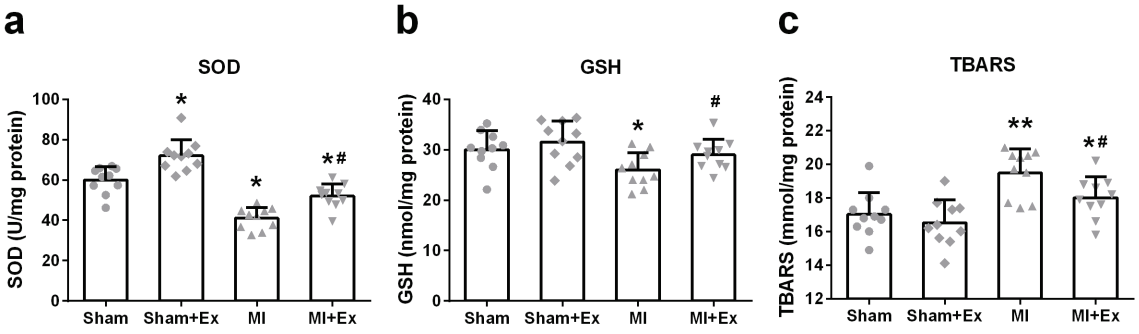

Fig. 4. Exercise suppressed oxidative stress induced by MI. SOD (**a**), GSH (**b**) and TBARS (**c**) activities were analyzed. Data were expressed as mean ± SD. n=10 for each group. * p<0.05, ** p<0.01 compared with sham group; # p<0.05 compared with MI group.

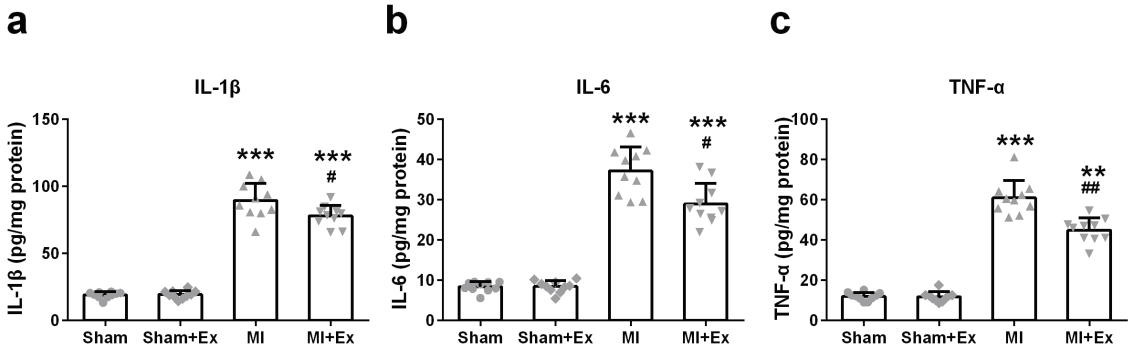

Fig. 5. Exercise suppressed inflammatory cytokines release in heart tissues following MI in aged rats. IL-1β (**a**), IL-6 (**b**) and TNF-α (**c**) levels were detected by ELISA. Data were expressed as mean ± SD. n=10 for each group. ** p<0.01, *** p<0.001 compared with sham group; # p<0.05, ## p<0.01 compared with MI group.

