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Original Article

Optimization of different intensities of exercise preconditioning in protecting exhausted exercise induced heart injury in rats

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ABSTRACT

This study was to optimize the exercise preconditioning (EP) intensity in protecting from exhaustive exercise-induced cardiac injury (EECI). A total of 98 male Sprague-Dawley rats were divided into 7 groups ($n = 14$): the control group (C), the exhaustive exercise group (EE) and the EP + EE groups, which include the V10 (53.0%$\dot{V}O_{2max}$), V15 (58.4%$\dot{V}O_{2max}$), V20 (67.0%$\dot{V}O_{2max}$), V26 (74.0%$\dot{V}O_{2max}$) and V30 (80.0%$\dot{V}O_{2max}$) groups. Except the C group, the other groups were subjected to treadmill running. The serum contents of N terminal pro B type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTn-I) were detected by the enzyme-linked immunosorbent assay method, ECG was recorded, heart function was detected by pressure volume catheter and the activities of mitochondrial electron transfer pathway (ET pathway) complexes I, II and IV were measured by high-resolution respiration instrument. Compared to the EE group, the EP groups have shown decrease of NT-proBNP and cTn-I, improvement of mitochondrial respiratory function and cardiac function. Compared to other EP groups, the V26 group has shown significant decrease of myocardial enzymes and improvement of mitochondrial function. The correlation analysis showed the EP effect was proportional to EP intensity in the range of 53.0%$\dot{V}O_{2max}$-74.0%$\dot{V}O_{2max}$. High intensity and long duration of exhaustive exercise caused cardiac injury and EP could decrease serum level of NT-proBNP and cTn-I, improve electrical derangement and the left ventricular function, and raise the activities of ET pathway complexes I, II and IV. The protection of EP on EECI was improved as the EP intensity was increased from 53.0%$\dot{V}O_{2max}$ to 74.0%$\dot{V}O_{2max}$ and when EP intensity was 74.0%$\dot{V}O_{2max}$, the effect was the most obvious among all the setting EP groups.

Introduction

Exercise training is a double-edged sword. Proper intensity exercise is beneficial to human health, whereas high-intensity endurance exercise can do harm to the body, especially to the heart. Recent reports suggest that prodigious amounts of exercise may increase markers for, and even the incidence of, cardiovascular disease.[1] The occurrence of arrhythmias or sudden cardiac death (SCD) during exercise are frequently heard in marathons or other long-distance running events.[2,3] An Italian study identified a 2.5-times relative risk for SCD in adolescents engaged in competitive sports versus an age-matched nonathletic population,[4] and a French investigation found the relative risk of sports-related SCD was 4.5 times greater in competitive young athletes compared with noncompetitive sports participants of the same age.[5] Exhaustive exercise induced cardiac injury (EECI) refers to the body continues to move in a state of fatigue which is beyond the limit of the body and leads to relative or absolute ischemia and hypoxia to the heart and generate myocardial damage.[6] In addition to SCD, the clinical manifestations of EECI also include changes in cardiac morphology, abnormal myocardial injury markers, exercise-induced arrhythmia, hypofunction and syncope, which are common in athletes, soldiers, and other groups that often engage in intense training.[7] It is reported high intensity or long duration of endurance exercise can lead to acute myocardial injury,[8] causing cardiac

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Abbreviations

EP	exercise preconditioning
EECI	exhaustive exercise-induced cardiac injury
SCD	sudden cardiac death
NT-proBNP	N terminal pro B type natriuretic peptide
cTn-I	cardiac troponin I
MFN2	mitochondrial fusion protein 2
DRP1	dynamin-related protein 1
HR	heart rate
CO	cardiac output
SV	stroke volume
Ves	end-systolic volume
Ved	end-diastolic volume
Pes	end-systolic pressure
Ped	end diastolic pressure
EF	ejection fraction
dp/dt_{max}	peak rate of pressure rise
dp/dt_{min}	peak rate of pressure decline
ESPVR	end-systolic pressure volume relationship
EDPVR	end-diastolic pressure volume relationship
Tau	relaxation time constant
PE	potential energy
SW	stroke work
CE	cardiac efficiency
$\dot{V}O_{2max}$	maximal oxygen consumption
ET pathway	electron transfer pathway
IR	ischemia reperfusion
IRI	ischemia reperfusion injury

troponin I concentration elevation,[9] atrial fibrillation,[10] myocardial fibrosis and coronary artery calcification.[11]

Exercise preconditioning (EP) can cause repeated and transient myocardial ischemia and hypoxia through continuous or intermittent intensive exercise in a short time, thus the tolerance of myocardial tissue is improved, and the myocardial injury that under the long time ischemia and hypoxia is reduced.[7] EP can increase vascular activity, reduce the area of myocardial infarction and reduce the occurrence of malignant ventricular arrhythmias.[12] EP can up-regulate the expression of mitochondrial biogenesis key pathway PGC-1α-NRF1/NRF2-TFAM, so the activities of mitochondrial respiratory complex I, II, and IV are elevated, and the energy metabolism of myocardial cells are improved. EP up-regulates mitochondrial fusion protein 2 (MFN2) expression and down-regulates the expression of dynamin-related protein 1 (DRP1), which reduce myocardial mitochondrial fragmentation.[7] EP activates the PI3K-Akt signal pathway, reduce myocardial cell apoptosis and fights against heart injury caused by EE.[13] EP down-regulates TXNIP/TRX/NF-κBp65/NLRP3 inflammatory signaling pathway, and reduces the content of downstream inflammatory factors.[14]

EP has important practical significance for preventing sports myocardial injury and formulating training plans due to its simplicity, safety and easy controllability. However, different intensities of EP have different protection on EECI. Lennon et al.[15] concluded that both moderate- (i.e., 60 min/day at 50% $\dot{V}O_{2max}$) and relatively high-intensity exercise (i.e., 60 min/day at 70%$\dot{V}O_{2max}$) performed during three consecutive days appear to be equally protective against IR-induced myocardial stunning. YM Li et al.[14] reported the moderate intensity EP(i.e., 32 m/min, at 65% -75% $\dot{V}O_{2max}$) had a better effect on regulating inflammatory pathways and protecting cardiac function. Studies have found that the activities of various myocardial mitochondrial complex enzymes were increased during moderate-intensity training, while low-intensity training modes had no significant effect on the activities of these complex enzymes.[16] Therefore, exploring an optimal intensity of EP training scheme is of great significance for guiding trainers to implement a reasonable and safe training program.

Analysis of mitochondrial function is central to the study of intracellular energy metabolism. In traditional mitochondrial function evaluation method, the organelles are extracted by centrifugation and the respiratory chain complexes are assessed individually. As important properties of mitochondria differ in *vivo* and in *vitro* and the isolation steps may affect the properties of mitochondria, that method has got some disadvantages.[17] In this study, we permeabilized myocardial fibers using saponin and analyzed the mitochondrial complex I, II and IV function without isolation of organelles, which allows the characterization of functional mitochondria in their normal intracellular position and assembly, preserving essential interactions with other organelles.

In this study, we intend to explore the protection relationship between EP intensity and cardiac protection and explore the optimal EP intensity through observing alterations in myocardial injury markers, electrophysiology, cardiac function, and myocardial mitochondrial respiratory function in response to different intensities of EP training and high intensity and long duration exhaustive exercise.

