Cardiac Effects of Treadmill Running With Different Intensities in a Rat Model

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Research Article

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Abstract

Purpose: Exercise is an effective intervention for both the prevention and treatment of cardiovascular diseases. In this study, we investigated the structural and functional changes that occur in cardiac response and how they differ with various exercise intensities, hence exploring the potential signal transduction.

Methods: Male Sprague–Dawley rats were randomly divided into the sedentary (SED), low-intensity running (LIR), medium-intensity running (MIR), and high-intensity running (HIR) groups. Each exercise group had 3 different operation time subgroups for 1, 4, and 8 weeks, respectively, and all rats observed a daily 1 h treadmill routine 5 days per week. Echocardiographic measurements were performed at the end of the experimental program. Additionally, myocardium samples and blood were collected for histological and biochemical examinations. Changes in the extracellular signal-regulated kinases 1/2 (ERK1/2) signal pathway were detected by Western blotting.

Results: After a week of running, ventricular myocyte size and the phosphorylation of ERK1/2 increased in the HIR group, while left ventricular (LV) diastolic diameter values and LV relative wall thickness increased in the LIR and MIR groups. In addition, we observed heart enlargement, cTnI decrease, and ERK1/2 signal activation in each of the exercise groups after 4 weeks of running. However, the HIR group displayed a substantial rupture and increased fibrosis in myocardial tissue. In addition, compared with the LIR and MIR groups, 8 weeks of HIR resulted in structural damage, fiber deposition, and increased cTnI. However, there was no difference in activation of ERK1/2 signaling between the exercise groups and SED group.

Conclusions: The effect of running on cardiac hypertrophy was intensity dependent. In contrast to LIR and MIR, 8 weeks of HIR-induced cardiac hypertrophy was characterized by potential cardiomyocyte injury, which increased the risk of pathological development. Furthermore, the ERK signaling pathway was mainly involved in the compensatory hypertrophy process of the myocardium in the early stage of exercise and was positively correlated with exercise load. However, long-term exercise may attenuate ERK signaling activation.

Introduction

Exercise and physical activity are effective ways to reduce the risk of cardiovascular disease, such as heart attack and stroke, and they can provide valuable benefits beyond medications. Improved cardiac performance, along with cardiac hypertrophy, is one major feature of endurance exercise, leading to a constellation of adaptations that affect the structure, electrical conduction, and function of the heart and that contribute to appropriate increases in cardiac output[1, 2]. Studies in animal models of exercise-induced cardiac hypertrophy—here in response to treadmill exercise, voluntary wheel running, and swim training—have shown a preserved or enhanced contractile function and relative cardiac hypertrophy[3-5]. The important differences that exist between these experimental methods may affect the interpretation of

the results. There is a plethora of studies demonstrating that treadmill running has become a preferred option in comparative studies of the effects of exercise training because of the precise control of exercise intensity and volume[6-8].

Physiological cardiac hypertrophy induced by endurance exercise is related to an increase in cardiac mass and individual cardiomyocyte growth in both length and width, with no interstitial or replacement fibrosis or cell damage; this is considered reversible progress and does not develop into heart failure[9]. In contrast, although pathological hypertrophy is initially induced as a compensatory response to growth of the ventricle, this kind of hypertrophy progresses to ventricular chamber dilatation, with wall thinning through loss of myocytes and contractile dysfunction, resulting in adverse cardiovascular events[10]. The impact of endurance exercise on heart health depends on the combination of intensity, time duration, frequency, and type[11]. However, less is known regarding cardiac adaptations to the different intensities of treadmill running in rats. Most studies have shown that low- and moderate-intensity exercise attenuates abnormal cardiac remodeling and myocardial dysfunction and improves functional capacity[5, 12]. Additionally, clinical studies also support this recommendation by identifying the benefits that can be derived from low- and moderate-intensity exercise[13]. Exercise performed at a high intensity appears to convey greater cardioprotective benefits than exercise of a moderate intensity, which may be because of the increase in aerobic fitness[14]. However, recent data show thathigh-intensity exercise hasan adverse effect on the heart, along with impairment of cardiomyocyte Ca^{2+} handling, mitochondrial respiration, and the activation of proapoptotic and profibrotic activity[15, 16]. Given that intensity dependence for cardiac function remains controversial, it is imperative to better understand how different treadmill running intensities alter the phenotype of the murine heart.

Exploring the molecular mechanisms of cardiac hypertrophy is helpful to better understand the adaptability of the heart. Among the numerous signaling factors, the extracellular signal-regulated kinases 1/2 (ERK1/2) pathway has been postulated as promoting cardiac hypertrophy[17]. For instance, transgenic mice overexpressing an activated MEK1 mutant under the transcriptional control of cardiac-specific α-myosin heavy chain promoter were found to induce cardiac hypertrophy in vivo, which constitutively activated ERK1/2 in the heart[18]. Furthermore, another study demonstrated that the ERK1/2 signaling pathway uniquely regulates the balance between eccentric and concentric growth of the heart[19]. Even though many studies conducted on pressure overload or mutagenesis have examined the role of the MEK1/2-ERK1/2 pathway, the regulatory mechanism for exercise-induced cardiac hypertrophy remains unclear.