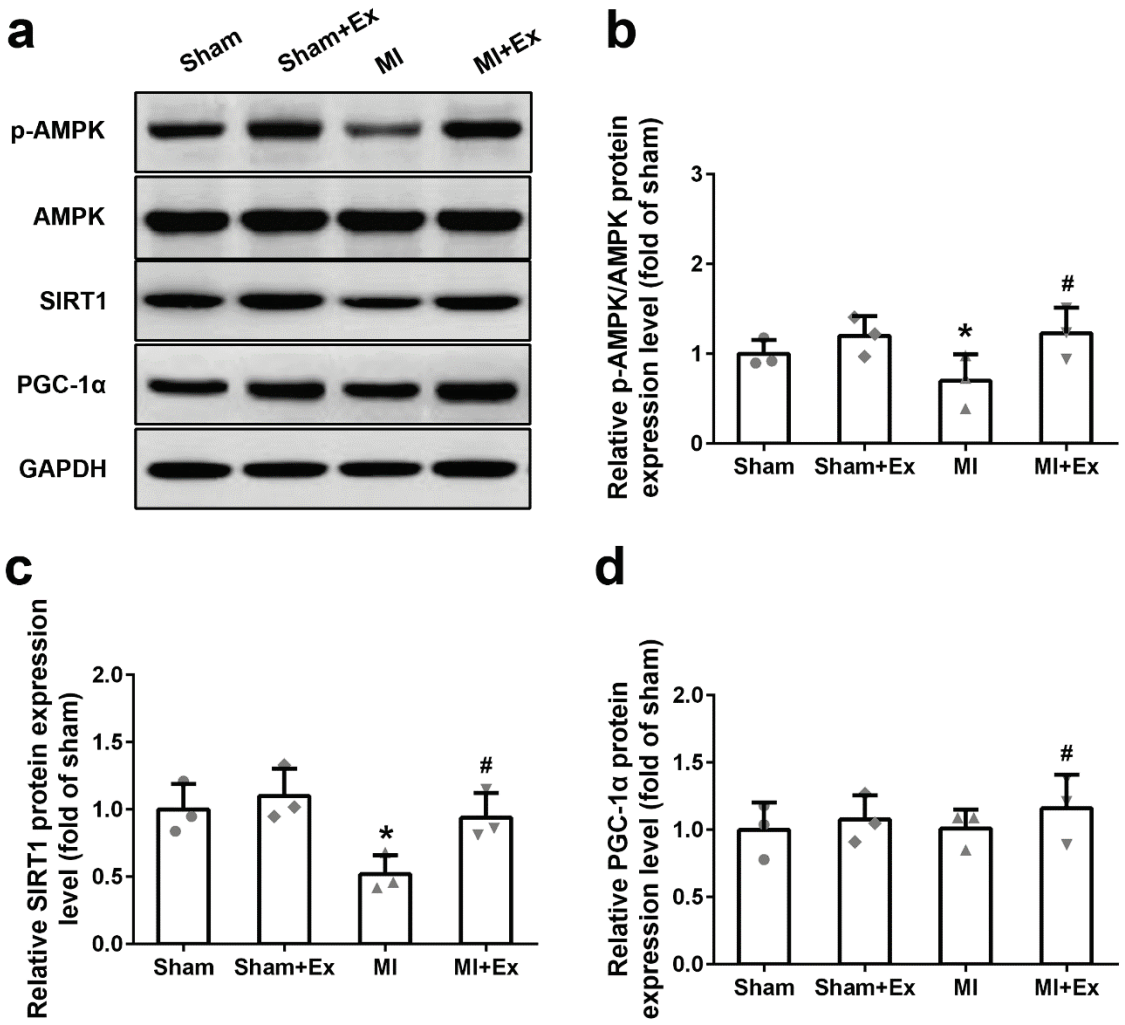

Fig. 6. Exercise training activates AMPK/SIRT1/ PGC-1α signaling pathway. The effects of exercise on p-AMPK, AMPK, SIRT1 and PGC-1α were analyzed by western blot (**a**) and relative expression levels of p-AMPK/AMPK (**b**), SIRT1 (**c**) and PGC-1α (**d**) were quantified. Data were expressed as mean ± SD. n=3 for each group. * $p<0.05$ compared with sham group; # $p<0.05$ compared with MI group.

Discussion

Myocardial infarction is a cardiovascular disease with the highest morbidity and mortality, and its incidence increases sharply with age in the world [1]. Studies have shown that exercise can improve the cardiovascular health of both healthy individuals and cardiovascular patients [30-32]. However, the mechanism contributing to its beneficial effect on elderly MI patients is still unclear. Here, we investigated the protective effect of post-MI exercise training on infarcted heart using the 20-week-old elderly MI rat model. We found that exercise training improved the MI-induced heart dysfunction and myocardial fibrosis significantly. The apoptosis, oxidative stress and inflammatory responses in the infarcted heart were decreased after four weeks of exercise. Mechanistically, AMPK/SIRT1/PGC-1α pathway was activated in the myocardial infarction area after exercise training, which might participate in the protection of cardiac function. Therefore, moderate exercise may become a new intervention method for the elderly patients with myocardial infarction.

Previous studies reported that MI result in pathological cardiac remodeling and cardiac dysfunctions, including the accumulation of cardiac fibrosis, decrease of LVSP and time derivatives of contraction (+dp/dt) and relaxation (-dp/dt), and increase of LVEDP [33-35]. In current study, MI surgery significantly reduced the value of LVSP and time derivatives of contraction/relaxation, and increased the LVEDP and cardiac fibrosis remarkably, which are in line with the previous findings observed in patients and animal models. With 4 weeks of post-MI exercise training, all the above pathological parameters were

significantly improved. Interestingly, cardiac function is improved not only in MI rats but also in Sham rats. In consideration of rats used in this study are elderly (20-month-old), our findings suggested that exercise training has significant benefits for both MI-injured and aged heart.

