Exercise training improves cardiac function in infarcted rabbits: involvement of autophagic function and fatty acid utilization

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Aims	To explore whether exercise can improve cardiac function in a post-myocardial infarction (MI) rabbit model and to determine contributing factors in the left ventricle (LV).
Methods and results	Adult male New Zealand White rabbits (2.5–3 kg) underwent MI by ligation of the left anterior descending coronary artery. For 8 weeks after surgery, sham-operated, and post-MI rabbits were housed under sedentary conditions or assigned to a 4-week treadmill exercise protocol at a speed of 1.0 km/h for 30 min 5 days per week, then sacrificed. The non-infarcted region of the LV was harvested for further analysis. MI decreased left ventricular ejection fraction (LVEF) and increased thiobarbituric acid reactive substances (TBARS) generation in the LV. Exercise improved the cardiac function of MI rabbits. Left ventricular LC3II/LC3I (microtubule-associated protein light chain 3) in the MI group was 2.1-fold higher than that of the sham group, exercise significantly decreased LC3II/LC3I in the MI group. MI down-regulated the expression of heart-type fatty acid binding protein (h-FABP), and exercise up-regulated h-FABP. In addition, LVEF had a significantly positive correlation with h-FABP and a negative correlation with LC3II/LC3I.
Conclusion	Exercise induced change in autophagic function and fatty acid utilization may contribute to the improvement in ventricular function in the infarcted heart.
Keywords	Exercise training • Myocardial infarction • Autophagic function • Fatty acid utilization • Oxidative status

Introduction

Myocardial infarction (MI) is one of the most important aetiologies of heart failure. Cardiac remodelling after MI consists of a series of changes in the left ventricle (LV), leading to a decline in LV performance.^{1–4} Importantly, severe MI can result in permanent disability or death. Recently, exercise has become a potential therapy for attenuating LV dysfunction. Numerous putative mechanisms of exercise-induced cardioprotection against myocardial injury have been proposed and investigated. These mechanisms include anatomical changes in the coronary arteries, induction of myocardial heat shock proteins, an increase in myocardial cyclooxygenase-2 activity, elevation of endoplasmic reticulum stress proteins,

improvement of function of sarcolemmal ATP-sensitive potassium channels and mitochondrial ATP-sensitive potassium channels, improvement of insulin resistance, promotion of angiogenesis, and attenuation of LV remodelling in the post-MI failing heart.^{2–4}

Exercise improves skeletal muscle lipid oxidation and plasma antioxidant enzyme activity in animals with chronic heart failure.^{5–8} Increasing the antioxidant enzyme activity of glutathione peroxidase and superoxide dismutase in the failing heart reduces cardiac injury.^{9,10} Since exercise induces higher myocardial oxygen demands^{11,12} and the failing heart has a limited ability for superoxide removal,¹ an important issue for exercise therapy is whether antioxidant capacity is enough to conquer the oxidative stress of the failing heart.

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Programmed cell death (PCD) is classified into typical apoptosis (PCD-1) and autophagic cell death (PCD-2), which differ in the predominance of caspase activation or autophagy, respectively.^{13,14} Both types of PCD have been observed in cardiac myocytes. Morphologically, PCD-1 is characterized by chromatin condensation and the fragmentation of the nucleus and cytoplasm into apoptotic bodies, although PCD-2 is associated with cell shrinkage and the formation of multiple autophagic vacuoles.^{13,14} In addition to its role in PCD-2, autophagy functions in the recycling of cytoplasmic organelles and proteins at low basal levels under normal conditions and is up-regulated in response to stresses such as ischaemia/reperfusion (I/R) and in cardiovascular diseases such as heart failure.^{15–17} Autophagy recycles the cytoplasmic portions in double-membrane sequestering vesicles called autophagosomes.¹⁷ Microtubule-associated protein light chain 3 (LC3), which is the main substrate for the autophagosome membrane, is synthesized and then cleaved at glycine 120 to form the cytosolic LC3I. Upon induction of autophagy, the C-terminal glycine of LC3I is conjugated with phosphatidylethanolamine and is presented in isolation on membranes of the autophagosomes as LC3II.¹⁸ The autophagosome is delivered to the lysosome compartments where it is broken down into its essential constituents and recycled back into the cytoplasm. Impairment of autophagic function causes protein to aggregate and accumulate as autophagosomes in the cytoplasm, leading to cell death.¹⁷

There is increasing evidence to show that exercise up-regulates autophagy, and increases the protein turnover rate;^{19–21} however, it is unknown whether exercise has the same effect on the failing heart. In the present study, we investigated the effect of exercise training on cardiac function in an infarcted rabbit model explored the possible mechanisms of this effect, by analysing biomarkers of oxidative stress, antioxidant capacity, autophagy, apoptosis, and fatty acid utilization.

