



Prehypertension exercise training attenuates hypertension and cardiac hypertrophy accompanied by temporal changes in the levels of angiotensin II and angiotensin (1-7)

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Received: 5 July 2018 / Revised: 7 April 2019 / Accepted: 2 June 2019 / Published online: 4 July 2019
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Abstract

Appropriate exercise training (ExT) has been shown to decrease high blood pressure. Accumulating data have indicated the beneficial effects of ExT on prehypertension. This study tested whether prehypertension ExT protects against hypertension and cardiac remodeling in spontaneously hypertensive rats (SHR) and explored the underlying mechanisms by examining the cardiac angiotensin-converting enzyme (ACE) and ACE2 signaling axes. Low-intensity ExT was started in male SHR and control Wistar-Kyoto rats prior to the onset of hypertension and maintained for 8 or 16 weeks. Blood pressure (BP) was measured biweekly by the tail-cuff method. Cardiac function and remodeling were assessed, and changes in the ACE and ACE2 axes were examined after the final ExT session. The results showed that prehypertension ExT slowed the onset and progression of hypertension in SHR. In parallel, hypertrophy in the hearts of hypertensive rats was attenuated, myocardial fibrosis was reduced, and impairment of left ventricular diastolic function was reduced. In the SHR myocardium, the levels of components involved in the ACE–Ang II–AT1 axis were homogeneously and progressively increased, whereas those involved in the ACE2–Ang(1-7)–MAS axis were heterogeneously decreased. Different temporal responses were observed for the key effectors Ang II and Ang(1-7). Myocardial Ang II levels were progressively increased in SHR and were consistently reduced by ExT. By contrast, Ang(1-7) decreased only after 16 weeks of sedentariness, and this decrease was abolished by ExT. In addition, 16 weeks of ExT increased the levels of Ang(1-7) in normotensive control rats. In summary, prehypertension ExT attenuates hypertension and cardiac remodeling. Downregulation of Ang II seems to serve as a protective mechanism during ExT, while upregulation of Ang(1-7) is induced after a relatively long period of ExT.

Keywords Exercise training · Prehypertension · Cardiac remodeling · Renin–angiotensin system · Hypertension

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Supplementary information The online version of this article (<https://doi.org/10.1038/s41440-019-0297-4>) contains supplementary material, which is available to authorized users.

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Introduction

Hypertension is a well-known cause of the steadily increasing incidence of heart failure. Hypertension not only increases the afterload in the heart but also activates multiple neurohumoral factors that contribute to cardiovascular dysfunction. Untreated or inadequately treated hypertension often leads to serious complications, typically including cardiac hypertrophy, which usually precedes the onset of heart failure.

A wealth of studies show that the renin–angiotensin system (RAS) plays a critical role in the pathogenesis of hypertensive cardiac hypertrophy [1]. In the RAS, angiotensin II (Ang II) serves as the key effector peptide, which is formed by the action of angiotensin converting enzyme (ACE). Emerging clinical evidence shows that

ACE inhibitors and Ang II receptor type 1 (AT1) antagonists can improve left ventricular (LV) hypertrophy and heart function, demonstrating that excessive activation of the ACE–Ang II–AT1 axis contributes to cardiac hypertrophy [2, 3]. Ang(1-7) is a newly recognized player in the RAS. Ang(1-7) is mainly derived from the degradation of Ang II by the ACE homolog ACE2 and acts through Mas receptors [4]. Accumulating data shows that the ACE2–Ang(1-7)–Mas axis is able to counteract most of the deleterious effects of the ACE–Ang II–AT1 axis, including the vasoconstrictive and proliferative effects of Ang II [5, 6]. Decreases in ACE2 were observed in animal models of hypertension, while ACE2 gene transfer and Ang(1-7) treatment have been shown to attenuate hypertension and associated LV hypertrophy and fibrosis [7–9]. There is a growing consensus that the balance between the ACE–Ang II–AT1 and ACE2–Ang(1-7)–Mas axes is crucial for maintaining cardiovascular stability. Hypertensive cardiac hypertrophy and remodeling may result from either an increase of ACE–Ang II–AT1 axis activity or loss of ACE2–Ang(1-7)–Mas axis activity.