MI is usually caused by myocardial ischemia that leads to cell death and cardiac remodeling, and then trigger and aggravate the oxidative stress and inflammatory responses in the injured and peri-infarcted areas. Hori *et al.* demonstrated that the ischemic myocardium generated ROS injure the cell membrane and lead to apoptosis directly, in addition, ROS release also trigger multiple signal transduction to promote the overproduction of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [9]. Bezerra *et al.* reported that suppression of the oxidative stress and inflammation *via* cholinergic stimulation can improve MI in rat model [36]. With post-MI exercise training, apoptosis markers, oxidative stress and inflammatory responses were reduced in MI heart significantly, which indicated that exercise improves MI-induced cardiac dysfunction through inhibition of oxidative stress and inflammation in heart.

Recent studies demonstrated that exercise increase SIRT1 expression and activate its downstream target PGC-1α, and then suppress myocardial apoptosis and fibrosis, eventually improve cardiac function in elderly rat hearts [37,38]. SIRT1/PGC-1α pathway plays important role in oxidative stress and inflammation modulation [39]. However, its function in infarcted heart, especially in exercise mediated post-MI heart recovery, is still unclear. Here, we reported for the first time that post-MI exercise training significantly improve MI-induced cardiac dysfunction, and AMPK/SIRT1/PGC-1α signaling might be one of molecular pathways involved in this pathophysiological alteration. The underlying molecular regulation and upstream factors of AMPK/SIRT1/PGC-1α signaling pathway in exercise mediated cardiac function improvement need to be addressed in the future studies. It's worth to note that the results of this study are based on animal data, elderly patients after MI do not have healthy coronary artery which may be the difference with respect to the outcomes of exercise intervention.

Conclusions

Post-MI exercise training improves cardiac function in the aged drat model through suppression of apoptosis, oxidative stress, inflammatory responses, and activation of AMPK/SIRT1/ PGC-1α signaling pathway. Our findings may shed lights in MI treatment *via* the non-pharmacological intervention, such as exercise training.

Conflict of Interest

There is no conflict of interest.

References

1. Chakhtoura Z, Canonico M, Gompel A, Scarabin PY, Plu-Bureau G. Progestogen-only contraceptives and the risk of acute myocardial infarction: a meta-analysis. J Clin Endocrinol Metab 2011;96:1169-1174. https://doi.org/10.1210/jc.2010-2065
2. Liu J, Elbadawi A, Elgendy IY, Megaly M, Ogunbayo GO, Krittanawong C, Tamis-Holland JE, ET AL. Age-stratified sex disparities in care and outcomes in patients with ST-elevation myocardial infarction. Am J Med 2020;133:1293-1301.e1. https://doi.org/10.1016/j.amjmed.2020.03.059
3. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1211-1259. https://doi.org/10.1016/S0140-6736(17)32154-2
4. Puerto E, Viana-Tejedor A, Martinez-Selles M, Dominguez-Perez L, Moreno G, Martin-Asenjo R, Bueno H. Temporal trends in mechanical complications of acute myocardial infarction in the elderly. J Am Coll Cardiol 2018;72:959-966. https://doi.org/10.1016/j.jacc.2018.06.031
5. Garza MA, Wason EA, Zhang JQ. Cardiac remodeling and physical training post myocardial infarction. World J Cardiol 2015;7:52-64. https://doi.org/10.4330/wjc.v7.i2.52
6. Haykowsky M, Scott J, Esch B, Schopflocher D, Myers J, Paterson I, Warburton D, Jones L, Clark AM. A meta-analysis of the effects of exercise training on left ventricular remodeling following myocardial infarction: start early and go longer for greatest exercise benefits on remodeling. Trials 2011;12:92. https://doi.org/10.1186/1745-6215-12-92