Methods

Animal model and experimental protocol

Male New Zealand White rabbits weighing 2.5–3 kg were subjected to MI by ligation of the left anterior descending coronary artery as previously described.²² One month later, the rabbits were randomly allocated to two groups: (i) exercise rabbits assigned to a 4-week training programme and (ii) sedentary rabbits confined to their cages during the same time period. Exercise training was performed on a low-speed, levelled, motorized treadmill (model LDM300D, Lee Dah Mei Industry Co, Ltd., Taiwan). The training programme was preceded by a 2-week period of adaptation to the treadmill exercise, during which the running time and speed of the treadmill were gradually increased from 10 min at 0.5 km/h to 30 min at 1 km/h. Exercise training consisted of a 4-week period of running at a speed of 1 km/h for 30 min 5 days per week. This exercise intensity and duration have been demonstrated to ameliorate endothelium-dependent vascular dysfunction and reduce pro-inflammatory changes in rabbits caused by a high-cholesterol diet.²³

At the end of the study period, all the rabbits were anaesthetized and sacrificed with a pentothal overdose. The non-infarcted region of the LV (counter part of the infarcted area, remote zone) from each rabbit was cut into small pieces and stored in liquid nitrogen until analysis. All experiments were approved by the Institutional

Animal Care and Use Committee of National Taiwan University and were carried out in accordance with the National Institutes of Health (NIH Publication no. 85-23, revised 1996) and the American Physiological Society's *Guide for the Care and Use of Laboratory Animals*.

Cardiac magnetic resonance imaging

In order to measure LV volume, mass, function, and infarct size, animals were examined using a 3-T magnetic resonance imaging (MRI) unit (Trio; Siemens, Erlangen, Germany) with an eight-channel cardiac phased-array coil for signal reception.²⁴ An ECG-gated turbo fast low angle shot (TurboFLASH) cine pulse sequence was acquired in two long-axis and five to seven short-axis views. After cine imaging was completed, an intravenous bolus dose of 0.20 mmol/kg of gadodiamide (Nycomed Imaging AS, Oslo, Norway) was administered, and late gadolinium enhancement images were acquired 10 min later using an inversion-recovery prepared segmented TurboFLASH sequence. LV volume, mass, and ejection fraction were assessed using cine MRI and an automated boundary detection algorithm. To assess infarct size, we quantified the late gadolinium enhancement using a signal intensity threshold criterion of >2 standard deviations above the mean signal intensity of the remote myocardium, and expressed it as a percentage of the total LV mass. Imaging analysis was performed using the Mathematica software package (Wolfram Research, Inc., IL, USA) and Matlab (MathWorks, Inc., Natick, MA, USA). The intra-observer variability in the LV volume results was 3%.

Oxygen radical absorbance capacity assay

Total antioxidant capacity was analysed by the oxygen radical absorbance capacity (ORAC) assay, which is based on the oxidation of fluorescein by peroxyl radicals via a classic hydrogen atom transfer mechanism and provides a direct measurement of antioxidant capacity against hydrophilic chain-breaking peroxyl radicals.²⁵ For analysis by the ORAC assay, the LV was pulverized under liquid nitrogen, weighed out (50 mg) as frozen material and added to 1 mL of ice-cold 75 mM K₂HPO₄/NaH₂PO₄ phosphate buffer (pH 7.0). The tissue samples were homogenized and centrifuged. The pellet was resuspended in homogenization buffer and centrifuged. The two supernatants were combined in a volumetric flask, adjusted to 2 mL of total volume, and used for the ORAC assay.