Exercise training (ExT) is believed to be a cornerstone therapy for the prevention and treatment of cardiovascular diseases, including hypertension and hypertensive hypertrophy [10]. Appropriate ExT can reduce hypertension and the risk of cardiovascular events [10, 11]. Additionally, pathological cardiac hypertrophy induced by hypertension may be reduced [12] or converted into physiological hypertrophy [13]. Inhibition of excessive cardiac ACE–Ang II activity and enhancement of cardiac ACE2–Ang(1-7)–Mas expression have been shown to be involved in the antihypertrophic effects of ExT [14, 15]. While there is general consensus that exercise lowers resting blood pressure (BP), existing recommendations differ in terms of the frequency, intensity, time, and type of exercise, as well as the reported reductions in the magnitude of BP that result from them [16]. Moreover, concerns have been raised that the current recommendations for exercise intervention increase the risk of sub-optimal management of hypertension in young adults and seem unlikely to reduce long-term cardiovascular risks [17]. There is a need for continued research to improve age-specific exercise strategies. Prehypertension exercise has shown promising effects on hypertension [11, 18]. The present study employed young spontaneously hypertensive rats (SHR) that were started on ExT prior to the onset of hypertension, and changes in blood pressure and cardiac pathology were tracked. Furthermore, to investigate whether ExT has time-dependent effects on the ACE–Ang II–AT1 and ACE2–Ang(1-7)–Mas axes, the present study examined both axes after relatively short- or long-term ExT.

Methods

Animal model and ExT program

Five-week-old male SHR and Wistar-Kyoto control rats (WKY) were purchased from Vital River Laboratory Animal Technology Ltd (Beijing, China) and maintained on a 12-h dark/light cycle in a specific pathogen-free (SPF) facility with controlled temperature and humidity. Water and standard chow were provided ad libitum. The experimental protocols were approved by the Animal Care and Use Committee of Fujian Medical University, China and were conducted in accordance with the Guidelines for Care and Use of Laboratory Animals issued by Fujian Medical University, China.

All WKY and SHR rats were randomly assigned to sedentariness or ExT for either 8 or 16 weeks. For ExT, rats were allowed to run on a treadmill (Techman FT-200, Chengdu, China) at a speed of 18–20 m/min and 0% grade for 60 min per day for 5 days per week. The training intensity was low (approximately 55% of maximal oxygen uptake) and was conducted as previously described [19, 20]. Sedentary rats were placed on the treadmill without movement during the training sessions.

Measurement of blood pressure

Systolic blood pressure (SBP) was measured during the conscious state using a computerized tail-cuff system (Techman BP-300A, Chengdu, China) prior to the first ExT session and then every 2 weeks as described previously [14, 20]. To minimize the stress that might be caused by treadmill exercise, all measurements were taken 24–48 h after termination of the ExT session.

Echocardiography and hemodynamic measurement

Twenty-four hours after the last ExT session, rats were anesthetized by 10% chloral hydrate (3 mL/kg, i.p.) and their heart function was assessed by echocardiography (Vivid E9, General Electric Company, CT, USA) [21]. Left ventricular end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), interventricular septal thickness (IVST), LV wall thickness (LVWT), and fractional shortening (FS) were determined according to M-mode tracings.

Twenty-four hours after echocardiography, the cardiac hemodynamic parameters were measured. Under anesthesia, the trachea of the rat was cannulated to facilitate breathing. A pressure transducer (Millar Inc., Houston, Texas, USA) was inserted into the right carotid artery and advanced into the LV. The LV end-diastolic pressure (LVEDP) and maximum rise/fall velocity of the LV

pressure ($\pm dP/dt_{\max}$) were measured by a biological signal acquisition and processing system (Techman BL-420N, Chengdu, China).

