EXPERIMENTAL STUDY

Changes in Autophagy Levels in Rat Myocardium During Exercise Preconditioning-Initiated Cardioprotective Effects

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Summary
The role of autophagy in the cardioprotection conferred by ischemic preconditioning (IPC) has been well described. This study aimed to investigate the changes in autophagy levels during the cardioprotective effects initiated by exercise preconditioning (EP).

Rats were randomly divided into 4 groups: group C (control), group EP, group EE (exhaustive exercise), and group EP + EE (EP pretreatment at 0.5 hours before EE). The EP protocol included 4 periods of 10 minutes of treadmill running each at 30 m/minute with intervening 10 minute periods of rest. Hematoxylin-basic fuchsin-picric acid (HBFP) staining and plasma levels of cardiac troponin I (cTnI) were used to evaluate the ischemia-hypoxia injury in rat myocardium. Alteration levels in several autophagy proteins in the left ventricular myocardium were analyzed by Western blot. The phasic alterations of autophagy levels during EP-initiated cardioprotective phase were also examined.

Compared with group C, the ischemia-hypoxia positive areas and IOD value in HBFP-staining and cTnI plasma levels increased significantly in group EE. Compared with group EE, the ischemia-hypoxia injury was markedly attenuated in group EP + EE. Compared with group C, the LC3-II/LC3-I ratio, a marker of autophagosome formation, was reduced in group EE, but the LC3-II/LC3-I ratio remained unaltered in group EP + EE. Furthermore, the LC3-II/LC3-I ratio increased significantly at 2 hours during the cardioprotective phase after EP.

These results suggest that the activated autophagy level during the EP-initiated cardioprotective phase may be partly involved in the cardioprotective effects by maintaining a normal autophagy basal level during the subsequent exhaustive exercise in rat myocardium.

Key words: Exhaustive exercise, Ischemia-hypoxia injury, Cardioprotective phase, LC3-II/LC3-I ratio

Multiple and brief ischemic episodes initiate the cardioprotective effects against a subsequent sustained ischemic insult, which was termed myocardial ischemic preconditioning (IPC). Exercise preconditioning (EP) established by an acute bout of high-intensity intermittent exercise before a severe, sustained myocardial ischemia episode could initiate remarkable intrinsic cardioprotective effects for strenuous and repetitive stimulations to cardiovascular systems. EP can be divided into EP-initiated cardioprotective effects and cardioprotective phase. Although the EP-initiated cardioprotective effects have been widely recognized and the evidences show the cardioprotective phase can last a long time, the potential mechanisms during EP-initiated cardioprotective effects and phase still need to be investigated.

Autophagy is a renovation process that occurs in the cytoplasm. The impaired cell organelles or aging proteins are engulfed by autophagosomes and then fused with lysosomes. Finally, acid hydrolase in the lysosomes degrades the internal materials of autophagosomes into monomeric units for cell reuse. Autophagy is maintained at low levels under normal conditions, but this process is altered by several pathological or physiological conditions, such as starvation, endoplasmic reticulum stress, oxidative stress, aging, and disease pathogenesis.

Previous studies reported autophagy levels were inhibited in the ischemic myocardium in both in vitro and in vivo models of heart I/R injury. Enhanced autophagic flux functions as a protective countermeasure against I/R injury in cardiac myocytes. Activated autophagy is an essential endogenous defense mechanism and may be required for the cardioprotective effects conferred by IPC. IPC triggers autophagy in cardiac muscle, which induces cardioprotective effects by removing damaged intercellular organelles and nonessential proteins. This process inhibits the accumulation of misfolded proteins in the endoplasmic reticulum and apoptosis in the...
myocardium.22) Three days of aerobic exercise training was reported to result in cardioprotection due to the preservation of basal autophagy levels in the I/R region in rat cardiac muscle.19) However, whether autophagy levels are altered during the EP-initiated cardioprotective effects remains unclear.

In mice, acute strenuous exercise can activate the autophagy level in the myocardium, and the increase in autophagosome numbers reaches a plateau at 80 minutes after exercise.23) Expression of the autophagy indicator LC3-II in rat myocardium showed a biphasic change after an acute bout of 30 minutes of aerobic running exercise.24) However, alterations in autophagy levels in the myocardium during the cardioprotective phase after EP remain unclear.