The ORAC assay was performed as described by Broke et al.²⁵ with minor modifications. Measurements were performed on a Perkin Elmer Luminescence Spectrometer LS50 (Perkin Elmer, Waltham, MA, USA) with 542-nm excitation and 576-nm emission filters, conducted at 37°C in 75 mM phosphate buffer (pH 7.0). The fluorescence probe was phycoerythrin (PE) and the peroxyl radical generator was 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). As a control standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used. Each sample was analysed in duplicate using a 'forward-then reverse' ordering in each plate row. To each well of the preheated microplate, 30 µL of sample (blank, phosphate buffer; Trolox, 1 M final conc. or tissue extract) and 100 µL PE (3.78 mg/L) were added, the plate was then shaken for 30 s and initial fluorescence was measured. AAPH solution (30 µL; 20 mM final conc.) was added to each well, and after shaking the plate measurements were taken every 15 min until the fluorescence was <5% of the initial reading. All fluorescence measurements were expressed relative to the initial reading. Results were calculated by using the differences of areas under the PE decay curves between the blank and each sample. The final results (ORAC values) were calculated by linear regression of the areas vs. sample concentration and expressed as mole Trolox equivalents (TE) per gram of myocardial tissue protein (mol TE/g).

Measurement of thiobarbituric acid reactive substances and 3-nitrotyrosine

The LV samples were homogenized in lysis buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris, pH7.4) and the tissue homogenates were prepared for thiobarbituric acid reactive substances (TBARS) and 3-nitrotyrosine (3-NT) analysis. TBARS were measured by fluorometric assay (excitation at 515 nm, emission at 552 nm) using 1,1,3,3-tetraethoxypropane as the standard, as described by Chen *et al.*²⁶ The linearity (R^2) of the standard curves was 0.991. The coefficients of variation (CV) of the intra- and inter-run from quality control samples were 5.02 and 6.98%, respectively. The levels of total 3-NT was measured by a competitive enzyme-linked immunosorbent assay using a commercial kit according to the manufacturer's instructions (Millipore, Billerica, MA, USA). The intra- and inter-batch CV from quality control samples were 6.09 to 6.43%, respectively. The amount of total nitrotyrosine in the LV tissue homogenates was estimated by comparing it with nitrated BSA as a standard.

Determination of reduced (GSH) and oxidized (GSSG) glutathione levels in the non-infarcted part of the left ventricle

The procedure for determining the total (GSH + GSSG) glutathione content in the non-infarcted part of the LV has been described previously.²⁶ All procedures were performed at 30°C. Twenty milligrams of tissue was lysed in 2 mL of 5% trichloroacetic acid, then 50 μ L of the lysate was added to 150 μ L of the glutathione assay mixture, which consisted of 0.6 nM 5,5'-dithiobis-(2-nitrobenzoic acid), 0.4 U of glutathione reductase, and 0.2 nM reduced nicotinamide adenosine dinucleotide phosphate, the mixture was then incubated for 1.5 min. The absorbance at 410 nm was then measured and the concentration calculated from a standard curve prepared using dilutions of a known GSSG or GSH concentration. For the GSSG assay, *N*-ethylmaleimide (0.02 N final concentration) was added to the lysate to remove GSH by forming a stable complex to prevent it from interfering with the assay. Following incubation for 60 min at 25°C, the solution was extracted with 10 volumes of ether to ensure complete removal of the unreacted sulfhydryl reagent which would inhibit glutathione reductase activity in the assay mixture. After the extraction process, the solution was added to the assay mixture for GSSG determination. GSH levels were calculated by subtracting the GSSG value from the GSSG + GSH value.

Western blotting

Tissue samples were homogenized in RIPA lysis buffer (Santa Cruz, Santa Cruz, CA, USA), and protein concentrations were measured using the protein assay kit (Bio-Rad, Hercules, CA, USA). Protein suspension from the tissue lysate (20 μ g) was loaded onto a SDS-PAGE gel for electrophoresis and then transferred to a PVDF membrane (Amersham Bioscience). The membrane was then incubated for 1–2 h at room temperature with primary antibodies against LC3, Bcl2, Bax, heart-type fatty acid binding protein (h-FABP) and actin (Santa Cruz), respectively. After washing with PBS-T, the membrane was incubated with secondary antibody against goat or mouse IgG and the immunoblotting was visualized using QUANTITY ONE (Bio-Rad).