Histological and morphological analysis of the LV

After completion of the hemodynamic measurements, rats were sacrificed under deep anesthesia, and aortic retrograde perfusion was performed with cold (4 °C) phosphate-buffered saline (PBS), followed by 4% polyformaldehyde for 30 min. Then, the heart was excised, postfixed with 10% formalin for 6 h, dehydrated with ethanol, embedded in paraffin blocks, and cross-sectioned into 5- μ m-thick slices.

To visualize collagen, the paraffin slices were stained with Sirius red solution. Under a high-magnification microscope (Olympus, Japan), the LV collagen content was measured in five visual fields of each section and four inconsecutive sections from each heart using Image Pro Plus 6.0 software (Media Cybernetics, Inc., Washington, USA). The interstitial collagen volume fraction (CVF) was calculated as the ratio of the stained collagen area to the total myocardial area, and the perivascular collagen area (PVCA) was calculated as the ratio of the perivascular collagen area to the luminal area of the vessel [22].

To visualize the cellular structure, the paraffin slices were stained with hematoxylin and eosin (HE). The diameters of the cardiac myocytes were measured in HE-stained slices using the method described by Silva et al. [15]. The diameters of myocytes with visible nuclei and intact cellular membranes were measured. The widths of the individual cardiomyocytes were manually measured across the middle of the nucleus with Image Pro Plus 6.0 software. Approximately 80 myocytes were measured for each rat, and the average diameter was recorded. The observer was blinded to the experimental groups.

Quantitative real-time PCR analysis

Total RNA was isolated from LV free wall tissue using TRIzol reagent (Takara, Japan) according to the manufacturer's instructions. Quantitative real-time PCR analysis was performed to measure the mRNA levels of ACE, ACE2, AT1, Mas, collagen I, collagen III, and β -actin using a One-Step SYBR Prime Script RT-PCR Kit II (Takara, Japan). The primer sets are listed in Supplementary Table 1. The relative levels of the target mRNAs were calculated by the $2^{-\Delta\Delta Ct}$ method, and β -actin was used as the loading control [23].

Western blot analysis

Protein lysates from LV tissue were prepared and separated by 8% sodium dodecyl sulfate polyacrylamide gel

electrophoresis and then transferred onto nitrocellulose membranes (GE Healthcare Amersham Biosciences, Oslo, Norway) as previously described [24]. The blotted membranes were incubated with primary antibodies against ACE2 (Santa Cruz, USA), ACE (Abcam, UK), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Santa Cruz, USA), followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:3000) (Jackson Immuno Res, USA). The membranes were then developed with chemiluminescence reagents (GENVIEW, USA). The western blotting signals were analyzed using a BioDocIt Imaging System (UVP, USA).

Measurement of Ang II and Ang1-7 in heart tissue

The apex of LV was quickly harvested, rinsed, homogenized in cold (4 °C) PBS containing protease inhibitors (25 mmol/L potassium EDTA, 0.44 mmol/L *o*-phenanthroline, 0.12 mmol/L pepstatin A, and 1 mmol/L 4-chloromercuribenzoic acid), and centrifuged at 12,000g for 10 min at 4 °C. The supernatant was divided into several aliquots and kept at -80 °C. The Ang II and Ang1-7 levels were determined using commercial ELISA kits [25] according to the manufacturer's instructions (Chuanxian, Shanghai, China). The protein concentration was determined using a bicinchoninic acid protein assay method as described by Huang et al. [26]. The amounts of cardiac Ang II and Ang1-7 are expressed as pg/mg protein.