In this study, we established an animal model that demonstrates the cardioprotective effects initiated by EP against ischemia-hypoxia injury in rat myocardium during a subsequent bout of exhaustive exercise. Since inhibited autophagy levels were observed in the ischemic myocardium in previous reports,2,4,10,20 and ischemia-hypoxia injury in rat myocardium could be induced after exhaustive exercise,5,9 we proposed that autophagy levels were inhibited in rat myocardium after exhaustive exercise, and that the alterations in autophagy levels during the cardioprotective phase after EP in rat myocardium may be involved in the EP-initiated cardioprotective effects. To test this hypothesis, we examined the changes in autophagy levels during the cardioprotective effects initiated by EP. Furthermore, phasic alterations in the autophagy levels were detected during the cardioprotective phase after EP in the rat myocardium.

Methods

Ethical approval and animals: Eight week old male Sprague-Dawley rats (body mass: 252 ± 11 g) were purchased from Shanghai Super-B&K Laboratory Animal Corp. Ltd. They were housed and bred in the SPF animal facility of The Shanghai University of Sport with food and water ad libitum (12-hour light-dark cycle). All animal experimental protocols were approved by the ethics committee on animal research of The Shanghai University of Sport and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Exercise experimental protocol: At one week before the exercise experiment, the rats were initially acclimatized to running on a motor-driven animal treadmill at 15 m/minute, 0% grade for 15 minutes every day. After the acclimatization period, a screening exercise test was performed to determine if the rats could run on the motor-driven animal treadmill at 28-30 m/minute, 0% grade, for 10-15 minutes. Animals that could not complete the screening test were excluded from the study. After two days of rest, the rats were randomly divided into two experimental protocols. The first protocol was to establish an animal model of EP-initiated cardioprotection against exhaustive exercise-induced ischemia and hypoxia injury in the myocardium (Figure 1A). Rats were randomly divided into 4 groups (n = 20 per group), as follows:

Group C (Control group): rats were kept on the animal treadmill without exercise.

Group EP (Exercise preconditioning group): an EP protocol was designed with minor modifications from previous studies.20 The rats were kept on the animal treadmill. After a warm-up protocol of 15 m/minute, 0% grade for 5 minutes, the speed of the treadmill was increased gradually to 30 m/minute within a 5 minute period. The speed was then maintained at 30 m/minute and 0% grade (approximately 70% of VO2max).26 The rats completed 4 repeated cycle periods of running for 10 minutes followed by 10 minutes of rest. The rats were then anesthetized and sacrificed at 0.5 hours after the last running period.

Group EE (Exhaustive exercise group): the exhaustive exercise was applied to induce ischemia-hypoxia injury in the myocardium.5,9 The rats warmed-up on the treadmill at 15 m/minute, 0% grade for 5 minutes, and then the treadmill speed was increased gradually to 30 m/minute, 0% grade within 15 minutes, and maintained at this running intensity until exhaustion. The rats were anesthetized and sacrificed at 0.5 hours after exhaustion.

Group EP + EE (Exercise preconditioning plus exhaustive exercise group): the EP pretreatment at 0.5 hours before subsequent exhaustive exercise was designed to attenuate the ischemia-hypoxia injury in the myocardium.5,9 First, the rats completed 4 repeated cycle periods of running for 10 minutes followed by 10 minutes of rest on the animal treadmill. Next, 30 minutes after the EP, the rats ran at 30 m/minute and 0% grade until exhaustion. The rats were then anesthetized and sacrificed at 0.5 hours after exhaustion.

To guarantee the equality of the extent of exhaustive exercise between group EE and group EP + EE, exhaustion was defined as the rat being unable to upright itself when placed on its back,27,28 and the duration and distance were recorded immediately after the exhaustive exercise.

To demonstrate the relationships between the autophagy level and the EP-initiated cardioprotective effects in the myocardium, phasic alterations of the autophagy level during the cardioprotective phase were designed (Figure 1B). The rats were anesthetized and sacrificed at 0.5 hours, 1 hour, 2 hours, 3 hours, 5 hours, and 24 hours after EP.

The rats from all groups were anesthetized with trichloroacetaldehyde monohydrate (40 mg/100 g by intraperitoneal injection) after which the abdominal cavity was opened rapidly, and blood was collected from the inferior vena cava. Heart perfusion fixation procedures were performed for histological analysis in a random selection of 10 rats from each group.29 The other 10 animal hearts from each group were rapidly removed, frozen in liquid nitrogen, and stored in a refrigerator at -80°C.