Materials and methods

Experimental animal

The male Sprague-Dawley rats (200 ± 20 g) were provided by Beijing Zhongke Dasheng Biological Technology Co., Ltd. (Beijing, China), certification number SCXK (Jing)-2016-0002. National standard rodent dry feed was provided *ad libitum*, and the indoor temperature was maintained at 25°C–26 °C under a 12 h dark and 12 h light cycle, and the relative humidity was maintained at 40%–55%. All experiments were conducted in compliance with the guide for the Care and Use of Laboratory Animals and were permitted by the Ethics Committee for the Use of Experimental Animals at the PLA 82nd Group Military Hospital (CA19-02).

Drugs and instruments

The main reagents used in the present study are listed below. The cardiac troponin I (cTn-I) and N terminal pro B type natriuretic peptide (NT-proBNP) enzyme-linked immunoassay kits were obtained from Cloud-Clone Corp. (Wuhan, China). ADP potassium salt, Cytochrome *c*, Lactobionate, ATP Na_2, Na_2Phosphocreatine, EGTA, Ascorbic acid, Taurine, Imidazole, DTT, HEPES, MES, Glutamate, Malate, Succinate, TMPD, Antimycin A and Rotenone were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The following main instruments were used in the present study: An animal treadmill (Taimeng, China), a PowerLab signal acquisition and analysis system, a MultiscanGO enzyme standard instrument (Thermo, USA), a pressure volume catheter (SPR-838, Millar Company, USA), a PowerLab data acquisition and analysis system (AD Instruments, Australia), a bioelectric amplifier (AD Instruments, Australia), a needle electrode (AD Instruments, Australia) and a high-resolution respirometry (Oroboros Instruments, Austria).

Establishment and grouping of animal models

A total of 98 rats ultimately entered the formal experiment. They were randomly divided into 7 groups ($n = 14$): the sedentary control group (C), the exhaustive exercise group (EE) and the EP + EE groups which were further divided into the V10 group, V15 group, V20 group, V26 group and V30 group. In the V10 group, the run slope was 5° and the speed was 10 m/min, equivalent to 53.0%$\dot{V}O_{2max}$. In the V15 group, the run slope

was 5° and the speed was 15 m/min, equivalent to 58.4%$\dot{V}O_{2max}$. In the V20 group, the run slope was 5° and the speed was 20 m/min, equivalent to 67.0%$\dot{V}O_{2max}$. In the V26 group, the run slope was 5° and the speed was 26 m/min, equivalent to 74.0%$\dot{V}O_{2max}$. In the V30 group, the run slope was 5° and the speed was 30 m/min, equivalent to 80.0%$\dot{V}O_{2max}$ (Table 1). All the EP + EE groups underwent 30 min of EP training for 6 days per week, and the scheme lasted for 3 weeks. The EP training intensity grading was referred to Bedford's quantified training maximum oxygen consumption relationship table.[18] Finally, the EE and EP + EE groups performed a one-time exhaustive treadmill running at a speed of 19–26 m/min and a run slope of 10° for about 4 h until the rats met the exhausted criteria as below: the running of the rats was significantly more difficult in the late stages of exercise than in the early stages, unable to bear the original running speed, speed gradually decreased, movement posture also changed from the ground running to half supine position running, even stayed on the back third of the runway as many as 10 times, and there was significant change in the motion state of the rats under the various stimulus.[7,19] Owing to the Millar catheter procedure was an invasive test, it may affect other experimental results, so each group of the rats were divided into two parts, one part was used for the pressure volume catheter detection of cardiac function ($n = 8$ animals per group), and the remaining animals ($n = 6$ animals per group) were used to detect the myocardial mitochondrial function and collect serum or myocardial specimens.

ECG tracing

Adaptive electrocardiography training was performed in all experimental rats. ECGs were recorded from the rats of C group in a quiet state for 5 min. In EE and EP + EE groups, ECGs were recorded for 5 min immediately after EE. Anesthetic rats were placed in the rat cage, and all the sets of limbs and the right forearm were routinely disinfected. Subcutaneous punctures in extremities were created to insert the electrodes (the left hind leg was used as the positive electrode, the right foreleg was used as the negative electrode, and the left foreleg was used as the grounding electrode), and the electrode needle was fixed. The dynamic ECG results were recorded by a PowerLab data acquisition and analysis system. The Heart Rate (HR), PR interval, QRS interval, ST height, and T amplitude were obtained.[7]

Determination of cardiac function parameters with a pressure volume catheter

The rats was weighted and anesthetized with pentobarbital sodium (40 mg kg^{-1}, intraperitoneal injection), and fixed on the operating table in supine position. After endotracheal intubation, we separated the right carotid artery, and calibrated pressure with MPVS control software. The Millar catheter (SPR-838, Millar, USA) was inserted into the left ventricle from the right carotid artery. The left ventricular pressure volume waveforms of the anesthetized rats were recorded with Chart 7 software in real-time. The basic waveform was recorded for 10 min. A ventral midline incision was performed on the abdomen, and the inferior vena cava was occluded and the waveform was recorded. 30% NaCl solution (30 μL) was injected in the left jugular vein and the pressure-volume waveform was recorded. Then the catheter tips were submerged in the holes of a calibration cuvette which was filled with fresh heparinized warm blood respectively and recorded the conductance changes in the volume channel, finally the volume can be calculated. The parameters: stroke work (SW), cardiac output (CO), stroke volume (SV), end-systolic volume (Ves), end-diastolic volume (Ved), end-systolic pressure (Pes), end diastolic pressure (Ped), ejection fraction (EF), peak rate of pressure rise (dp/dt_{max}), peak rate of pressure decline (dp/dt_{min}), potential energy (PE), cardiac efficiency (CE), end-systolic pressure volume relationship (ESPVR), end-diastolic pressure volume relationship (EDPVR), relaxation time constant (Tau) were detected.[7]

Table 1
The exercise protocols of each group.

Groups	*n*	Run slope, °	Speed, $m.min^{-1}$	% of $\dot{V}O_{2max}$	Duration
C	14	0	0	33.3	
EE	14	10	19–26		4–5 h
V10	14	5	10	53.0	EP for 30 min, and EE for 4–5 h
V15	14	5	15	58.4	EP for 30 min, and EE for 4–5 h
V20	14	5	20	67.0	EP for 30 min, and EE for 4–5 h
V26	14	5	26	74.0	EP for 30 min, and EE for 4–5 h
V30	14	5	30	80.0	EP for 30 min, and EE for 4–5 h

C: Sedentary control group. EE: Exhaustive exercise. EP: Exercise preconditioning. V10, V15, V20, V26, and V30: exercise preconditioning performed at 53.0%$\dot{V}O_{2max}$, 58.4%$\dot{V}O_{2max}$, 67.0%$\dot{V}O_{2max}$, 74.0%$\dot{V}O_{2max}$ and 80.0%$\dot{V}O_{2max}$ respectively. $\dot{V}O_{2max}$: Maximal oxygen consumption.