The present study was designed to develop a rat model of three treadmill running programs to test whether different types of intensity training can induce cardiac structural and functional changes. Our hypothesis was that different exercise loads will result in myocardial hypertrophy based on intensity dependence, which might be associated with different LV functional consequences in rats. Therefore, we aimed to provide LV structural and functional characterization of exercise-induced hypertrophy in rats. Additionally, we investigated molecular alterations in exercise-induced cardiac hypertrophy.

Materials And Methods

Experimental animals and exercise protocols.

A total of 72 male Sprague–Dawley rats at 8 weeks of age, 200–220 g in weight, were randomized into four even groups: (1) sedentary control (SED, n = 18), (2) low-intensity running (LIR, n=18), (3) medium-intensity running (MIR, n=18), and (4) high-intensity running (HIR, n=18). Each exercise group had three different operation time subgroups (1, 4, and 8 weeks following the end of the last training) (n=6 for each time subgroup). The rats were housed in cages under controlled temperature (22±1°C), humidity (50%) conditions, and light/dark (12/12 h) cycle, and they were allowed free access to standard rodent chow and water. This study was approved by the Animal Ethics Committee of Fujian Medical University.

All animals were first acclimatized to run on a treadmill at a speed of 10 m/min for 30 min/day for 1 week. Subsequently, animals in the LIR, MIR, and HIR groups were regularly exercised according to the previously described running protocols for 1, 4, and 8 weeks[20]. Training speed and inclination varied as follows: LIR, 15.2 m/min with 0˚ incline for 60 min, 5 days/week; MIR, 19.3 m/min with 5˚ incline for 60 min, 5 days/week; and HIR, 26.8 m/min with 10˚ incline for 60 min, 5 days/week. The exercise-trained rats were encouraged to run by mild electrical stimulation. Meanwhile, the rats in the SED group maintained a sedentary lifestyle.

Echocardiography

Transthoracic echocardiogram was performed for all rats 24 h after the last bout of exercise, and heart function was assessed by echocardiography (Vivid E9, General Electric Company, CT, USA). The rats were anesthetized using 10% chloral hydrate (3 mL/kg, i.p.). The following structural variables were measured according to M-mode tracings: LV end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LV posterior wall thickness (LVPWT), ejection fraction (EF), fractional shortening (FS), stroke volume (SV), heart rate (HR), and cardiac output (CO).

Biochemical measurements

After completion of the transthoracic echocardiogram, the abdominal cavity was quickly opened, and blood samples were collected from the ventral aorta. The blood samples were kept at room temperature for 1 h, centrifuged at 300×g for 15 min, and stored at -80 °C until analysis. Cardiac troponin T (cTnI) was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Histological evaluation

After completing echocardiography, the whole hearts were perfused with 250~300 ml normal saline, rapidly excised, and weighed on an electronic balance to calculate HM/BM. Subsequently, the hearts were either fixed in 4% paraformaldehyde for histological analysis or frozen in liquid nitrogen for a protein

analysis. After 24 h fixation in paraformaldehyde solution, the atria were removed, and the ventricles were dehydrated with ethanol, embedded in paraffin, and cut transversely into 5 μm sections. Hematoxylin-eosin (HE) staining and wheat germ agglutinin were performed on the heart sections to measure myocyte cross-sectional area. Picrosirius red (PSR) staining was performed to define the average volume of collagen deposition in the heart. The captured images were analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Western blot analysis

Proteins were extracted from snap-frozen heart tissue using a RIPA buffer. A BCA protein analysis kit (P0010S; Beyotime Biotechnology) was used to evaluate the protein amounts, and then, the protein concentration was normalized before all Western blot experiments. Equal amounts of protein were separated by 10% SDS-PAGE and transferred onto a nitrocellulose membrane. After that,these membranes were blocked with nonfat milk for 1 h at room temperature, followed by overnight incubation at 4°C with primary antibodies against MEK (ab178876, 1:20000; Abcam), ERK (ab36991, 1:2000; Abcam), p-ERK (Thr202/Tyr204) (#4376, 1:1000; CST), and GAPDH (60004-1-Ig, 1:2000; Proteintech). The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The membranes were developed with chemiluminescence reagents (P0019; Beyotime Biotechnology). The Western blotting signals were analyzed using an Azure Biosystems C600 (Azure Biosystems Inc., CA, USA).

Statistical analysis

All statistical analyses were carried out using SPSS 23.0 software. Data are expressed as the mean ± standard deviation. Differences between multiple groups were tested by one-way ANOVA. Post hoc LSD or Kruskal–Wallis H tests were used for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

Body weight and heart weight

We expected different exercise intensities to be associated with cardiac hypertrophy. At the gross level, the hearts of exercise rats were remarkably larger than those of the sedentary animals and presented with typical hypertrophic changes after 4 and 8 weeks of exercise (Fig. 1A). The data in Table 1 show that there was a significant decrease in body weight in the MIR and HIR groups after 1, 4, and 8 weeks of exercise and a lower body weight in the LIR group compared with the SED group after 8 weeks of exercise ($P < 0.05$). A significant difference in heart weight was identified in the MIR and HIR groups at 8 weeks of exercise ($P < 0.05$). When comparing the heart weight to body weight ratios, although 4 and 8 weeks of different treadmill running intensities resulted in a significant increase ($P < 0.05$), no significant differences were found between the individual SED group and other exercise groups after 1 week, which

was consistent with gross morphologic examination. At the tissue level, we found that the HIR group displayed significantly greater ventricular myocyte size compared with the SED group, and this held true as well between the HIR group and other exercise groups after 1 week of exercise (Fig. 1B **and C**). Compared with the ventricular myocytes of the individual sedentary controls, the cross-sectional area of the ventricular myocytes of the rats subjected to different treadmill running intensities for 4 and 8 weeks was significantly larger ($P < 0.01$).