HBFP staining: HBFP staining was applied to examine the alterations of ischemia-hypoxia injury in rat myocardium.30 After routine dewaxing treatment, the paraffin slices were immersed in deionized water for 3 minutes. The hematoxylin stained the nucleus for 5 minutes, and was then washed out under a stream of water for 3 minutes. The slices were placed into 1% hydrochloric acid in alcohol solution for differentiation for 2 seconds, and then rinsed with water for 3 minutes. Paraffin slices were immersed in 0.1% basic fuchs in for 3 minutes and then...
rinsed 3 times with deionized water for 5 minutes. The slices were rinsed in pure acetone for 5 seconds, differentiated with 0.1% picric acid in pure acetone solution for 5 seconds, and then rinsed in acetone for 5 seconds. For histological analysis, 5 slices from each group were selected randomly for analysis, and an optical microscope (Olympus Corporation, Tokyo) was used to obtain 5 random images per slice. Therefore, a total of 25 images were analyzed in each group. The integral optical density (IOD) and positive areas from each image were analyzed by Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA).

**Detection of cTnI levels in plasma:** The blood samples were centrifuged for 15 minutes at 3000 rpm to collect the plasma. An automated chemiluminescent immunoassay system of AccuTnI+3 troponin I assay (Beckman Coulter, Brea, CA, USA) was used to detect cTnI levels in the plasma. This detection was based on a two-site enzyme immunoassay method: the solid phase was designed by anti-cTnI monoclonal antibody paramagnetic coated particles and the anti-cTnI monoclonal antibody combined with alkaline phosphatase to conjugate with different antigen sites of cTnI. Lum-Phos*530 (Lumigen, Southfield, MI, USA) was applied as a chemiluminescent substrate to produce a brown-colored signal, followed by counterstaining with hematoxylin. The same locations from myocardium near the endocardial layer of cTnI immunohistochemistry and HBFP staining in adjacent slices were imaged using an optical microscope (Olympus Corporation, Tokyo).

**Protein extraction:** Cardiac muscle tissues from the left ventricles (20 mg) were homogenized in an ice-chilled buffer including 500 μL of radioimmunoprecipitation assay (RIPA) cell lystate and 5 μL of the protease inhibitor phenylmethanesulfonfyl fluoride (PMSF). After mincing and homogenizing on ice for three 15-second cycles, the homogenates were centrifuged at 15,000 g for 20 minutes at 4°C. The supernatants were then collected. After centrifugation once again, the supernatants were collected for bicinchoninic acid (BCA) detection. A BCA protein assay kit (No. P0010, Beyotime Biotechnology) was used to determine the protein concentrations of the supernatants.

**Western blot:** Total protein lysates (30 μg) from each group were loaded into a 10% or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis system (Bio-Rad Laboratories Inc) with constant voltage at 70 V for 60-90 minutes at room temperature and then transferred to polyvinylidene fluoride membranes (Beyotime Biotechnology) with constant voltage at 70 V for 90 minutes at 4°C. After being rinsed in Tris-buffered saline with 0.1% Tween-20 (TBST), the membranes were blocked by 5% nonfat dry milk in TBST for 1 hour at room temperature.
The membranes were then probed with a primary rabbit antibody (1:1000) solution with 5% albumin from bovine serum (BSA) in TBST overnight at 4°C. The membranes were rinsed with TBST and then probed with Anti-rabbit IgG, HRP-linked secondary antibody (1:3000) by 5% non-fat dry milk in TBST for 1 hour at room temperature. After the membranes were rinsed with TBST, the immunoblots of antigen were exposed by an enhanced chemiluminescence reagent (Merck Millipore Company) and captured (Tonon Chemiluminescent Imager system). Each band was analyzed using computer software in our laboratory (Tonon Chemiluminescent Imager Analysis System). Data for immunoblots were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exposure. Protein expression levels after exercise are expressed in fold-change compared with their control group values.