The test of myocardial mitochondrial respiratory function in situ

The rat hearts were removed in icy BIOPS to clean the blood. Then a part of the left ventricular apex was extracted for mitochondrial respiratory function tests by a sharp blade. Myocardial fibers were isolated by dissecting muscle tissue (left ventricle) in BIOPS solution on ice followed by saponin permeabilization. Cell membrane permeabilization with saponin enables the study of organelle function while maintaining cellular architecture and controlling the intracellular milieu. Mitochondrial function was measured at 37 °C by high-resolution respirometry (Oroboros Instruments). The respiration medium (MiR-05) included 110 mM sucrose, 60 mM K-lactobionate, 0.5 mM EGTA, 1 g/L bovine serum albumin (essentially fatty acid-free), 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO_4, and 20 mM HEPES (pH 7.1). DatLab software was used for data acquisition and analysis. Cytochrome *c* (10 μM) was added after the addition of ADP and the substrates of complex I to test the intactness of mitochondrial outer membrane. We used 1 mM adenosine diphosphate (ADP) to stimulate respiration (state 3) and measured it sequentially through complex I (10 mM glutamate and 2 mM malate), complex II (10 mM succinate and 0.5 M rotenone), and complex IV (0.5 mM TMPD, 5 mM ascorbate, and 2.5 M antimycin A). Respiratory rates were expressed per mg wet weight.[20]

Collection and preparation of serum

After exhaustion, all rats were anesthetized by the intraperitoneal injection of pentobarbital (30 mg kg^{-1}). The chest was opened, and blood was collected from the inferior thoracic vena cava. The blood was centrifuged at 3000 $r.min^{-1}$ for 20 min, and the supernatant was collected and stored in a −80 °C freezer.[7]

Enzyme-linked immunoassays for N terminal pro B type natriuretic peptide and cardiac troponin I levels in the serum

The serum was removed from the −80 °C freezer. Enzyme-linked immunosorbent assays were performed according to the instructions of the kits. The OD value of each sample was measured at 450 nm. The OD value for the standard was measured, and a standard curve was constructed with the OD value on the y-axis and the concentration on the x-axis. The concentration of the indicated marker in each sample was obtained from the standard curve.[7]

Statistical analyses

SPSS version 22.0 was used for statistical analysis. The results were expressed as means ± *SD*. One-way analysis of variance (ANOVA) was

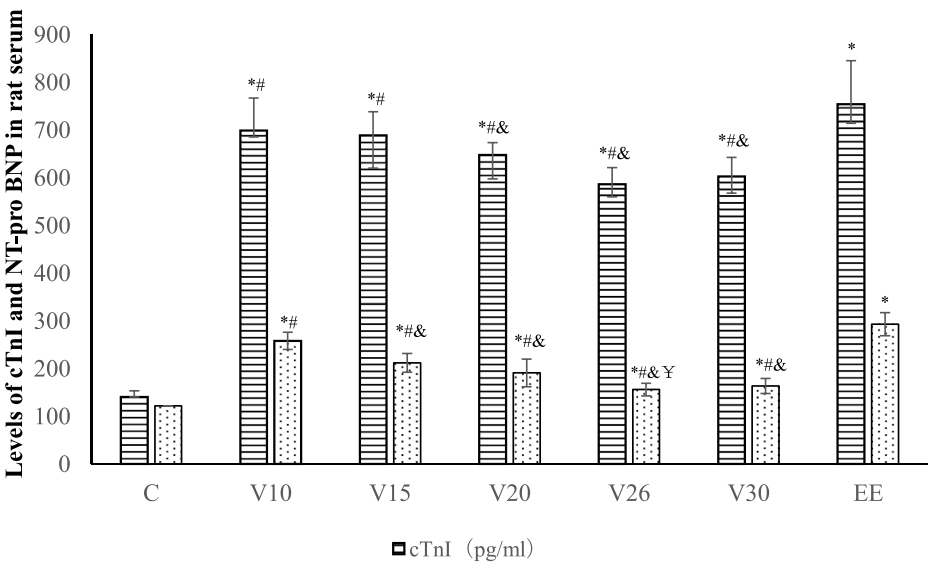

Fig. 1. Comparison of the contents of cTn-I and NT-pro BNP in rat serum.
Data are expressed as *means* ± *SD*; $n = 6$ for each group; $^{*}p < 0.05$ versus the C group, $^{\#}p < 0.05$ versus the EE group, $^{\&}p < 0.05$ versus the V10 group, $^{¥}p < 0.05$ versus the V15 group, $^{@}p < 0.05$ versus the V20 group, $^{\triangle}p < 0.05$ versus the V26 group. NT-proBNP: N terminal pro B type natriuretic peptide. cTn-I: cardiac troponin I. C: Sedentary control group. EE: Exhaustive exercise group. V10, V15, V20, V26, and V30: exercise preconditioning performed at 53.0%$\dot{V}O_{2max}$, 58.4%$\dot{V}O_{2max}$, 67.0%$\dot{V}O_{2max}$, 74.0%$\dot{V}O_{2max}$ and 80.0%$\dot{V}O_{2max}$ respectively. SD: Standard deviation.

used to compare data among multiple groups. Dunnett *t*-test or SNK *q*-test was used for multiple comparisons between groups. Levene method was used to test the homogeneity of variance. Difference was considered significant statistically when $p < 0.05$. A correlation analysis was performed by calculating Spearman's correlation coefficients, and $p < 0.05$ was considered to indicate a significant difference.

Results

Different intensities of EP decreased the serum N terminal pro B type natriuretic peptide and cardiac troponin I levels in exhausted rats

As shown in Fig. 1, compared with the C group, the content of cTn-I in EE group was increased ($p < 0.001$). Compared with the EE group, the content of cTn-I in the V26 and V30 groups were decreased significantly ($p = 0.014$ and $p = 0.027$ respectively). Compared with the V20 group, the V26 group was reduced insignificantly. Compared with the V26 group, the V30 group was increased insignificantly.

Compared with the C group, the serum level of NT-pro BNP was increased in the EE group significantly ($p < 0.001$). Compared with the EE group, the level of NT-pro BNP in the V10, V15, V20, V26 and V30 groups were reduced significantly ($p = 0.003$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). Compared with the V10 group, the V15 groups was decreased significantly ($p < 0.001$). Compared with the V15 group, the V26 and V30 groups were significantly decreased ($p < 0.001$, $p < 0.001$). Compared with the V26 group, the V30 group was increased insignificantly.

Different intensities of EP improved electrocardiogram indicators in exhausted rats

As shown in Table 2 and Fig. 2, compared with the C group, the ST segment of rats in EE group was significantly elevated ($p < 0.001$). Compared with the EE group, the ST segment in the V10, V15, V20, V26 and V30 groups were significantly depressed ($p = 0.049$, $p = 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$). Compared with the V10 group, the V15 group, V20 group, V26 group and V30 group were depressed ($p = 0.005$, $p < 0.001$, $p < 0.001$, $p < 0.001$). Compared with the V15 group, the V20, V26, and V30 groups were depressed significantly ($p = 0.034$, $p < 0.001$, $p = 0.016$).

Compared with the C group, the QT interval of EE group was wider ($p < 0.001$). Compared with the EE group, the QT interval of each EP group was significantly shortened ($p < 0.001$ respectively). Compared with the V10 group, the V20 and V26 groups were decreased ($p = 0.042$, $p = 0.071$).

Different intensity of EP improved cardiac function of exhausted rats

As shown in Table 3, compared with the C group, the SV of the EE group was significantly reduced ($p < 0.001$). Compared with the EE group, the CO in the V26 groups was significantly increased ($p = 0.042$). Compared with group C, the EF of the EE group was significantly smaller ($p < 0.001$). Compared with the EE group, the EF of the V20 group and V26 group was significantly increased insignificantly ($p < 0.001$, $p < 0.001$).