Table 1
Average body weight and heart weight along with heart weight to body weight ratio for the different groups studied (n = 6 in each case)

	1 week			
	SED	LIR	MIR	HIR
BW(g)	326.5 ± 15	323.3 ± 11.9	304.5 ± 15.3[a]	294.8 ± 20.8[bd]
HW(mg)	1039 ± 46	1055.2 ± 100.3	986 ± 82.8	982.4 ± 82.5
HW/BW(mg/g)	3.2 ± 0.2	3.3 ± 0.2	3.2 ± 0.3	3.3 ± 0.1
		4 weeks		
	SED	LIR	MIR	HIR
BW(g)	435 ± 25.5	405.5 ± 25.6	384.2 ± 36.2[b]	368.3 ± 24.9[b]
HW(mg)	1126.5 ± 127.1	1233.8 ± 85.1	1247.2 ± 134.4	1281.5 ± 130.7
HW/BW(mg/g)	2.6 ± 0.1	3.1 ± 0.3[b]	3.3 ± 0.3[b]	3.5 ± 0.2[bc]
		8 weeks		
	SED	LIR	MIR	HIR
BW(g)	479.8 ± 27.4	448.2 ± 29.8[a]	419.2 ± 15.4[b]	407.3 ± 17.2[bc]
HW(mg)	1192.8 ± 87.8	1192.8 ± 87.8	1351.2 ± 106.7[a]	1398.7 ± 121.5[b]
HW/BW(mg/g)	2.5 ± 0.1	2.9 ± 0.1[b]	3.2 ± 0.2[bc]	3.4 ± 0.2[bd]
BW body weight, HW heart weight, HW/BW heart weight/body weight ratio.				
Data are presented as means ± SEM. [a]$P<0.05$ vs. SED, [b]$P < 0.01$ vs. SED, [c]$P<0.05$ vs. LIR, [d]$P<0.01$ vs. LIR. (at the same age)				

Morphometry and histology

Myocardial structure and fibrosis are the hallmarks of cardiac hypertrophic remodeling. Compared with the myocardial structure of the individual sedentary group, the cardiomyocytes in the HIR group began to occasionally and slightly rupture after 4 weeks of exercise, while those after 8 weeks of exercise were

irregular in shape and disorganized in arrangement (Fig. 2A). Similar to the changes in myocardial structure, collagen deposition in the myocardium was also detected in the HIR group following 4 and 8 weeks of exercise, showing a significant increase compared with the individual sedentary group and other exercise groups ($P < 0.01$; Fig. 2B and 2C). However, the fibrosis of the ventricular myocytes decreased significantly in the MIR group after 8 weeks ($P < 0.05$). After 1 week, all trained rats showed no changes of myocardial structure and no collagen deposition in the myocardium.

Morphological and functional parameters

To determine if cardiac hypertrophy caused by exercises of varying intensities was different in their morphology and function, we subjected the heart to echocardiography (Fig. 2D). The data in Table 2 show that LVEDD, LVESD, LVEDV, and LVESV were significantly increased in the LIR group and MIR group compared with the sedentary group after 1 week, but there were no significant changes in the left ventricle internal diameter or volumes in the HIR group. However, the resting cardiac function in rats as assessed by FS and EF was significantly lower in the LIR and MIR groups. After 4 weeks of exercise, the data in Table 3 revealed that medium-intensity running decreased LVEDV and increased LVESD and LVESV (all $P < 0.05$). Moreover, the decrease in LVEDV and HR resulted in the lower CO in the HIR group compared with the LIR group and MIR group. The data in Table 4 showed that 8-week low-to-medium-intensity running exhibited a significant increase in LVEDD compared with the SED group ($P < 0.05$). HR in the LIR group was significantly higher than that in the SED group, resulting in a significant increase in CO ($P < 0.05$).

Table 2
Echocardiographic analysis of SD rats subjected to different treadmill running intensities for 1 week.