Statistics

All data are expressed as the mean $\pm$ SD. Differences between groups were assessed by analysis of variance followed by a Tukey's test. Statistical analyses were performed using SAS (version 9.1; SAS Institute Inc., Cary, NC, USA). A *P*-value <0.05 was considered significant.

Results

A total of 40 rabbits underwent surgery, of these 26 were subjected to MI and 14 to sham surgery. Of the rabbits with MI, six died within 24 h of the operation and four died after the operation (38.5% post-operative mortality). The surviving 16 rabbits with MI were either left untreated (M, *n* = 8) or assigned to an exercise training programme for 4 weeks (ME, *n* = 8). Sham rabbits were assigned equally as untreated (S, *n* = 7) or treated with exercise training (SE, *n* = 7). There were no significant differences between the M and ME groups in terms of body weight, cardiac structure and function, or infarct size at 2 months after coronary ligation (data not shown). All rabbits survived until sacrificed three months post-MI. The non-infarcted region of the LV (counter part of the infarcted area, remote zone) from each rabbit was used to analyse biomarkers of oxidative stress, total antioxidant capacity, PCD, and fatty acid utilization.

Cardiac function

Left ventricular ejection fraction (LVEF) was used to evaluate cardiac function. No significant differences in cardiac function were observed between S and SE rabbits (LVEF for S and SE: 64.3 ± 3.4 and $66.0 \pm 3.9\%$, respectively; Figure 1). The cardiac function of MI rabbits had deteriorated 12 weeks after surgery; however, exercise training significantly increased the cardiac function of the MI rabbits (LVEF for M and ME: 40.1 ± 3.6 and $53.4 \pm 3.6\%$, respectively; *P* < 0.05). These results indicate that MI impaired the cardiac function of the rabbits and that exercise training improved the cardiac function of post-MI rabbits.

Oxidative status

Total antioxidant capacity was analysed by ORAC assay (Table 1). Myocardial infarction tended to decrease left ventricular total antioxidant capacity but this difference was not significant. Exercise training has a different effect on sham and MI rabbits: it kept antioxidant capacity at a low level in the MI rabbits, although it

Figure 1 Effect of myocardial infarction and exercise training on left ventricular function of rabbit hearts. Data are means $\pm$ SD (S and SE: *n* = 7; M and ME: *n* = 8, respectively; *, *P* < 0.05). S, adult rabbits with sham operation; M, adult rabbits with myocardial infarction for three months; E, rabbits assigned to a 4-week treadmill exercise protocol at a speed of 1.0 km/h for 30 min 5 days per week.

Table 1 Effect of myocardial infarction and exercise training on the antioxidative system in the rabbit left ventricle

	S (n = 7)	SE (n = 7)	M (n = 8)	ME (n = 8)
Total antioxidant capacity ($\mu\text{M TE/mg protein}$)	3594 $\pm$ 189	4091 $\pm$ 219*	3244 $\pm$ 242	3060 $\pm$ 212 [§]
TBARS (nmol/mg protein)	13.5 $\pm$ 1.9	14.2 $\pm$ 2.2	17.1 $\pm$ 2.0*	18.4 $\pm$ 2.2 [§]
3-Nitrotyrosine ($\mu\text{g nitrated BSA/mg protein}$)	2.61 $\pm$ 0.44	1.41 $\pm$ 0.44*	1.36 $\pm$ 0.47*	2.10 $\pm$ 0.42
GSH (ng/mg tissue)	385.8 $\pm$ 21.3	427.4 $\pm$ 22.7	406.6 $\pm$ 23.3	422.1 $\pm$ 27.8
GSSG (ng/mg tissue)	94.9 $\pm$ 3.0	94.3 $\pm$ 3.9	93.4 $\pm$ 4.8	91.9 $\pm$ 3.2
GSH + GSSG (ng/mg tissue)	480.7 $\pm$ 22.7	521.7 $\pm$ 26.9	500.0 $\pm$ 24.8	513.9 $\pm$ 25.2

Data show the means $\pm$ SD. TE, trolox equivalents; TBARS, thiobarbituric acid reactive substances; BSA, bovine serum albumin; GSH, reduced glutathione; GSSG, oxidized glutathione. S, adult rabbits with sham operation; M, adult rabbits with myocardial infarction for 3 months; E, rabbits assigned a 4-week treadmill exercise protocol at a speed of 1.0 km/h for 30 min 5 days per week.