Table 2
Effects of different intensities of exercise preconditioning on ECG indexes of exhausted rats.

	C	EE	V10	V15	V20	V26	V30
HR(bpm)	356.88 ± 9.54	438.88 ± 5.28*	428.37 ± 14.82*	412.63 ± 15.06*&	405.98 ± 6.41*&	406.38 ± 6.70*&	424.50 ± 14.57*¥
P wave(ms)	16.00 ± 2.73	24.00 ± 2.07	22.75 ± 3.45	19.75 ± 3.41	18.63 ± 3.62	18.38 ± 2.88	19.50 ± 5.26
PR intervalms)	41.50 ± 3.59	57.13 ± 3.56*	52.50 ± 3.59*#	48.88 ± 3.36*#&	47.00 ± 4.04*#&	46.38 ± 1.69*#&	50.75 ± 2.38*#&¥
R Amplitude(mv)	74.25 ± 3.24	87.13 ± 3.64*	83 ± 3.63*	85.5 ± 3.30*	83.88 ± 4.32*	86.38 ± 3.16*	87.38 ± 1.19*
QRS Duration(ms)	15.75 ± 2.75	26.50 ± 1.41*	24.38 ± 2.67*#	23.75 ± 2.60*#	22.19 ± 3.16*#	18.75 ± 2.12#&	17.69±2.7#&
QT Interval(ms)	51.88 ± 3.72	70.63 ± 2.83*	65.25 ± 2.49*#	62.00 ± 3.70*#	61.00 ± 2.93*#&	59.25 ± 3.54*#&	64.88 ± 2.23*#¥
ST Height(mV.10^3)	34.85 ± 5.17	246.53 ± 15.56*	219.93 ± 11.40*#	194.07 ± 9.78*#&	177.56 ± 6.98*#&¥	165.79 ± 7.80*#&¥	167.35 ± 14.34*#&¥

Data are expressed as *means* ± *SD*, $n = 8$. $^{*}p < 0.05$ versus the C group, $^{\#}p < 0.05$ versus the EE group, and $^{\&}p < 0.05$ versus the V10 group, $^{¥}p < 0.05$ versus the V15 group, $^{@}p < 0.05$ versus the V20 group, $^{\triangle}p < 0.05$ versus the V26 group, $^{\star}p < 0.05$ versus the V26 group. HR: Heart rate. C: Sedentary control group. EE: Exhaustive exercise. V10, V15, V20, V26, and V30: exercise preconditioning performed at 53.0%$\dot{V}O_{2max}$, 58.4%$\dot{V}O_{2max}$, 67.0%$\dot{V}O_{2max}$, 74.0%$\dot{V}O_{2max}$ and 80.0%$\dot{V}O_{2max}$ respectively. SD: Standard deviation.

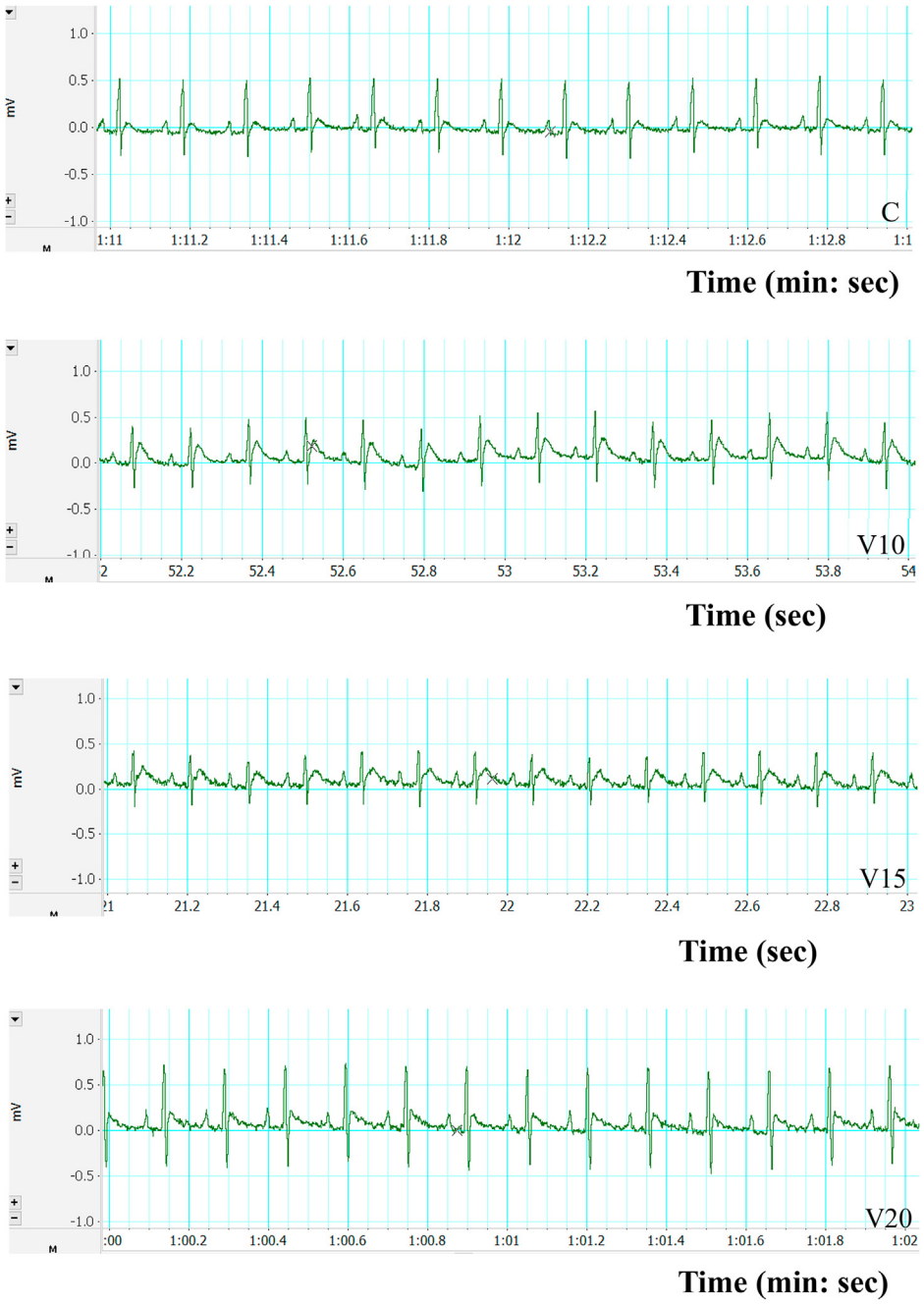

Fig. 2. Original recording showing the ECG.
C: Sedentary control group. EE: Exhaustive exercise group. V10, V15, V20, V26, and V30: exercise preconditioning performed at 53.0%$\dot{V}O_{2max}$, 58.4%$\dot{V}O_{2max}$, 67.0%$\dot{V}O_{2max}$, 74.0%$\dot{V}O_{2max}$ and 80.0%$\dot{V}O_{2max}$ respectively.

As shown in Table 3, compared with the C group, the Tau, -dp/dtmin and EDPVR of the EE group showed no statistical changes insignificantly.