**Antibodies:** The primary antibodies applied in Western blot analysis were p62 (1:500, No. p0067), LC3 (LC3A/B, 1:1000, No. 12471), Beclin1 (1:1000, No. 3495), Atg5 (1:1000, No. 12994), Atg7 (1:1000, No. 8558), and GAPDH (1:1000, sc-25778). GAPDH was purchased from Santa Cruz Biotechnology, and p62 was purchased from Sigma-Aldrich. Other antibodies were all purchased from Cell Signaling Technology, Inc. The anti-rabbit IgG, HRP-linked second antibody (1:3000, No. 7074) was also purchased from Cell Signaling Technology, Inc.

**Statistics:** All data are expressed as the mean ± SD. The duration and distance of exhaustive exercise between group EE and group EP + EE were compared using the one-way analysis of variance (ANOVA) followed by post hoc analysis. All statistical analyses were performed using computer software in our laboratory (Tonon Chemiluminescent Imager Analysis System). Data for immunoblots were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exposure. Protein expression levels after exercise are expressed in fold-change compared with their control group values.

**Results**

**Cardioprotective effects initiated by EP:**

Duration and distance of exhaustive exercise The duration and distance of exhaustive exercise were 85.28 ± 16.92 minutes and 2554.94 ± 503.27 meters in group EE and 77.50 ± 20.83 minutes and 2291.30 ± 596.19 meters in group EE and EP, respectively. Compared with group EE, both the positive areas and IOD value in group EP + EE were compared using the one-way analysis of variance (ANOVA) followed by post hoc analysis. All statistical analyses were performed using computer software in our laboratory (Tonon Chemiluminescent Imager Analysis System). Data for immunoblots were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exposure. Protein expression levels after exercise are expressed in fold-change compared with their control group values.

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I n t H e a r t J
March 2019 423

AUTOPHAGY IN EP-INITIATED CARDIOPROTECTION

FIGURE 2. Cardioprotective effects initiated by EP. A: The ischemic and hypoxic changes in rat myocardium shown by HBF staining (scale bar = 20 μm). B: Quantification of data (n = 25 images per group) in ischemic and hypoxic positive areas and IOD value by HBF staining. C: Alterations in plasma cTnI levels during EP-initiated cardioprotection (n = 20 group C; n = 19 group EP; n = 18 group EE; n = 20 group EP + EE). Values are expressed as the mean ± SD. *P < 0.05: versus group C. #P < 0.05: versus group EE. D: cTnI immunohistochemistry and HBF staining in adjacent slices. Powerful and interesting evidence in adjacent slices of myocardium demonstrated that the ischemia-hypoxia cardiomyocytes in HBF staining exhibited greater loss of cTnI expression in immunohistochemistry (scale bar = 20 μm).

Discussion

In this study, we found that prolonged exhaustive exercise can induce ischemia-hypoxia injury in rat myocardium, and that pretreatment with EP at 0.5 hours before exhaustive exercise attenuated the ischemia-hypoxia injury. An acute bout of exhaustive exercise and EP pretreatment before the exhaustive exercise resulted in different changes in autophagy levels in the rat myocardium.

Cardioprotective effects initiated by EP: Although a regimen of regular exercise training is widely recognized for its powerful cardiovascular health benefits and promotion of optimal living and longevity, adverse effects could be induced by excessive exercise training or sustained exhaustive exercise events. Specifically, elevated plasma levels of cardiac biomarkers and functional impairments of the heart could be found in participants after several strenuous endurance competitions. In animal studies, elevated levels of the cardiac biomarkers cTnT and creatine kinase in plasma, localized myocyte damage, func-
tional impairment, increased levels of malondialdehyde (MDA) in the myocardium, and enhanced apoptotic signaling and dysregulation of the matrix metalloproteinase system were observed after sustained exhaustive swimming in rats.37-39 In this study, HBFP staining and the plasma levels of cTnI were used to evaluate ischemia-hypoxia injury in rat myocardium after exhaustive exercise. HBFP staining is sensitive enough to detect the early stages of myocardial ischemia by different affinity levels of basic fuchsin to ischemic or normal myocardium.30 In this study, the significant increases in ischemic and hypoxic positive areas and IOD value in group EE suggested that ischemia-hypoxia injury in rat myocardium was induced after exhaustive exercise. cTnI is located in myofibrils in cardiomyocytes, and the plasma cTnI level can not only be used as a highly cardiac-specific and sensitive biomarker for the early diagnosis of ischemic-hypoxic myocardial injury in clinic,40,41 but also for the detection of small myocardial damage.42 Furthermore, the highly sensitive cTnI level could also be used as an indicator for strenuous exercise-induced sub-clinical myocardial injury.43-45 In this study, we also observed significantly increased plasma levels of cTnI after high-intensity exhaustive exercise. Sanchis-Gomar, et al reported transient mild elevation of plasma cTnT levels in professional soccer players throughout a competitive season and recovery to normal conditions after the detraining period, which would reflect an adaptive response of cardiomyocytes to the physiological milieu.46 In this study, the areas of cTnI positive immunoreactivity loss in myocardium were greater in group EE, which indicated the loss of cTnI in cardiomyocytes released into the circulation. Thus, the increase in cardiac troponin after prolonged exercise regarded as an adaptive response of cardiomyocytes to the physiological milieu is likely due to increased myocardial membrane permeability.47 The ultrastructural evidence showed that exhaustive exercise could lead to disruption of myofibrils in cardiomyocytes, which might result in more cTnI being leaked into the circulation.48 In this study, the ischemia-hypoxia cardiomyocytes demonstrated more loss of cTnI expression in adjacent slices of myocardium, suggesting that the elevation of cTnI levels in plasma, at least in part, was caused by the ischemia-hypoxia injury after exhaustive exercise. Therefore, both the increased myocardial membrane permeability and ischemia-hypoxia injury could be regarded as the potential mechanisms for the release of cytosolic cTnI after exhaustive exercise.49