*P-value for the S vs. other group <0.05 .

[§]P-value for the SE vs. ME <0.05 .

significantly elevated cardiac antioxidant capacity in the sham rabbits. TBARS was applied as the indicator of lipid peroxidation. Myocardial infarction caused a significant increase in cardiac TBARS levels (TBARS for S and M: 13.5 ± 1.9 and 17.1 ± 2.0 nmol/mg protein, respectively). Exercise training did not affect cardiac TBARS production either in the MI rabbits or in the sham rabbits. Tyrosine is a common target for nitrosative stress and 3-NT generation represents a marker for nitrosative stress.²⁷ MI rabbits had a lower level of cardiac 3-NT than did sham rabbits. Exercise training significantly decreased cardiac 3-NT generation in the sham group, whereas it tended to increase 3-NT generation in the MI group but this difference was not significant. The cardiac levels of GSSG and GSH were not significantly different among the four groups. These results show lower antioxidant capacity and higher TBARS in the failing heart. Exercise training increased cardiac antioxidant capacity and attenuated nitrosative stress in the sham group, but not in the MI group.

Programmed cell death

Apoptosis

Bcl2 and Bax were analysed as indicators of anti-apoptotic properties and pro-apoptotic properties, respectively. Twelve weeks after MI, cardiac Bcl2 expression was similar between the sham and MI groups (Figure 2A and B). Exercise training slightly increased Bcl2 expression in the sham rabbits but this tendency did not reach a significant difference. In the MI group, exercise training did not cause any differences in Bcl2 expression. A comparable pattern in cardiac Bax expression among treatments was observed. Bax expression in the LV was similar between the sham and MI groups. In the MI rabbits, exercise training did not cause a significant change in Bax expression, whereas it significantly up-regulated Bax expression in the sham group. The up-regulation of Bax and slightly increased expression of Bcl2 in the sham rabbits by exercise training resulted in a lower ratio of Bcl2 to Bax, and therefore there were no differences between treatments. These results indicate that exercise training did not affect apoptosis either in the sham group or in the MI group.

Autophagy

The amount of LC3-II and the ratio of LC3II to ILC3I are closely correlated with the number of autophagosomes, and serve as indicators of autophagosome formation.¹⁸ Myocardial infarction did not cause any change in left ventricular LC3I expression (Figure 2A and C). Exercise training significantly increased LC3I expression in the sham rabbits (1.6-fold), although it did not regulate LC3I expression in the MI rabbits, indicating that exercise training enhanced the autophagic function in the sham group, but not in the MI group. Cardiac LC3II expression was higher in the MI group than in the sham group. Exercise training significantly decreased LC3II expression in the MI group; however, it tended to increase LC3II expression in the sham group but this difference was not significant. The MI group had a higher ratio of LC3II to LC3I than the sham group. Exercise training caused a lower ratio of LC3II to LC3I in the MI group but did not alter the ratio in the sham group. Taken together, these data imply that there were cardiac autophagosome accumulations in the MI group, and exercise training promoted degradation of these autophagosomes.

Fatty acid utilization

Heart-type h-FABP was used as the indicator of fatty acid utilization in the heart (Figure 2A and D). Compared with the sham rabbits, the MI rabbits had down-regulated cardiac expression of h-FABP. Exercise training elevated h-FABP expression in the MI group, whereas it had no effect on h-FABP expression in the sham group. These result imply that MI decreased cardiac fatty acid utilization, and that exercise training could improve it.

Correlation between biomarkers and left ventricular ejection fraction

We evaluated the relationship between several biomarkers and LVEF in the present study, and found that LVEF had a significantly negative correlation with the ratio of LC3II to LC3I, but was positively correlated with h-FABP expression (Figure 3A and B; $r = -0.509$ and 0.598 , respectively). These results suggest that left ventricular dysfunction is related more to autophagosome accumulation and less to fatty acid utilization.