Statistics

All data are expressed as the means \pm standard deviation (SD). The statistical significance of the differences among groups was evaluated by one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls test for post hoc analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Prehypertension ExT lowered blood pressure

The time course of SBP is shown in Fig. 1a, b. At 5 weeks of age, SBP was comparable between the WKY and SHR groups. ExT was started at 6 weeks of age and lasted for 8 or 16 weeks. SBP in SHR progressively increased over the observation period. However, significant reductions in SBP appeared in SHR-E after 4 weeks of exercise and persisted until the end of the experiment. Mild reductions were also observed in WKY-E, which became significant after 10 weeks of exercise. Specifically, compared with the sedentary groups, SBP decreased by 12 and 7 mmHg at

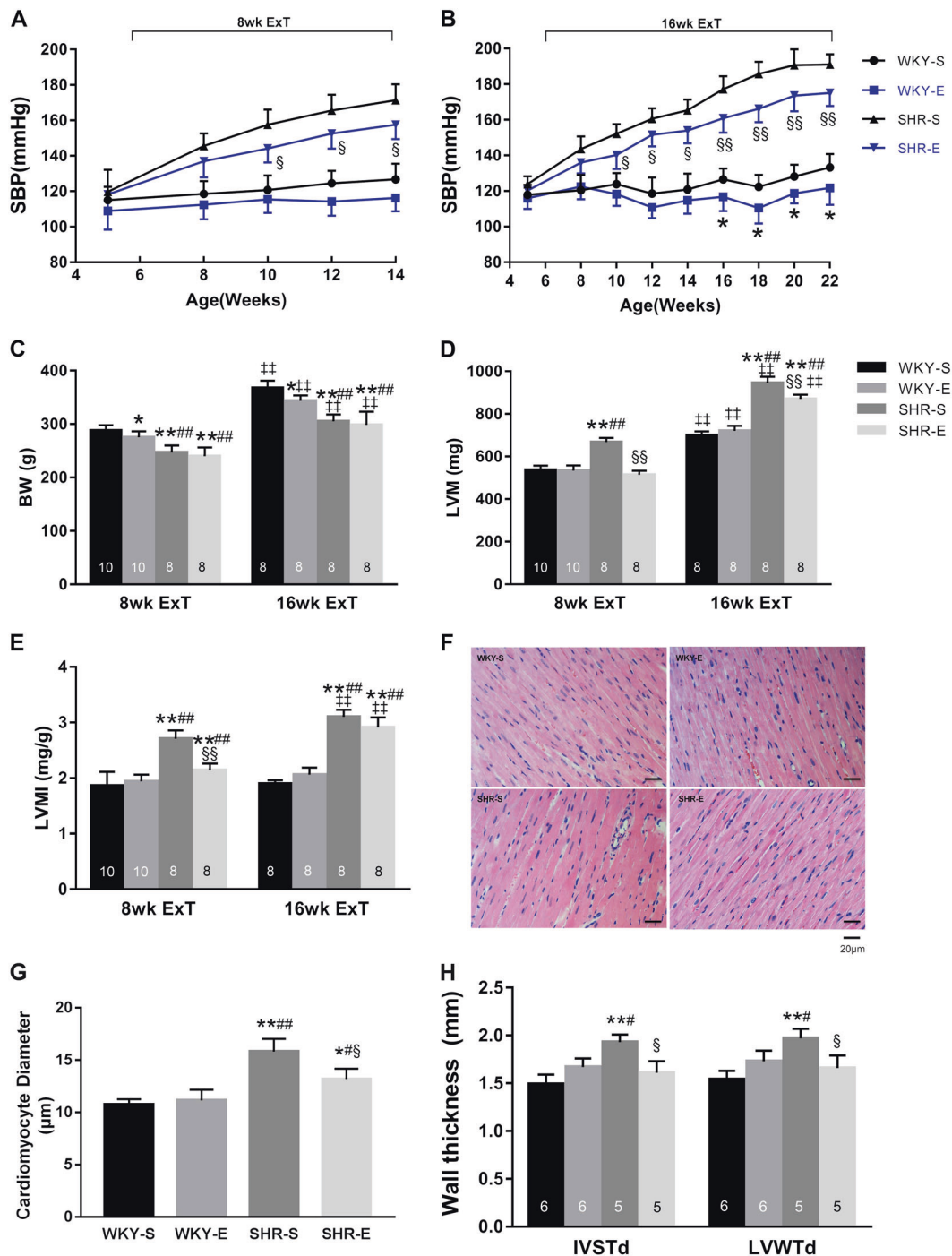


Fig. 1 Effects of prehypertension exercise training (ExT) on systolic blood pressure (SBP) and cardiac hypertrophy in spontaneously hypertensive rats (SHR), with Wistar-Kyoto (WKY) rats serving as the control. ExT was started at the age of 6 weeks. SBP was monitored biweekly over 8 (a) or 16 (b) weeks of sedentariness (-S) or exercise training (-E). Cardiac hypertrophy-related indexes, measured after 16 weeks of “-S” or “-E”, are shown: **c** body weight (BW), **d** left ventricular mass (LVM), **e** LVM index (LVMI), **f** representative

images of hematoxylin–eosin staining (scale bars = 20 μm), **g** cardiomyocyte diameter, **h** interventricular septal thickness diameter (IVSTd), and left ventricular wall thickness diameter (LVWTd). $n = 8–10$ /group unless otherwise indicated. Data are the means \pm SD. * $P < 0.05$, ** $P < 0.01$ vs WKY-S; # $P < 0.05$, ### $P < 0.01$ vs WKY-E; § $P < 0.05$, §§ $P < 0.01$ vs SHR-S; † $P < 0.05$, †† $P < 0.01$ vs 8 weeks of the same treatment