As shown in Table 3, Compared with the C group, PE of rats in the EE group was significantly increased ($p = 0.006$). Compared with the EE group, the PE of rats in the V20, V26 and V30 groups were decreased significantly ($p = 0.083$, $p = 0.069$, $p = 0.074$). Compared with the C group, SW in the V10 group and the V15 group were significantly smaller ($p = 0.044$, $p = 0.042$). Compared with the EE group, the SW in the V20, V26, and V30 groups were significantly increased ($p < 0.001$, $p < 0.001$, $p < 0.001$).

Different intensities of EP improved myocardial mitochondrial respiration function of exhausted rats

As shown in Fig. 3, the respiratory flux was not increased significantly after cytochrome *c* test, indicating the intactness of the mitochondrial outer membrane. With glutamate and malate as electron donors for complex I, compared to the C group, the state 3 respiration rate of the EE group was reduced significantly ($p < 0.001$). Compared with the EE group, the state 3 respiration rates of the V15, V20, V26 and V30 groups were increased significantly ($p = 0.039$, $p = 0.001$, $p < 0.001$, $p < 0.001$). Compared with the V26 group, the V15 group were decreased significantly ($p = 0.002$).

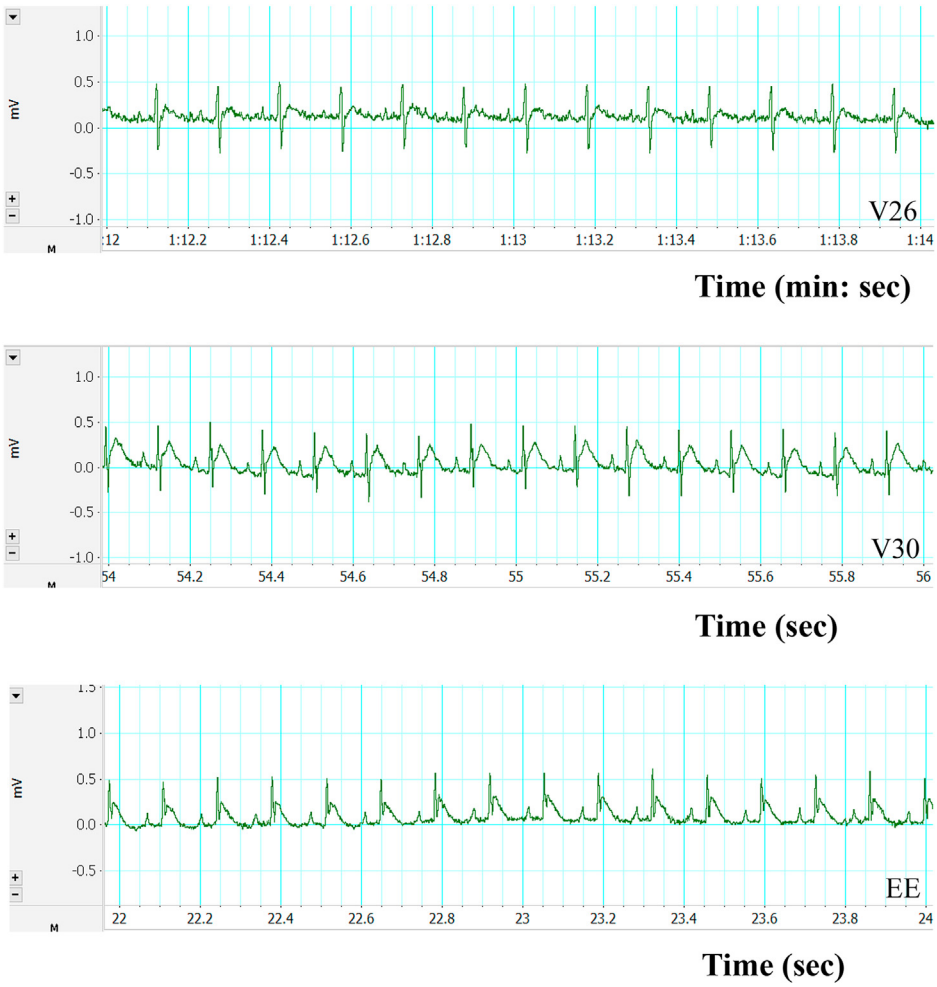

Fig. 2. (*continued*).

Table 3
Comparison of effects of exercise preconditioning intensity on cardiac function of exhausted rats.

	C	EE	V10	V15	V20	V26	V30
Systolic function parameters							
SV(ul)	170.88 ± 31.13	103.16 ± 32.34*	107.34 ± 14.44*	107.81 ± 25.94*	118.56 ± 40.43*	123.65 ± 21.77*	119.40 ± 34.20*
CO(ul/min)	54.43 ± 12.06#	34.79 ± 11.24*	35.46 ± 6.01*	35.90 ± 9.44	40.10 ± 16.47	46.30 ± 9.27#	35.86 ± 10.00*
EF(%)	69.20 ± 4.78#△	43.12 ± 5.93*	44.15 ± 3.27*△	48.80 ± 3.70*△	53.28 ± 3.59*#	57.54 ± 6.33*#	49.87 ± 8.31*△
Ves(ul)	108.49 ± 19.56	157.51 ± 43.28	148.48 ± 18.66	141.69 ± 17.93	130.25 ± 36.54	121.82 ± 28.39	127.00 ± 18.23
Pes(ul)	127.15 ± 14.81	87.51 ± 43.28	90.90 ± 31.71	103.81 ± 24.55	108.57 ± 27.77	115.07 ± 28.26	112.40 ± 25.02
dp/dt_{max}(mmHg.10^3/s)	9.15 ± 1.77	7.09 ± 2.76	7.18 ± 3.73	7.22 ± 3.45	7.29 ± 2.95	7.36 ± 2.27	7.29 ± 2.65
ESPVR	2.53 ± 0.67	1.30 ± 0.52*	1.35 ± 0.31*	1.52 ± 0.42*	1.62 ± 0.40*	1.63 ± 0.63*	1.64 ± 0.65*
Diastolic function parameters							
Ved(ul)	231.25 ± 41.77	246.61 ± 71.44	239.84 ± 33.39	239.16 ± 39.85	238.14 ± 27.76	231.70 ± 75.42	238.48 ± 33.84
Ped(ul)	4.63 ± 3.70	6.62 ± 2.42	6.17 ± 2.51	5.93 ± 3.56	5.55 ± 4.75	5.27 ± 1.89	5.25 ± 4.06
$-dp/dt_{min}$(mmHg.10^3/s)	7.54 ± 1.35	5.16 ± 2.81	5.35 ± 4.69	5.81 ± 3.80	5.69 ± 3.60	6.21 ± 3.63	6.59 ± 2.73
EDPVR	0.021 ± 0.005	0.051 ± 0.019	0.047 ± 0.023	0.044 ± 0.011	0.041 ± 0.044	0.028 ± 0.023	0.029 ± 0.020
Tau(ms)	12.11 ± 0.90	14.35 ± 1.47	13.45 ± 3.87	13.26 ± 2.53	11.80 ± 1.89	11.66 ± 2.29	12.00 ± 2.71
Mechanoenergetics parameters							
PE(mmHg·uL/10^3)	8.53 ± 3.37	15.16 ± 2.39*	13.40 ± 7.50*	12.58 ± 3.64	11.12 ± 2.65#	10.91 ± 3.07#	10.99 ± 6.57#
SW(mmHg·uL/10^3)	15.92 ± 4.04	8.08 ± 3.32	8.85 ± 1.84*	10.15 ± 4.46*	11.55 ± 4.37#	11.59 ± 3.41#	12.12 ± 1.37#
CE(%)	61.82 ± 14.08#	43.38 ± 6.01	58.95 ± 13.55#	47.29 ± 10.66#	46.22 ± 6.10#	53.97 ± 13.77#	46.21 ± 7.83#