Figure 2 Effect of myocardial infarction and exercise on protein expression in the non-infarcted region of the left ventricle (counter part of the infarcted area, remote zone) by western blots (A). Bar charts showing the relative density measurements of bands obtained from all treatments. (B) Apoptotic marker (anti-apoptotic: Bcl2; pro-apoptotic: Bax). (C) Marker of autophagosome formation. (D) Fatty acid utilization in the rabbit hearts (h-FABP). Data show the means $\pm$ SD (S and SE: $n = 7$; M and ME: $n = 8$, respectively). S, adult rabbits with sham operation; M, adult rabbits with myocardial infarction for 3 months; E, rabbits assigned to a four-week treadmill exercise protocol at a speed of 1.0 km/h for 30 min 5 days per week; ADU, arbitrary densitometric units (B, C, and D; *, $P < 0.05$).

Figure 3 Correlation between left ventricular ejection fraction and other biomarkers. (A) Correlation with autophagosome formation (the ratio of LC3II to LC3I) in the rabbit hearts. (B) Correlation with fatty acid utilization (h-FABP). ADU, arbitrary densitometric units.

Discussion

The major findings in the present study are that MI caused LV dysfunction, increased lipid peroxidation, decreased autophagic function, and lower fatty acid utilization in the LV. Exercise training increased antioxidant capacity, decreased nitrosative stress, and promoted autophagic function in the sham rabbits. However, exercise training had different effects on post-MI rabbits. It improved LV function, and interestingly, it increased autophagic function and fatty acid utilization which were decreased by MI. However, exercise training did not alter the antioxidant capacity or nitrosative stress in the MI rabbits.

In accordance with our findings, exercise significantly improved LV function in post-infarction hearts. Giannuzzi *et al.*²⁸ have reported concordant results, showing that a 6-month exercise programme improved LVEF in heart failure patients. Moreover, a recent meta-analysis demonstrated that aerobic training significantly improved LVEF and decreased both end-diastolic volume and end-systolic volume.²⁹ However, another study showed that exercise induced LV dilation and wall thinning following MI.³⁰ Currently, MRI is the most precise method for assessment of the remodelling process. Data from our previous study using MRI showed that exercise training in patients with reduced left ventricular function following MI did not cause further myocardial damage such as wall thinning, infarct expansion or an increase in ventricular volume.³¹ In the current study, although an increase

in the LV end-diastolic volume and a decrease in the LV end-systolic volume were noted in the exercise trained rabbits, statistical analysis did not find any significant differences in LV geometry.

It is clear that MI induces oxidative stress and concordant results were seen in this study such as high TBARS in the infarcted heart. Unexpectedly, we found that the 3-NT level was significantly decreased in the infarcted hearts, and that exercise slightly increased the 3-NT level in the infarcted heart. Nitric oxide (NO) is cardioprotective via its inhibition of I/R injury, suppression of inflammation, and prevention of LV remodelling.³² Some NO metabolites, such as nitrite and nitrate, have vasculoprotective properties; whereas peroxynitrite, an oxidant metabolite of NO, reacts with tyrosine in proteins to form 3-NT and aggravates myocardial damage in I/R injury and in chronic heart failure.^{32,33} Compared with healthy subjects, chronic heart failure patients have a lower basal nitrite concentration and NO production, as well as a lower enhancement of exercise-induced NO production.^{34,35} Therefore, we speculated that the lower NO production in the failing heart might mean that there was less NO available for peroxynitrite formation resulting in less 3-NT generation. In addition, it has been reported that 3-NT generation is greater in the border zone than in the remote zone of infarcted mice hearts.³⁶ This might explain the reduced 3-NT level in the remote LV in the MI group in the present study. In addition to the change in 3-NT level, it is important to identify what kinds of proteins are modified to affect cardiac function. Lokuta et al.³⁷ found that increased nitration of sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2a) is seen in the idiopathic dilated cardiomyopathy (DCM) heart, which contributes to the Ca pump failure and hence heart failure in DCM. Therefore, we need to clarify the kind of 3-NT in proteins and to elucidate their effects, in future experiments.