Regular exercise training is widely recognized for its role in the prevention of myocardial infarction induced ischemic injury and its superiority regarding simplicity, economy, and lack of side effects.49,50 Pretreatment consisting of high-intensity intermittent exercise that includes 5 periods of 5 minutes each at 6 km/hour with intervening

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**Figure 3.** Changes in autophagy level in rat myocardium during EP-initiated cardioprotection. Changes in the autophagy levels of protein Beclin1 (A), Atg7 (B), Atg5 (C), autophagy marker LC3-II/LC3-I (D) and p62 (E) during EP-initiated cardioprotective effects. Values are expressed as the mean ± SD (n = 10 per group). *P < 0.05: versus group C, #P < 0.05: versus group EE.
5 minute periods of rest before a coronary occlusion can initiate a significant protective effect with respect to the cardiac infarct size in dogs.2-4) Pretreatment of EP established by an acute bout of high-intensity intermittent exercise which strenuously and repeatedly stimulates cardiac work was also found to initiate cardioprotective effects against isoproterenol injection or exhaustive exercise-induced ischemia-hypoxia injury in rat myocardium.5,6) In this study, pretreatment of EP at 0.5 hours before exhaustive exercise attenuated the ischemia-hypoxia injury in rat myocardium and decreased the release of cTnI.

Changes in autophagy level after exhaustive exercise: As an effective stimulation of the cardiovascular system, exercise can cause changes in coronary flow and energy metabolism in cardiomyocytes. Alteration of the autophagy level could be observed by examining autophagy protein expression after exhaustive exercise. An acute bout of strenuous exercise activated the autophagy level in mice myocardium, upregulated Beclin1 and LC3-II levels, and degraded autophagy substrate protein p62 after exercise.23) The expression of the autophagy indicator LC3-II in rat myocardium exhibited a biphasic change after an acute bout of 30 minutes of aerobic running exercise, with an initial immediate downregulation after exercise and a subsequent upregulation at 1 hour after the exercise.24) Increases in the expression levels of Atg7 and the LC3-II/LC3-I ratio and LC3-II, but repressed expression levels in Beclin1 and p62 were observed at 1 hour after a single bout of 0% grade treadmill running for 60 minutes at 12 m/minute in mice.25) In this study, we found the LC3-II/LC3-I ratio, an autophagosome formation marker, was reduced after exhaustive exercise, but observed no obvious changes in the expression levels of Beclin1, Atg7, Atg5, and p62. A repressed autophagy level was observed in early morphological research after intraperitoneal isoproterenol injection.26) Similar severe ischemia-hypoxia injuries were observed in rat myocardium after exhaustive exercise and intraperitoneal isoproterenol injection in previous studies.27,28) Inhibited autophagy levels were also found in ischemic myocardium in both in vitro and in vivo models of heart I/R injury.24,29) In this study, we also observed induced ischemia-hypoxia injury in the myocardium and a reduced LC3-II/LC3-I ratio in group EE, which indicates that the autophagy level was inhibited after prolonged exhaustive exercise. The differences in animal species, gender, mode of exercise, exercise intensity and duration, or post-exercise heart harvest time points may contribute to the inconsistent results (relative to other investigations) of this study. The degradation process of metabolic wasting proteins or damaged organelles may be inhibited due to reduced autophagy activity after exhaustive exercise. Thus, the inhibited autophagy levels may cause adverse effects and ischemia-hypoxia injury were induced in rat myocardium after exhaustive exercise.