Data are expressed as *means* ± *SD*, $n = 8$. *$p < 0.05$ versus the C group, #$p < 0.05$ versus the EE group, &$p < 0.05$ versus the V10 group, ¥$p < 0.05$ versus the V15 group, @$p < 0.05$ versus the V20 group, △$p < 0.05$ versus the V26 group. SV: Stroke volume. CO: Cardiac output. EF: Ejection fraction. Ves: End-systolic volume. Pes: End-systolic pressure. dp/dt_{max}: Peak rate of the increase in pressure. ESPVR: Slope of end-systolic pressure volume relationship. Ved: End-diastolic volume. Ped: End-diastolic pressure. $-dp/dt_{min}$: Peak rate of the decrease in pressure. Tau: Relaxation time constant. EDPVR: Slope of end-diastolic pressure volume relationship. PE: potential energy. SW: stroke work. CE: cardiac efficiency. C: Sedentary control group. EE: Exhaustive exercise. V10, V15, V20, V26, and V30: exercise preconditioning performed at 53.0%$\dot{V}O_{2max}$, 58.4%$\dot{V}O_{2max}$, 67.0%$\dot{V}O_{2max}$, 74.0%$\dot{V}O_{2max}$ and 80.0%$\dot{V}O_{2max}$ respectively. SD: Standard deviation.

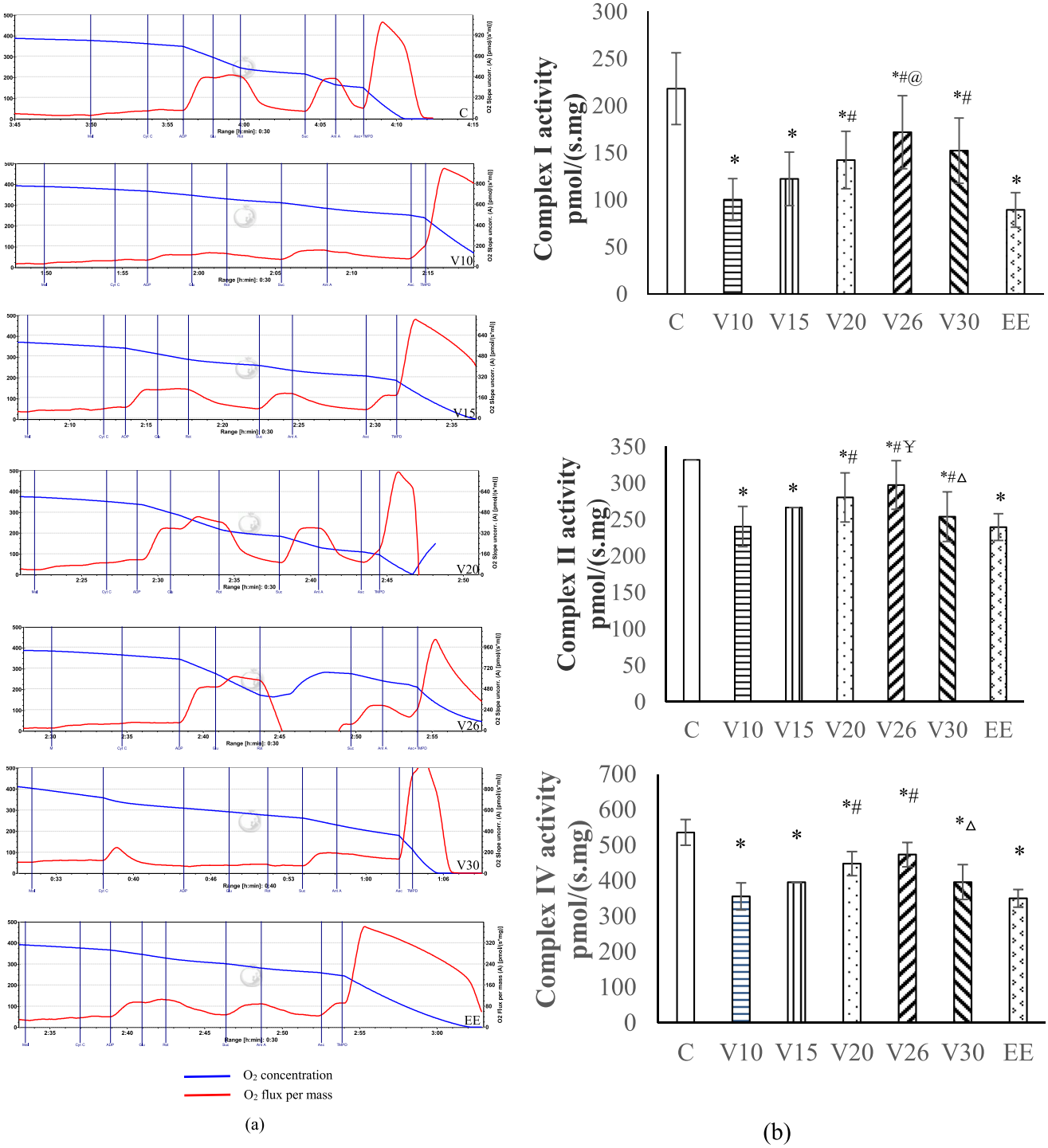

Fig. 3. Maximal respiratory capacity in permeabilized myocardial fibers. (a) Original recording. (b) Comparison. Data are expressed as *means* ± *SD*, $n = 6$. $^{*}p < 0.05$ versus the C group, $^{\#}p < 0.05$ versus the EE group, and $^{\&}p < 0.05$ versus the V10 group, $^{¥}p < 0.05$ versus the V15 group, $^{@}p < 0.05$ versus the V20 group, $^{\triangle}p < 0.05$ versus the V26 group. C: Sedentary control group. Glu, glutamate; Mal, malate; Cyt C, cytochrome *c*; Suc, succinate; Rot, rotenone; Ant A, antimycin A; and Asc, ascorbate. EE: Exhaustive exercise group. V10, V15, V20, V26, and V30: exercise preconditioning performed at 53.0%$\dot{V}O_{2max}$, 58.4%$\dot{V}O_{2max}$, 67.0%$\dot{V}O_{2max}$, 74.0%$\dot{V}O_{2max}$ and 80.0%$\dot{V}O_{2max}$ respectively. SD: Standard deviation.

Using succinate as a substrate for complex II, compared to the C group, the state 3 respiration rate of the EE group was reduced significantly ($p < 0.001$). Compared with the EE group, the state 3 respiratory rate of the V20, V26 and V30 groups were all increased significantly ($p = 0.021$, $p < 0.001$). Compared with the V26 group, the V10, V15 and V30 groups were all reduced significantly ($p = 0.002$, $p = 0.076$, $p = 0.014$).