The present study showed that exercise increased antioxidant capacity in the sham heart, but not in the MI heart despite an improvement in cardiac function. Accordingly, this suggests that other factors may contribute to this improvement. In the healthy animal model, exercise training increases myocardial oxygen consumption and it also increases the activity of antioxidant enzymes, thus maintaining a low oxidative status in the heart.^{11,38} A similar pattern was found in this study with increased antioxidant capacity in the sham heart following exercise training. Similarly, exercise increases the antioxidant enzyme activity in the skeletal muscle of heart failure patients.³³ However, we found that exercise had no effect on total antioxidant capacity or levels of reduced GSH in the MI heart, but interestingly exercise did not worsen the cardiac oxidative status despite high myocardial oxygen consumption, implying that there might be another antioxidant supply from outside of the heart, such as reduced GSH via the circulation. The major source of plasma GSH is the liver; and after its synthesis, GSH is transported to supply other tissues.³⁹ A high blood reduced GSH concentration has been reported in chronic heart failure patients compared with healthy subjects.⁴⁰ Therefore, we speculate that plasma GSH was targeted to the MI heart to compensate for the oxidative stress, and thus no further oxidative damage was caused in the MI heart. This might explain why there was no change in reduced GSH levels among the treatment groups in this study.

Autophagy is a major catabolic pathway by which mammalian cells degrade and recycle macromolecules and organelles.^{17,41} It plays a critical role in removing protein aggregates, as well as damaged or excess organelles, in maintaining intracellular homeostasis and in keeping the cell healthy. In the heart, autophagy occurs at low levels to maintain cellular homeostasis under normal conditions, and increased autophagy can be seen in conditions of starvation, I/R, and heart failure.^{15–17} Impairment in both formation and downstream lysosomal degradation of autophagosomes triggers mitochondrial and lysosomal pro-apoptotic pathways, and therefore culminates in cardiomyocyte death.^{15,17} There is increasing evidence showing that exercise activates autophagy in muscle.^{19–21} Resistance exercise causes a significant increase in protein degradation activities, such as lysosomal hydrolyase and Vps34,^{19,20} and increases the content of myocardial protein synthesis,^{20,21} thus stimulating protein turnover. In this study, exercise training caused an increase in LC3I and maintained a similar LC3II expression in the sham group; although in the MI group it decreased LC3II expression and caused a lower ratio of LC3II to LC3I. Therefore, we hypothesize that in the MI rabbits exercise training increased protein turnover, promoted the recycling of autophagosomes, and then enhanced the autophagic degradation. Additionally, autophagosome formation was negatively correlated with cardiac function in this study (Figure 3A), implying that increasing autophagosome degradation by exercise training correlates with the improvement in cardiac function. Taken together, exercise training induced cardioprotection even partially through increasing autophagic function in the LV of the failing hearts.

Exercise has been demonstrated to induce metabolic rates and increase lipid utilization as an energy source by elevating total muscle plasma FABP content and inducing the whole body capacity for fat oxidation in healthy and post-MI subjects.^{42,43} In the present study, high level of TBARS and increased expression of h-FABP in the LV due to exercise training were observed, and thus we speculate that exercise training elevated myocardial lipid uptake and exposure of fatty acids to a hyper-oxygen environment might have caused the increased lipid peroxidation observed in this study. Additionally, the positive correlation between h-FABP expression and LVEF suggests that this might be involved in the improvement in cardiac function (Figure 3B).

Some experiments which were important for verifying exact mechanisms were limited in this study. Autophagy involves the delivery of the autophagosomes and their contents to lysosomes that contain the degradative enzymes needed to complete the catabolic processes of autophagy. Therefore, the reduced LC3II/LC3I may reflect either decreased autophagosome formation, or increased turnover of autophagosomes due to activation in the degradation pathway, or a combination of the two. The best way to distinguish between 'decreased autophagosome formation and increased turnover of autophagosomes' is to measure the kinetics of autophagic flux.¹⁵ Without the kinetics of autophagic flux, less evidence could be obtained to elucidate the exact mechanism. The exploration of fatty acid utilization had similar limitations. High cardiac expression of h-FABP implies an increase in fatty acid uptake, but a better measure is the evaluation of real-time fatty acid uptake kinetics.⁴⁴ The available method uses a

fluorescently labelled fatty acid agent which is still not suitable for our animal model; this therefore limits the findings of this study.

To conclude, exercise training improved cardiac function in a post-MI rabbit model. Exercise training promoted autophagic function and increased fatty acid utilization as well, and these factors may have contributed to the improvement in cardiac function. Exercise did not improve antioxidant capacity or oxidative stress in the MI rabbits.

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Conflict of interest: none declared.

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