Criteria	SED	LIR	MIR	HIR
LVEDD(mm)	6.493 ± 0.979	7.495 ± 0.48[a]	7.628 ± 0.677[a]	6.8 ± 0.456
LVESD(mm)	3.07 ± 0.814	4.46 ± 0.576[b]	4.285 ± 0.701[b]	3.602 ± 0.538[c]
LVEDV(lL)	0.657 ± 0.222	0.942 ± 0.168[a]	0.998 ± 0.219[b]	0.72 ± 0.142[e]
LVESV(lL)	0.085 ± 0.062	0.225 ± 0.079[b]	0.207 ± 0.089[a]	0.124 ± 0.054[c]
SV(lL)	0.57 ± 0.186	0.717 ± 0.129	0.82 ± 0.206[a]	0.626 ± 0.117
IVST(mm)	1.567 ± 0.121	1.533 ± 0.216	1.417 ± 0.147	1.48 ± 0.311
LVPWT(mm)	2 ± 0.237	1.767 ± 0.137[a]	1.75 ± 0.164[a]	1.88 ± 0.217
EF(%)	86.923 ± 4.504	76.211 ± 5.784[b]	79.323 ± 6.731[a]	82.571 ± 6.55
FS(%)	51.733 ± 5.072	38.446 ± 7.29[b]	43.678 ± 7.186[a]	46.719 ± 6.314
HR(bpm)	440.167 ± 31.141	413.5 ± 45.465	405.167 ± 23.609	427.4 ± 25.706
CO	251.675 ± 83.315	276.854 ± 53.919	354.382 ± 30.831[a]	254.583 ± 87.635[e]

Data are presented as the means ± SEM. [a]P<0.05 vs. SED, [b]P < 0.01 vs. SED, [c]P<0.05 vs. LIR, [d]P<0.01 vs. LIR, [e]P<0.05 vs. MIR, [f]P<0.01 vs. MIR.

Table 3
Echocardiographic analysis of SD rats subjected to different treadmill running intensities after 4 weeks.

Criteria	SED	LIR	MIR	HIR
LVEDD(mm)	6.697 ± 0.847	6.952 ± 0.407	7.552 ± 0.329	6.528 ± 0.712
LVESD(mm)	4.015 ± 0.827	3.614 ± 0.539	4.458 ± 0.508[c]	3.883 ± 0.553
LVEDV(lL)	0.707 ± 0.25	0.766 ± 0.12	0.96 ± 0.113[a]	0.653 ± 0.19
LVESV(lL)	0.175 ± 0.096	0.124 ± 0.047	0.218 ± 0.062[c]	0.153 ± 0.066
SV(lL)	0.532 ± 0.158	0.64 ± 0.9	0.742 ± 0.07	0.5 ± 0.158
IVST(mm)	1.582 ± 0.116	1.512 ± 0.232	1.567 ± 0.186	1.4 ± 0.237
LVPWT(mm)	1.903 ± 0.152	1.86 ± 0.163	1.933 ± 0.121	1.783 ± 0.232
EF(%)	78.347 ± 4.861	83.461 ± 5.951	76.589 ± 6.245	75.89 ± 7.862
FS(%)	42.306 ± 4.308	47.809 ± 6.952	40.696 ± 5.733	40.209 ± 6.849
HR(bpm)	383 ± 39.542	411.2 ± 42.222	360.833 ± 56.926	341 ± 71.828[c]
CO	205.042 ± 70.87	263.764 ± 46.099	266.84 ± 44.624	166.91 ± 54.841[cf]

Data are presented as the means ± SEM. [a]P<0.05 vs. SED, [b]P < 0.01 vs. SED, [c]P<0.05 vs. LIR, [d]P<0.01 vs. LIR, [e]P<0.05 vs. MIR, [f]P<0.01 vs. MIR.

Table 4
Echocardiographic analysis of SD rats subjected to different treadmill running intensities after 8 weeks.

Criteria	SED	LIR	MIR	HIR
LVEDD(mm)	6.458 ± 0.761	7.117 ± 0.624[a]	7.202 ± 0.635[a]	7.065 ± 0.618
LVESD(mm)	3.197 ± 0.849	3.72 ± 0.952	4.128 ± 0.724	3.993 ± 0.722
LVEDV(lL)	0.64 ± 0.187	0.828 ± 0.203	0.85 ± 0.205	0.805 ± 0.201
LVESV(lL)	0.097 ± 0.079	0.15 ± 0.118	0.186 ± 0.088	0.17 ± 0.086
SV(lL)	0.498 ± 0.14	0.68 ± 0.121[a]	0.664 ± 0.144	0.635 ± 0.127
IVST(mm)	1.508 ± 0.165	1.643 ± 0.074	1.512 ± 0.223	1.637 ± 0.181
LVPWT(mm)	2.01 ± 0.222	1.998 ± 0.085	1.856 ± 0.134	1.995 ± 0.157
EF(%)	85.355 ± 7.926	82.997 ± 8.031	79.036 ± 6.454	79.405 ± 6.301
FS(%)	50.387 ± 8.344	47.633 ± 8.177	43.156 ± 6.244	43.2 ± 5.843
HR(bpm)	365.833 ± 61.029	421.5 ± 43.284[a]	410.4 ± 28.676	400 ± 23.401
CO	203.713 ± 69.378	288.195 ± 67.943[a]	272.106 ± 63.302	253.157 ± 46.626

Data are presented as the means ± SEM. [a]P<0.05 vs. SED, [b]P < 0.01 vs. SED, [c]P<0.05 vs. LIR, [d]P<0.01 vs. LIR, [e]P<0.05 vs. MIR, [f]P<0.01 vs. MIR.