8 weeks in exercised SHR and WKY, respectively. SBP further decreased at 16 weeks by 17 and 10 mmHg in exercised SHR and WKY, respectively. These results

showed that prehypertension ExT markedly slowed the development and progression of hypertension in SHR and mildly reduced SBP in WKY.

ExT alleviated cardiac hypertrophy and remodeling in SHR

As shown in Fig. 1c, both WKY and SHR naturally gained weight as the observation period increased from 8 to 16 weeks. Whether exercised or not, SHR had lower body weights than WKY. ExT slightly but significantly reduced body weight in WKY but barely changed it in SHR.

Left ventricular mass (LVM) in sedentary SHR was greatly increased compared to that in WKY (Fig. 1d). However, LVM was remarkably decreased by ExT after either 8 or 16 weeks. Meanwhile, LVM of WKY remained unchanged after exercise. As for the LVM index (LVMI) of exercised SHR (Fig. 1e), it tended toward an overall decrease due to the decreased LVM. A significantly decreased LVMI was observed for SHR that received 8 weeks of exercise (2.14 ± 0.12 vs 2.71 ± 0.15 mg/g, $P < 0.01$). However, since the body weights of SHR exercised for 16 weeks were relatively low, their LVMI showed no difference from that of sedentary SHR (2.91 ± 0.18 vs 3.1 ± 0.13 mg/g, $P > 0.05$). However, the cardiomyocyte diameter in exercised SHR was significantly smaller than that in sedentary SHR (Fig. 1f, g), suggesting that ExT reduced cardiac hypertrophy.

In line with the LVM and cardiomyocyte diameter data, an echocardiographic examination showed that inter-ventricular septal thickness at end-diastole (IVSTd) and LV wall thickness at end-diastole (LVWTd) were significantly larger in SHR-S than those in WKY-S (Fig. 1h). ExT reduced IVSTd and LVWTd in SHR-E to levels comparable to those found in WKY-E. Regarding WKY, although ExT showed a tendency to increase IVSTd and LVWTd, no statistical significance was detected. Overall, the results suggested that ExT attenuated LV hypertrophy in SHR without inducing significant hypertrophy in WKY.