Changes in autophagy level during EP-initiated cardioprotective effects: Activated autophagy is an important

**Figure 4.** Phasic alterations in the autophagy level in rat myocardium during the cardioprotective phase after EP. Alterations in the levels of autophagy protein Beclin1 (A), Atg7 (B), Atg5 (C), autophagy marker LC3-II/LC3-I (D) and p62 (E) in rat myocardium during the cardioprotective phase after EP. Values are expressed as the mean ± SD (n = 9 per group). *P < 0.05: versus group C.
endogenous defense mechanism and is required for the cardioprotective effects conferred by IPC. 18) Cardiac autophagy levels were decreased in ischemic injury myocardium caused by cardiac ischemia reperfusion (IR), and 3 days of aerobic exercise training induced cardioprotection via the preservation of basal autophagy levels in I/R regions of cardiac muscle. 19) Similar results for the LC3-II/LC3-I ratio in group EP + EE in this study suggests that normal basal autophagy levels were preserved under EP pretreatment before the subsequent exhaustive exercise. Thus, the preserved normal degradation and renovation process in cardiomyocytes would likely prevent the adverse effects on the myocardium during a subsequent bout of exhaustive exercise.

To gain further insight into the changes in autophagy in EP-initiated cardioprotective effects, we determined the phasic alterations of autophagy level during the cardioprotective phase after EP. We found several autophagy formation proteins were elevated in different groups during the cardioprotective phase after EP. The up-regulation of Atg7 at 1 hour, the LC3-II/LC3-I ratio at 2 hours, and Atg5 at 3 hours and 5 hours indicated the autophagy level was activated in the rat myocardium during the cardioprotective phase after EP. This was especially the case at 2 hours post-EP as we observed a significant increase in the LC3-II/LC3-I ratio, an autophagosome formation marker, even though no significant changes were found in autophagy substrate protein p62. The greatest decline in the mean value was at 2 hours after EP (versus group C, \( P = 0.09 \), Figure 4E). Activated autophagy levels in the myocardium during the cardioprotective phase could promote the quick degradation of metabolic waste proteins or damaged organelles in the cytoplasm and ensure normal activity and function of cardiomyocytes. Thus, reduced ischemia-hypoxia injury with an enhanced degradation and renovation process within the myocardium during the cardioprotective phase may attenuate the inhibition of autophagy levels during a subsequent bout of exhaustive exercise (Figure 5).

Amino acids produced by autophagy can be utilized for energy production, and ATP is one of the important effectors for cell survival. 53) The improved myocardial ATP levels were associated with the cardioprotective effects conferred by exercise training. 54) Further investigations on the alterations of ATP levels and other functional pathways produced by autophagy would be important topics to confirm the final effectors for cardioprotection induced by exercise preconditioning. Only modest physiological perturbations of cardiac biomarkers, and no obvious evidence of myocyte injury, have been found in the blood of athletes following intermittent bouts of exercise. 55) Thus, we recommend EP as an efficient and safe protocol for the prevention of myocardial ischemia and hypoxia changes prior to engaging in sustained high-intensity exercise training as it may promote normal intracellular substance turnover and metabolite degradation in the myocardium.
Conclusions

Exhaustive exercise could induce ischemia-hypoxia injury and inhibit autophagy levels in rat myocardium, and pretreatment with EP prior to the exhaustive exercise attenuates the ischemia-hypoxia injury. The activated autophagy level during the cardioprotective phase after EP may be partly involved in the cardioprotective effects by maintaining a normal autophagy basal level during subsequent exhaustive exercise in rat myocardium. The current results have demonstrated a potential cardioprotective role for warming up activity like EP protocol prior to subsequent high-intensity exercise training by preserving the normal intracellular substance turnover and metabolite degradation in the myocardium.

Disclosures

Conflicts of interest: The authors have no conflicts of interest to report.

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