Serum cTnI levels

Figure 3A shows the levels of the serum cTnI of different treadmill running intensity groups. Compared with the SED group, no significant difference was found in cTnI content in the exercise groups with varying intensities following 1 week of exercise. However, after 4 weeks of exercise, the levels of cTnI in the LIR group, MIR group, and HIR group were significantly lower than those in SED group. After 8 weeks of exercise, the levels of cTnI in the LIR and MIR groups were significantly lower than those in the SED group, whereas there was no significant difference between the HIR and SED groups.

Protein analysis

We conducted MEK-ERK1/2 signaling to investigate the differences in exercise-induced hypertrophy. As shown in Fig. 3 **(B and C)**, after 1 week of exercise, the phosphorylation of ERK1/2 was significantly increased in the HIR group compared with the SED group (P < 0.01), as well as the other exercise groups (P < 0.05), whereas there was no difference between the LIR, MIR, and SED groups. After 4 weeks of exercise, there was a significant increase in the phosphorylation of ERK1/2 in the LIR, MIR, and HIR groups compared with the SED group (P < 0.01). However, exercise after 8 weeks did not bring about significant changes between the groups of varying exercise intensities and sedentary controls.

Discussion

In the present study, we have provided an in vivo structural and functional comparison of exercise-induced hypertrophy in a rat model, depicting that the changes that occur in cardiac response differ based on exercise intensity and on the potential signal transduction. Here, our findings indicate that running leads to cardiac hypertrophy in an intensity-dependent manner. In contrast to LIR and MIR, 8 weeks of HIR-induced cardiac hypertrophy was characterized by potential cardiomyocyte injury, which increased the risk of pathological development. Furthermore, the ERK signaling pathway was mainly involved in the compensatory hypertrophy process of the myocardium in the early stage of exercise and was positively correlated with exercise load. However, long-term exercise may attenuate ERK signaling activation.

In the process of long-term adaptation to regular exercise, myocardial hypertrophy leads to an increased blood pumping ability to meet the increased needs of the whole body [9]. Cardiac hypertrophy is characterized by myocyte hypertrophy, structural arrangement, and the accumulation of cardiac collagen[21]. Macroscopically, we found that the size of the hearts in the running group was more obvious after 4 and 8 weeks. Moreover, the ratio of heart mass to body mass (HM/BM), which is widely used to assess hypertrophy in the heart, has shown similar results. Although the ratio of heart mass to tibia length would seem to be more reliable, the data from other studies[22, 23] have indicated that there was no significant difference between HM/BM and HM/TL. Microscopically, our study showed that being subjected to 1 week of running induced cardiac hypertrophy in high-intensity running, while myocyte hypertrophy was the same as the macro situation after 4 and 8 weeks of running; these cardiac dimensions are consistent with previous experimental results[8], which have suggested that myocardial hypertrophy occurred in tandem with an increase of exercise intensity and extension of exercise time. To induce a hypertrophic heart, a longer period of exercise may be required.

We found that low-to-medium-intensity running led to proper cell morphological characteristics and decreased fibrosis, which likely contributed to overall myocardial tissue integrity and homeostasis for cardiac beneficial effects[24, 12]. High-intensity running, however, may have a special impact on the heart. From the week 1 to week 8, cardiomyocytes undergo several morphogenetic changes, including occasional rupture, irregular shape, and, eventually, disorganized arrangement. In line with this, collagen deposition in the myocardium also increased significantly. These pathological structural changes correspond with a previous rat model[25] and athlete's heart[26] after long-term high-intensity exercise.

Transthoracic echocardiogram is accurate and widely used in the field to assess cardiac structure and function. The present study found that low-to-medium intensity running induced a significant increase in absolute LV diastolic diameter values and in LV relative wall thickness after 1 week. These results indicate a shift to LV eccentric remodeling. However, in contrast, other studies have demonstrated that short-duration exercise appeared to have little in the way of a negative impact on ventricular function[27]. The present study showed that FS and EF significantly decreased in the LIR and MIR groups but not in the HIR group. This disparity might be a consequence of myocyte hypertrophy in the HIR group. Moreover, the

impact of prolonged running on EF and FS was not significantly altered between the exercise groups and the SED group after weeks 4 and 8. The observed unchanged cardiac function is in line with recent findings on rat and athlete hearts[28, 29]. Although transthoracic echocardiography is accurate and widely used to evaluate cardiac structure and function in this field, magnetic resonance imaging (MRI) technology does provide more accurate and reproducible measurement of cardiac function[30, 31].

cTnI is a highly sensitive and specific marker of myocardial injury and is suitable for early and late diagnoses[32]. In the present study, no obvious myocardial injury was observed after treadmill running with varying intensities. In contrast to previous observations of animal models[33] and a large number of human exercise studies[34], the release of cTnI was markedly decreased after 4 weeks of running, and the same state also occurred after 8 weeks for the LIR and MIR groups (Fig. 3A), indicating the leakage of cTnI decreased after exercise. The reason for this situation is that cardiac biomarker concentrations tend to return to baseline at 24–48 h postexercise. However, running for 8 weeks without significant changes in the HIR group may cause potential myocardial damage, which is in accordance with the present data. Our HE and PSR staining of myocardium showed signs of sporadic cardiomyocyte damage and increased fibrosis. However, this probably represents a standard exercise intensity-dependent response rather than a pathological response.