With ascorbate/TMPD being used as substrates for complex IV, compared to the C group, the state 3 respiratory rate of the EE group was reduced significantly ($p < 0.001$). Compared with the EE group, the state 3 respiration rates of the V15, V20, V26 and V30 groups were increased significantly ($p = 0.028$, $p < 0.001$, $p < 0.001$, $p = 0.027$). Compared with the V26 group, the V10 group, V15 group and V30 group were decreased significantly ($p < 0.001$).

Table 4
Analysis of correlation between exercise preconditioning intensity and the effect parameters.

The effect parameters	Correlation coefficients r (p)
Serum levels of myocardial enzymes ($n = 6$)	
cTn-I	−0.705*(<0.001)
NT-proBNP	−0.881*(<0.001)
ECG parameters	
HR	−0.455*(0.001)
P wave	0.227(0.12)
PR	0.379*(0.008)
R Amplitude	0.161(0.274)
QRS Duration	0.346*(0.016)
ST Height	0.905*(<0.001)
QT Interval	0.386*(0.007)
Cardiac function parameters ($n = 8$)	
SW	0.141(0.339)
CO	0.138(0.349)
SV	0.243(0.096)
Ves	0.081(0.584)
Ved	−0.031(0.835)
Pes	0.178(0.226)
Ped	−0.197(0.18)
EF	0.524*(<0.001)
EA	0.278(0.055)
dp/dt_{max}	0.156(0.290)
dp/dt_{min}	0.068(0.647)
PE	−0.360*(0.012)
CE	0.477*(0.001)
Tau	−0.473*(0.001)
ESPVR	0.176(0.231)
EDPVR	0.151(0.306)
Mitochondrial function parameters ($n = 6$)	
ComplexIactivity	0.662*(<0.001)
Complex II activity	0.359*(0.012)
Complex IV activity	0.536*(<0.001)

*$p < 0.05$. EP: Exercise preconditioning. NT-proBNP: N terminal pro B type natriuretic peptide. cTn-I: cardiac troponin I. HR: Heart rate. SW: stroke work. SV: Stroke volume. CO: Cardiac output. EF: Ejection fraction. Ves: End-systolic volume. Pes: End-systolic pressure. dp/dt_{max}: Peak rate of the increase in pressure. ESPVR: Slope of end-systolic pressure volume relationship. Ved: End-diastolic volume. Ped: End-diastolic pressure. $-dp/dt_{min}$: Peak rate of the decrease in pressure. Tau: Relaxation time constant. ESPVR: End-systolic pressure volume relationship. EDPVR: Slope of end-diastolic pressure volume relationship. CE: cardiac efficiency.

Cardiac protection of EP is proportional to EP intensity

According to Spearman's correlation analysis, the serum level of cTn-I was strongly and negatively correlated with the EP intensity ($r = -0.705$, $p < 0.001$), and the serums levels of NT-proBNP was obviously and negatively correlated with the EP intensity ($r = -0.881$, $p < 0.001$). The HR of electrocardiogram parameters was significantly and negatively correlated with the EP intensity ($r = -0.455$, $p = 0.001$), and the PR ($r = 0.379$, $p = 0.008$), QRS Duration ($r = 0.346$, $p = 0.016$) and QT interval ($r = 0.386$, $p = 0.007$) were strongly and positively correlated with the EP intensity. The EF ($r = 0.524$, $p < 0.001$) and CE ($r = 0.477$, $p = 0.001$) of heart function parameters were significantly and positively correlated with the EP intensity. The PE ($r = -0.360$, $p = 0.012$) and Tau ($r = -0.473$, $p = 0.001$) of heart function parameters were significantly and negatively correlated with the EP intensity. The state 3 respiration rate of mitochondrial ET pathway complex I ($r = 0.662$, $p < 0.001$), Complex II ($r = 0.359$, $p = 0.012$) and Complex IV ($r = 0.536$, $p < 0.001$) were significantly and positively correlated with the EP intensity (Table 4).

Discussion

The occurrence of cardiovascular diseases during exercise are often heard, especially in the group engaged in intense training such as army soldiers and competitive athletes.[2,3] To discuss the consequence and risk of exhaustive exercise and the prevention measure for that is of great importance. In this study, we have established the exhaustive exercise rat model to simulate the intense military training or long-distance running exercise for human and observed the consequence of that to the heart. We have also discussed the influence of different intensities of exercise preconditioning on EECI by observing alterations of myocardial enzymology, electrophysiology, cardiac function, and myocardial mitochondrial respiratory function.

The serum marker analyses suggest that cTn-I and NT-proBNP were released into blood after exhaustive exercise, so the contents of these enzymes in the serum increased significantly, indicating that the cardiomyocytes were injured, which is in line with numerous studies on animals models[14,20,21] and human studies.[22,23] After EP, the contents of cTn-I and NT-proBNP in serum of exhausted rats were decreased, indicating that EP had the ability to resist the myocardial injury. The results were consistent with our previous results that the content of myocardial enzyme in serum of exhausted rats decreased after EP.[14] Different intensities of EP can reduce the serum levels of cTn-I and NT-proBNP to different degrees, and with the increase of EP intensity, the levels of serum cTn-I ($r = -0.705$, $p < 0.001$) and NT-proBNP ($r = -0.881$, $p < 0.001$) have shown a correlative decreasing trend. The protection of EP on myocardial injury of exhausted rats was most obvious when the EP intensity is 74.0%$\dot{V}O_{2max}$, and the protective effect declines when the intensity reached 80.0%$\dot{V}O_{2max}$. YM Li et al.[14] also reported the effect of moderate intensity EP training can be more obvious in reducing serum levels of cardiac injury enzymes than the lower and the higher intensity of EP.

EE induced abnormal electrocardiographic activity in rats, including prolonged atrial depolarization time and extension of the time from atrium to ventricle. EE also increased ventricular depolarization and repolarization time, and caused acute myocardial ischemia, which might potentially induce arrhythmia. Different intensities of EP training were shown to improve electrical disturbances in the heart caused by EE. This was consistent to Li's report EP could enhance the tolerance level of myocardium to ischemia and hypoxia and reduce the influence of myocardial injury caused by EE on electrical activity.[13] The protection of EP on cardiac electrophysiological activity is improved with the increase of EP intensity (HR: $r = -0.455$, $p = 0.001$; PR: $r = 0.379$, $p = 0.008$; QRS Duration: $r = 0.346$, $p = 0.016$; QT interval: $r = 0.386$, $p = 0.007$), among which the protection of V26(74.0%$\dot{V}O_{2max}$) and V30(80.0%$\dot{V}O_{2max}$) is the most obvious.