Figure 2 shows the CVF and PVCA in the left ventricle, as calculated from images in which collagen fibers were stained red with Sirius red. The CVF of SHR was approximately threefold that of WKY after 16 weeks of sedentariness. However, the CVF of SHR dramatically dropped to a level close to that of WKY after ExT (Fig. 2a, b). Similarly, the PVCA of SHR was much higher than that of WKY but was reduced by ExT (Fig. 2c, d). In addition, no difference in CVF or PVCA was observed between sedentary and exercised WKY, suggesting that ExT reduces pathological fibrosis without affecting physiological collagen metabolism.

Furthermore, the expression of collagen type I and type III mRNA was quantitatively determined (Fig. 2e, f), as the myocardial collagen matrix primarily consists of type I and type III collagens. The results showed that collagen type I and type III mRNA were highly overexpressed in SHRs and

were both suppressed by ExT. Nevertheless, in accordance with the data derived from Sirius red staining, ExT showed no effects on collagen synthesis in WKY.

ExT improved LV diastolic function

The indexes of LV function are shown in Fig. 3. Compared to WKY-S, SHR-S had significantly higher LVEDP, larger LVEDD, and $-dP/dt_{\max}$. Changes were predominantly observed for LVEDP, which increased from approximately 2.8 to 6.4 mmHg after 8 weeks and further increased to approximately 12.6 mmHg after 16 weeks. Concomitant to the increase in LVEDP and LVEDD, $-dP/dt_{\max}$ decreased in SHR-S, strongly indicating dysfunction of the LV diastole, which evidently resulted from myocardial hypertrophy and fibrosis.

While SHR-S suffered from diastolic dysfunction, it was reduced in SHR-E. ExT abolished the increase in LVEDP after 8 weeks and reduced LVEDP after 16 weeks. In parallel, ExT prevented the decrease in $-dP/dt_{\max}$ in SHR after 16 weeks. Interestingly, ExT for 16 weeks but not for 8 weeks increased $-dP/dt_{\max}$ in WKY, suggesting that relatively long-term exercise may improve diastolic function in normotensive rats. $-dP/dt_{\max}$ in WKY-S after 16 weeks was higher than that in WKY-S after 8 weeks, likely due to the physiological growth and maturation of the rats. Overall, the above results showed that diastolic dysfunction in SHR was alleviated by ExT.

In contrast to impaired diastolic function in SHR-S, no significant changes were observed for systolic parameters, including LVESD and $+dP/dt_{\max}$, and no reduction was observed in FS. These results indicate that LV systolic function in SHR-S remained relatively normal during our observation period and that compensation mechanisms were likely involved, as discussed later.

ExT resulted in temporal regulation of the ACE and ACE2 axes

The present study examined the ACE–Ang II–AT1 and ACE2–Ang(1-7)–Mas axes at 8 and 16 weeks after ExT.

As shown in Fig. 4, the ACE and AT1 mRNA and ACE protein levels in WKY rats remained constant. Neither 8 nor 16 weeks of exercise had effects on ACE and AT1 expression in WKY. For SHR, however, the ACE and AT1 mRNA and ACE protein levels were elevated at 8 weeks and were further increased at 16 weeks in the presence or absence of exercise. Over the same period of time, SHR-E showed lower levels of ACE and AT1 than SHR-S. It is shown here that ACE and AT1 expression in SHR increased over time and was attenuated but not prevented by ExT.