It is generally accepted that ERK1/2 activation is essential for cardiac hypertrophy[35]. However, several partially contradictory studies have indicated that ERK1/2 can lead to maladaptive cardiac hypertrophy[36, 37], but also physiological hypertrophy[18, 38], or that is has no effect on cardiac hypertrophy[39]. In addition, Izhak et al. demonstrated that the ERK1/2 signaling pathway uniquely regulates the balance between eccentric and concentric growth of the heart[19]. It has been theorized that the model, circumstances, and upstream signals may trigger the outcome of these mouse studies on ERK1/2-mediated cardiac hypertrophy. In our rat model, we found that only high-intensity running resulted in the activation of ERK1/2 after 1 week. The amount of phosphorylated ERK1/2 increased significantly by 134.3%, 137.1% and 237.1% when the rats ran at low, medium and high intensity running for 4 weeks. However, there were no significant changes in ERK1/2 activation between the running groups of varying intensities and the sedentary control group at week 8. As reported, exercise induces the activation of multiple MAPK pathways in the heart, an effect that gradually declines with the development of exercise-induced cardiac hypertrophy[40]. Therefore, the combined results from previous studies and our present evidence suggested that ERK1/2 represented a positive regulator in the progression of cardiac hypertrophy and was correlated with running intensity, which was, however, alleviated by running duration. Our research may provide a feasible signal observation on the intensity point and time point in terms of exercise-induced cardiac hypertrophy.

In summary, we confirmed that the effect of running on cardiac hypertrophy is intensity dependent. Compared with LIR and MIR, myocardial hypertrophy induced by HIR at 8 weeks was characterized by sporadic heart injuries, cumulative fibrosis, and increased myocardial enzymes, increasing the risk of pathological development. In addition, the ERK signaling pathway was mainly involved in the

compensatory hypertrophy process of early exercise and was positively correlated with exercise load. However, long-term exercise may weaken the activation of the ERK signal.

Study Limitations

There are several potential limitations of the current study that need to be acknowledged. First, although we applied the previous exercise regimens to differentiate running loading, the percentage of VO_2max might better evaluate the real cardiac capability of different intensities, not only running distance, slope, and speed alone. The possible influence of running intensity should be assessed in future studies. Moreover, our data on the decreased cardiac parameters of EF and FS suggest initiation of ventricular function degradation; however, the impact of the observed functional changes on the short duration remains unanswered. Further detection methods, such as MRI, are needed to clarify the possibility of myocardial dysfunction described in the present study.

Declarations

Authors' contributions ZPY and GXN conceived research concept; ZPY and NZ designed andperformed the experiments, analyzed the data, and prepared the figures;NZ, JTL, and TL contributed to the sample collection and sample storage; and GXN and ZPY drafted, revised, and edited the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials The necessary data were provided to support the assumption of this study (data will be made available on demand).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interests.

Ethical Statement This study was approved by Animal Ethics Committee of Fujian Medical University (2017-061).