7. Pagan LU, Gomes MJ, Martinez PF, Okoshi MP. Oxidative stress and heart failure: mechanisms, signalling pathways, and therapeutics. Oxid Med Cell Longev 2022;2022:9829505. https://doi.org/10.1155/2022/9829505
8. Nakamura K, Kusano K, Nakamura Y, Kakishita M, Ohta K, Nagase S, Yamamoto M, ET AL. Carvedilol decreases elevated oxidative stress in human failing myocardium. Circulation 2002;105:2867-2871. https://doi.org/10.1161/01.CIR.0000018605.14470.DD
9. Hori M, Nishida K. Oxidative stress and left ventricular remodelling after myocardial infarction. Cardiovasc Res 2009;81:457-464. https://doi.org/10.1093/cvr/cvn335
10. Hanif W, Alex L, Su Y, Shinde AV, Russo I, Li N, Frangogiannis NG. Left atrial remodeling, hypertrophy, and fibrosis in mouse models of heart failure. Cardiovasc Pathol 2017;30:27-37. https://doi.org/10.1016/j.carpath.2017.06.003
11. Vilahur G, Juan-Babot O, Pena E, Onate B, Casani L, Badimon L. Molecular and cellular mechanisms involved in cardiac remodeling after acute myocardial infarction. J Mol Cell Cardiol 2011;50:522-533. https://doi.org/10.1016/j.yjmcc.2010.12.021
12. Gabriel-Costa D. The pathophysiology of myocardial infarction-induced heart failure. Pathophysiology 2018;25:277-284. https://doi.org/10.1016/j.pathophys.2018.04.003
13. Cheng S, Zhang X, Feng Q, Chen J, Shen L, Yu P, Yang L, Chen D, Zhang H, Sun W, Chen X. Astragaloside IV exerts angiogenesis and cardioprotection after myocardial infarction via regulating PTEN/PI3K/Akt signaling pathway. Life Sci 2019;227:82-93. https://doi.org/10.1016/j.lfs.2019.04.040
14. Raish M. Momordica charantia polysaccharides ameliorate oxidative stress, hyperlipidemia, inflammation, and apoptosis during myocardial infarction by inhibiting the NF-kappaB signaling pathway. Int J Biol Macromol 2017;97:544-551. https://doi.org/10.1016/j.ijbiomac.2017.01.074
15. Yan L, Guo N, Cao Y, Zeng S, Wang J, Lv F, Wang Y, Cao X. miRNA145 inhibits myocardial infarctioninduced apoptosis through autophagy via Akt3/mTOR signaling pathway in vitro and in vivo. Int J Mol Med 2018;42:1537-1547. https://doi.org/10.3892/ijmm.2018.3748
16. Zeng Q, He H, Wang XB, Zhou YQ, Lin HX, Tan ZP, He SF, Huang GZ. Electroacupuncture preconditioning improves myocardial infarction injury via enhancing AMPK-dependent autophagy in rats. Biomed Res Int 2018;2018:1238175. https://doi.org/10.1155/2018/1238175
17. Tang J, Lu L, Liu Y, Ma J, Yang L, Li L, Guo H, Yu S, Ren J, Bai H, Yang J. Quercetin improve ischemia/reperfusion-induced cardiomyocyte apoptosis in vitro and in vivo study via SIRT1/PGC-1alpha signaling. J Cell Biochem 2019;120:9747-9757. https://doi.org/10.1002/jcb.28255
18. Alleman RJ, Tsang AM, Ryan TE, Patteson DJ, McClung JM, Spangenburg EE, Shaikh SR, Neufer PD, Brown DA. Exercise-induced protection against reperfusion arrhythmia involves stabilization of mitochondrial energetics. Am J Physiol Heart Circ Physiol 2016;310:H1360-H1370. https://doi.org/10.1152/ajpheart.00858.2015
19. Tao L, Bei Y, Lin S, Zhang H, Zhou Y, Jiang J, Chen P, Shen S, Xiao J, Li X. Exercise training protects against acute myocardial infarction via improving myocardial energy metabolism and mitochondrial biogenesis. Cell Physiol Biochem 2015;37:162-175. https://doi.org/10.1159/000430342
20. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, ET AL. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation 2015;131:e29-322. https://doi.org/10.1161/CIR.0000000000000152
21. Thirupathi A, de Souza CT. Multi-regulatory network of ROS: the interconnection of ROS, PGC-1 alpha, and AMPK-SIRT1 during exercise. J Physiol Biochem 2017;73:487-494. https://doi.org/10.1007/s13105-017-0576-y
22. Jia D, Hou L, Lv Y, Xi L, Tian Z. Postinfarction exercise training alleviates cardiac dysfunction and adverse remodeling via mitochondrial biogenesis and SIRT1/PGC-1alpha/PI3K/Akt signaling. J Cell Physiol 2019;234:23705-23718. https://doi.org/10.1002/jcp.28939
23. Liang Q, Cai M, Zhang J, Song W, Zhu W, Xi L, Tian Z. Role of muscle-specific histone methyltransferase (Smyd1) in exercise-induced cardioprotection against pathological remodeling after myocardial infarction. Int J Mol Sci 2020;21:7010. https://doi.org/10.3390/ijms21197010
24. Cai M, Wang Q, Liu Z, Jia D, Feng R, Tian Z. Effects of different types of exercise on skeletal muscle atrophy, antioxidant capacity and growth factors expression following myocardial infarction. Life Sci 2018;213:40-49. https://doi.org/10.1016/j.lfs.2018.10.015