During exercise, myocardial oxygen consumption increased, and stronger contract ability of the myocardium was required to deliver more oxygen to the muscles.[7] Under the overload pressure induced by exhaustive exercise, the left ventricular diastolic function and contractile function were impaired in rats. EP can improve the damage of EE to the rats' cardiac function, enhance the elasticity of the ventricular wall, increase cardiac output and increase myocardial systolic and diastolic ability. Among all the EP intensities, the medium intensity (74.0%$\dot{V}O_{2max}$ and 67.0%$\dot{V}O_{2max}$) of EP have better protection on cardiac systolic function than high intensity (80.0%$\dot{V}O_{2max}$, $p < 0.05$) and low intensity (53.0%$\dot{V}O_{2max}$, $p < 0.05$). Similar to our conclusion, Li Y et al.[14] report that moderate intensity of rat treadmill running EP (65%-75%$\dot{V}O_{2max}$) has the best protection in rats and Starnes et al.[24] showed that exercise training for 16 week, 5 days/week 40 min/day below 55%–60%$\dot{V}O_{2max}$ did not achieve protection against cardiac ischemia reperfusion injury (IRI). Lennon et al.[15] concluded that both moderate- (i.e., 60 min/day at 50%$\dot{V}O_{2max}$) and high-intensity exercise (i.e., 60 min/day at 70%$\dot{V}O_{2max}$) performed during three consecutive days appear to be equally protective against ischemia reperfusion induced myocardial stunning. The discrepancy in results may be due to the use of different exercise protocols in regards to exercise training duration and methodology for imposing intensity (continuous or interval) that could interfere in the amount of cardioprotection afforded.[25]

The PE was increased, and SW and CE were decreased, indicating the myocardium of the exhausted rats was damaged and the resistance of

ventricular wall movement was increased. Olah et al.[26] also demonstrated that CE and SW were reduced in rats after exhaustive exercise. After 3 weeks of EP, the PE of the V20, V26 and V30 groups ($p = 0.083$, $p = 0.069$, $p = 0.074$ respectively) were decreased and the SW of that ($p < 0.001$, $p < 0.001$, $p < 0.001$)were increased significantly, indicating the myocardium underwent adaptive changes and the cardiac efficiency were enhanced. The correlation analysis showed the PE ($r = -0.360$, $p = 0.012$, $p < 0.05$) and CE ($r = 0.477$, $p = 0.001$, $p < 0.05$) were correlated with the EP intensity, indicating the cardiac efficiency were improved with the increase of speed. Among the EP groups, the V26 and V30 were close to the C group.

Mitochondria are best known for harboring pathways involved in ATP synthesis through the tricarboxylic acid cycle and oxidative phosphorylation(OXPHO). In the process of OXPHO, oxygen is consumed and an electron-chemical gradient is established that drives the synthesis of ATP.[27] The consumption of oxygen in respiration is a measurable parameter reflecting mitochondrial function. Chance and Williams[28] defined five states of mitochondrial respiration (states 1–5). Commonly, a state 3 rate is measured primarily to determine an approximation of the maximal respiratory capacity of the mitochondrion with a large, non-limiting, amount of ADP present within the chamber for a fixed amount of mitochondria and O_2.

Muscle oxidative capacities have been assessed by measuring respiration of mitochondria in permeabilized fibres, with no limitation of substrates, ADP, or oxygen.[29] Results show that EE has reduced the state 3 respiration rates of myocardial mitochondrial respiratory complex I, II, and IV and the production of ATP was decreased. Different intensities of EP could improve the activities of mitochondrial function to varying degrees, indicating EP promotes changes in myocardial mitochondrial energy metabolism which may contribute to the cardiac protection induced by EE. With the increase of EP intensity in the range of 58.4%$\dot{V}O_{2max}$ to 74.0%$\dot{V}O_{2max}$, the mitochondrial function has been improved proportionally (Complex I: $r = 0.662$, $p < 0.001$; Complex II: $r = 0.359$, $p = 0.012$; Complex IV: $r = 0.536$, $p < 0.001$), among which the 74.0%$\dot{V}O_{2max}$ is most obvious. While when EP reached 30 m/min, the respiratory rates of electron transfer pathway (ET pathway) complexes I, II, and IV decline, making the 74.0%$\dot{V}O_{2max}$ a turning point. Studies have found that the activities of various myocardial complex enzymes were increased during moderate-intensity training, while low-intensity training modes had no significant effect on the activities of these complex enzymes.[16]

The correlation analysis results showed that EP protection is proportional to EP intensity in the range of 53.0%$\dot{V}O_{2max}$-74.0%$\dot{V}O_{2max}$, including reducing serum levels of cTn-I ($r = -0.705$, $p < 0.001$) and NT-proBNP ($r = -0.881$, $p < 0.001$), improving electrocardiogram activity(PR: $r = 0.379$, $P = 0.008$; QRS Duration: $r = 0.346$, $p = 0.016$; QT interval: $r = 0.386$, $p = 0.007$), improving cardiac function (EF: $r = 0.524$, $p < 0.001$; CE: $r = 0.477$, $p = 0.001$; PE: $r = -0.360$, $p = 0.012$; Tau: $r = -0.473$, $p = 0.001$) and elevating mitochondrial ET pathway complex I ($r = 0.662$, $p < 0.001$), II ($r = 0.359$, $p = 0.012$) and IV ($r = 0.536$, $p < 0.001$) activity. The protection of EP on serum myocardial injury enzymes, heart function and mitochondrial respiration function is most obvious when EP intensity is 74.0%$\dot{V}O_{2max}$. Starnes et al.[24] exercised rats on a treadmill at an intensity of 55%–60%$\dot{V}O_{2max}$, 40 min per day, 5 days per week for 16 weeks indicating that exercise training at 55%–60%$\dot{V}O_{2max}$ is below the threshold intensity necessary to induce intrinsic cardiac protection against ischemia reperfusion injury, which is not contradictory to our results.

In summary, high intensity and long duration exhaustive exercise caused myocardial damage, including myocardial injury, electrical derangement, cardiac dysfunction and decreased mitochondrial respiratory function. EP could protect heart from EECI by ameliorating myocardial injury, improving electrical derangement and the left ventricular function and raising the activity of ET pathway complexes I, II and IV. Different intensities of EP had different protection on EECI, and the protection was improved as the EP intensity was increased from 53.0%$\dot{V}O_{2max}$ to 74.0%$\dot{V}O_{2max}$. The optimal EP intensity in this experiment was shown to be 74.0%$\dot{V}O_{2max}$. Although this conclusion is only based on the animal experiment, it reminds us moderate intensity of "warm-up" before intensive exercise is necessary and beneficial for protecting from intense exercise induced heart injury.

Conclusion

High intensity and long duration exhaustive exercise caused myocardial damage, including myocardial injury, electrical derangement, cardiac dysfunction and decreased mitochondrial respiratory function. EP could decrease the serum contents of NT-proBNP and cTn-I, improve the electrical derangement and the left ventricular function, and enhance myocardial mitochondrial ET pathway I, II, and IV activity in exhausted rats. The protection of EP to heart injury induced by EECI was improved as the EP intensity increased from 53.0%$\dot{V}O_{2max}$ to 74.0%$\dot{V}O_{2max}$. When EP intensity was 74.0%$\dot{V}O_{2max}$, the effect was most obvious among all the setting EP groups.

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Ethical approval statement

All experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals and permitted by the Ethics Committee for the Use of Experimental Animals at the PLA 82nd Group Military Hospital (CA19-02).

Authors' contributions

Zheng Ping designed and carried out the experiment, analyzed data and draft the manuscript. Weijia Qiu performed the experiment and analyzed data. Mei Yang contributed significantly to analyses and manuscript preparation. Xiaoli Zhang established the animal model and performed the data analyses. Dongying Wang performed the experiment. Peng Xu determined the heart function and analyzed the data. Ziwen Wang designed the experiment, analyzed the data and draft the manuscript. Xuebin Cao designed and carried out the experiment, analyzed data and revised the draft.

Submission statement

We certify that the work reported in this paper is original and has not been submitted elsewhere for consideration of publication. All authors have read the final version of the manuscript and approved to submit it to your journal.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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