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Figures

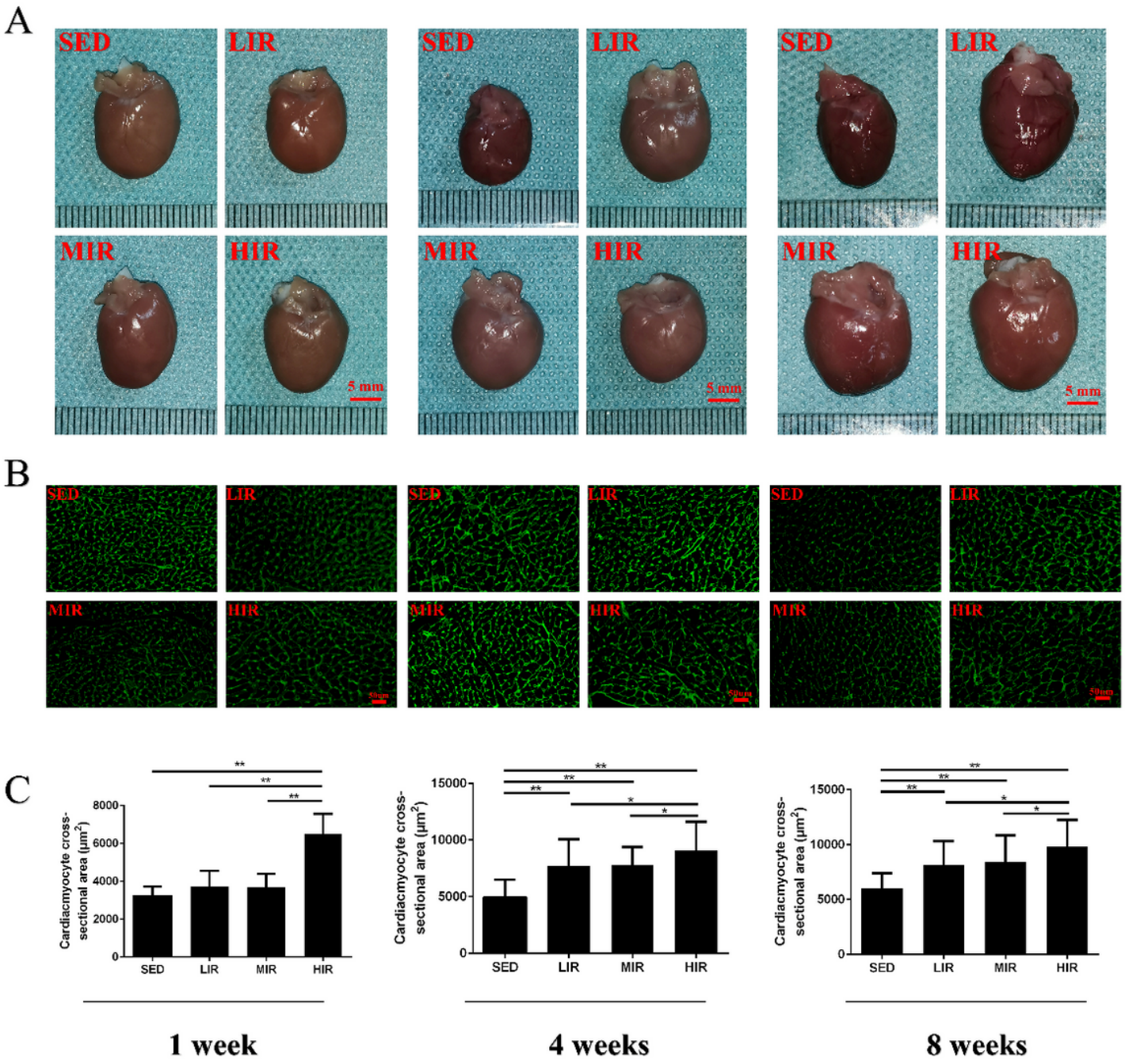

Figure 1

SD rats were subjected to different treadmill running intensities for 1, 4, and 8 weeks. (A-C) Hypertrophic changes of the hearts were observed by gross morphologic examination (A) and WGA staining (B and C). Scale bar: 5 mm (A), 50 μm (B). Data are presented as the means ± SEM. *P<0.05, **P<0.01.