25. Yao L, Chen H, Wu Q, Xie K. Hydrogen-rich saline alleviates inflammation and apoptosis in myocardial I/R injury via PINK-mediated autophagy. Int J Mol Med 2019;44:1048-1062. https://doi.org/10.3892/ijmm.2019.4264
26. Biernacka A, Frangogiannis NG. Aging and Cardiac Fibrosis. Aging Dis 2011;2:158-173.
27. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci 2014;71:549-574. https://doi.org/10.1007/s00018-013-1349-6
28. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002;53:31-47. https://doi.org/10.1016/S0008-6363(01)00434-5
29. Ferrari R, Guardigli G, Mele D, Percoco GF, Ceconi C, Curello S. Oxidative stress during myocardial ischaemia and heart failure. Curr Pharm Des 2004;10:1699-1711. https://doi.org/10.2174/1381612043384718
30. Maessen MF, Eijsvogels TM, Stevens G, van Dijk AP, Hopman MT. Benefits of lifelong exercise training on left ventricular function after myocardial infarction. Eur J Prev Cardiol 2017;24:1856-1866. https://doi.org/10.1177/2047487317728765
31. Medeiros WM, de Luca FA, de Figueredo Junior AR, Mendes FAR, Gun C. Heart rate recovery improvement in patients following acute myocardial infarction: exercise training, beta-blocker therapy or both. Clin Physiol Funct Imaging 2018;38:351-359. https://doi.org/10.1111/cpf.12420
32. Qin R, Murakoshi N, Xu D, Tajiri K, Feng D, Stujanna EN, Yonebayashi S, ET AL. Exercise training reduces ventricular arrhythmias through restoring calcium handling and sympathetic tone in myocardial infarction mice. Physiol Rep 2019;7:e13972. https://doi.org/10.14814/phy2.13972
33. Disatnik MH, Ferreira JC, Campos JC, Gomes KS, Dourado PM, Qi X, Mochly-Rosen D. Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. J Am Heart Assoc 2013;2:e000461. https://doi.org/10.1161/JAHA.113.000461
34. Feriani DJ, Coelho-Junior HJ, de Oliveira J, Delbin MA, Mostarda CT, Dourado PMM, Caperuto EC, Irigoyen MCC, Rodrigues B. Pyridostigmine improves the effects of resistance exercise training after myocardial infarction in rats. Front Physiol 2018;9:53. https://doi.org/10.3389/fphys.2018.00053
35. Matsuura K, Honda A, Nagai T, Fukushima N, Iwanaga K, Tokunaga M, Shimizu T, ET AL. Transplantation of cardiac progenitor cells ameliorates cardiac dysfunction after myocardial infarction in mice. J Clin Invest 2009;119:2204-2217. https://doi.org/10.1172/JCI37456
36. Bezerra OC, Franca CM, Rocha JA, Neves GA, Souza PRM, Teixeira Gomes M, Malfitano C, ET AL. Cholinergic stimulation improves oxidative stress and inflammation in experimental myocardial infarction. Sci Rep 2017;7:13687. https://doi.org/10.1038/s41598-017-14021-8
37. Bindu S, Pillai VB, Gupta MP. Role of sirtuins in regulating pathophysiology of the heart. Trends Endocrinol Metab 2016;27:563-573. https://doi.org/10.1016/j.tem.2016.04.015
38. Lira VA, Benton CR, Yan Z, Bonen A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am J Physiol Endocrinol Metab 2010;299:E145-E161. https://doi.org/10.1152/ajpendo.00755.2009
39. Tanno M, Kuno A, Yano T, Miura T, Hisahara S, Ishikawa S, Shimamoto K, Horio Y. Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure. J Biol Chem 2010;285:8375-8382. https://doi.org/10.1074/jbc.M109.090266