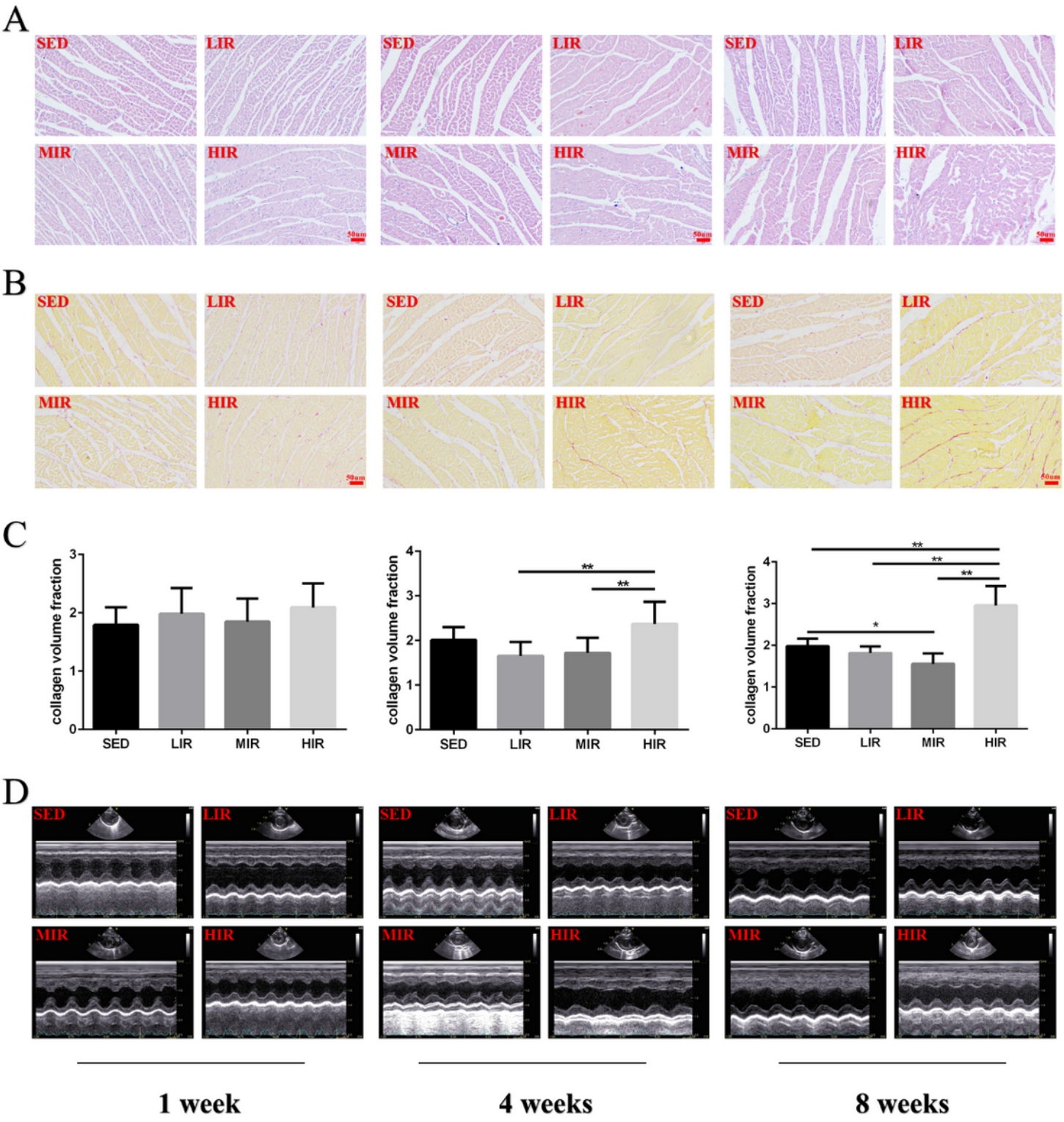

Figure 2

SD rats were subjected to different treadmill running intensities for 1, 4, and 8 weeks. (A-C) Histological changes of the hearts were observed by HE staining (A), PSR staining (B and C). Scale bar: 50 μm (A and B), (D) Typical M-mode images of echocardiogram in the left ventricle. Data are presented as the means ± SEM. *P<0.05, **P<0.01.

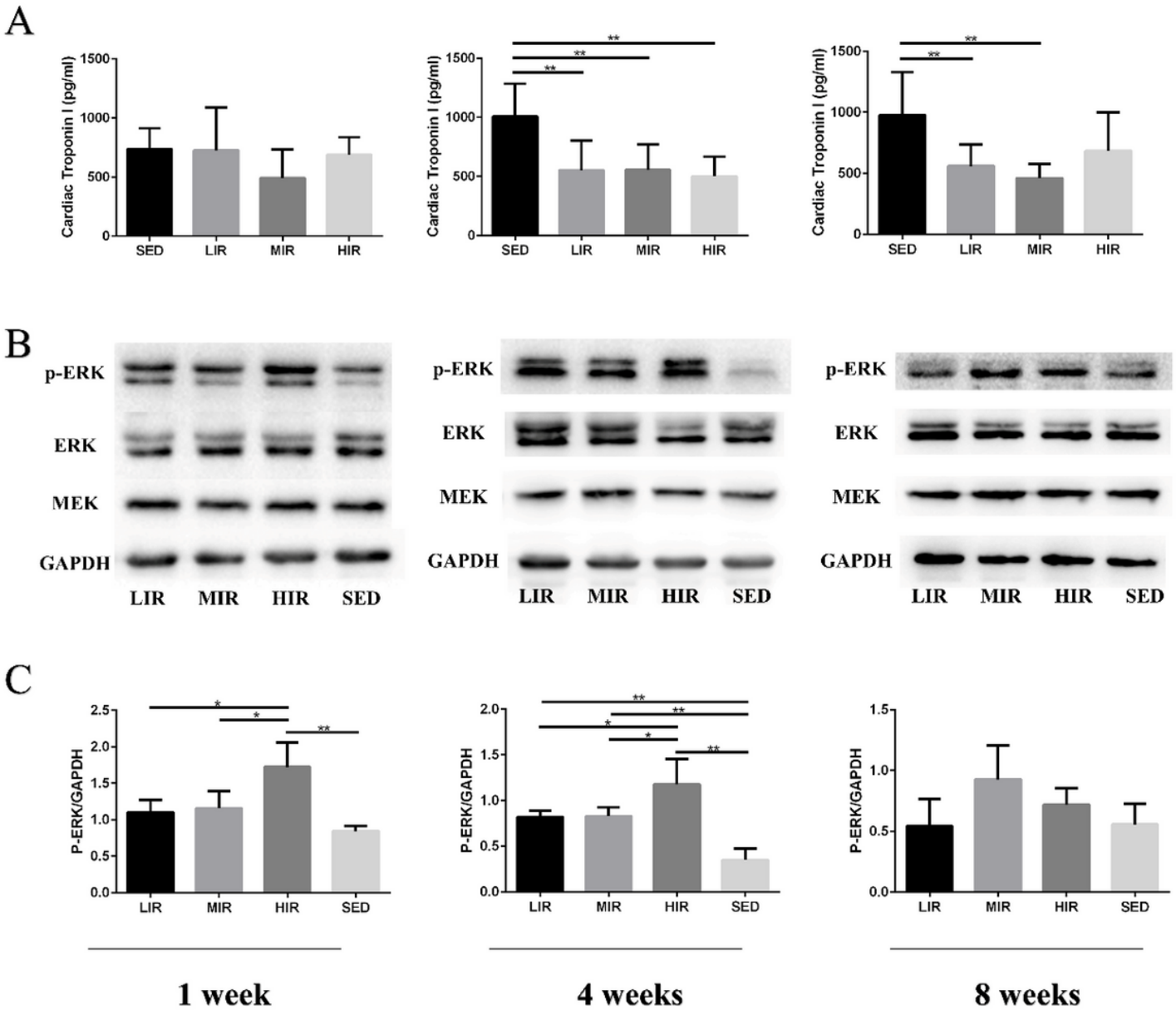

Figure 3

Effects of different treadmill running intensities after 1, 4, and 8 weeks on serum cTnI levels (A) and ERK phosphorylation (B and C) in rats. Data are presented as the means ± SEM. *P<0.05, **P<0.01.