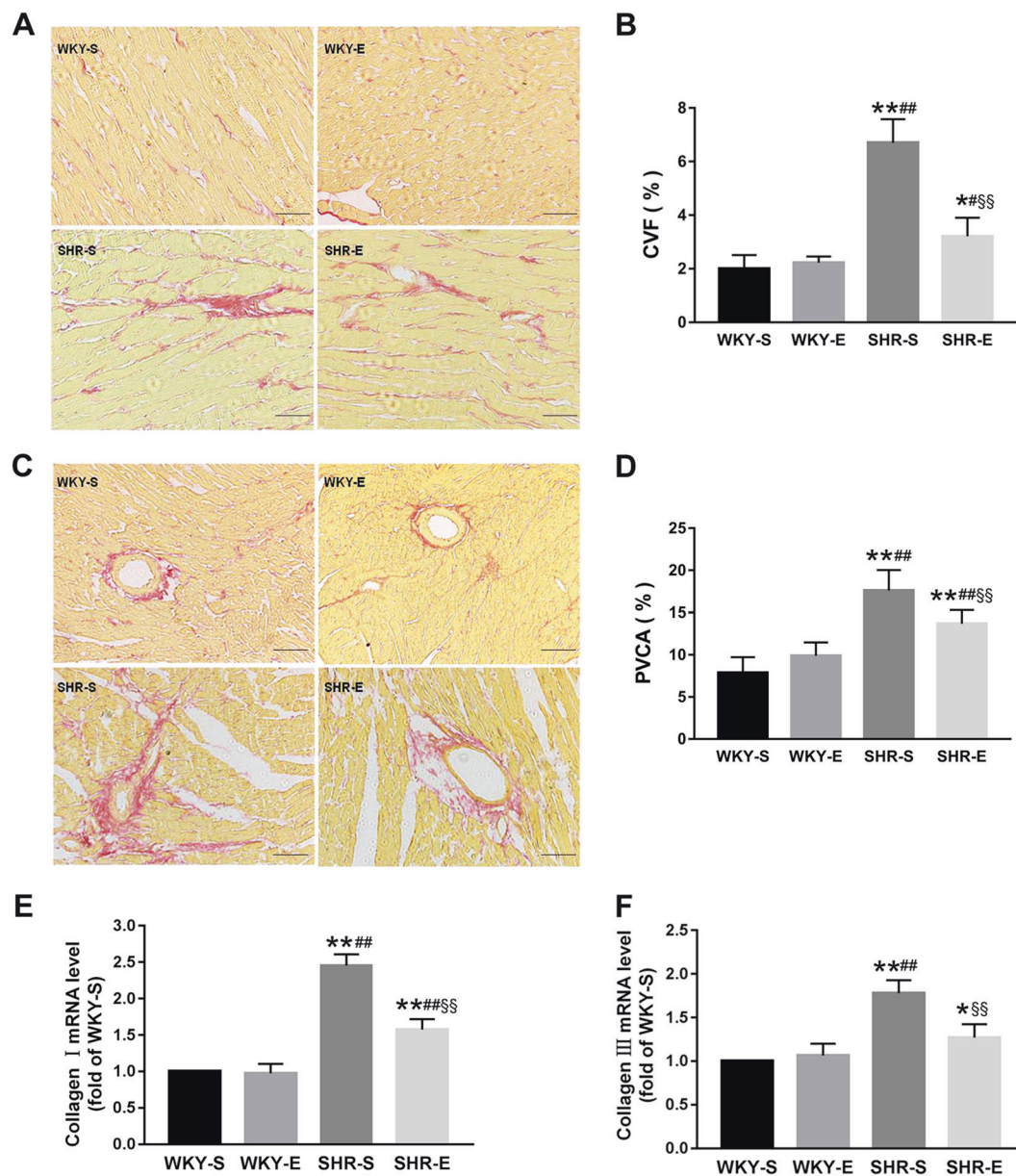


Fig. 2 Effects of prehypertension exercise training on cardiac fibrosis and collagen mRNA expression. Measurements were taken from the left ventricles of WKY and SHR after 16 weeks of sedentariness (-S) or exercise training (-E). **a, b**) Representative images with Sirius red staining and statistical data regarding the interstitial collagen volume fraction (CVF), scale bars = 50 μ m. **(c, d)** Representative images with

Sirius red staining and statistical data regarding the perivascular collagen area (PVCA), scale bars = 20 μ m. **(e, f)** mRNA levels of collagen type I and III, as determined by real-time RT-PCR. $n = 4-6$ /group. Data are the means \pm SD. * $P < 0.05$, ** $P < 0.01$ vs WKY-S; # $P < 0.05$, ## $P < 0.01$ vs WKY-E; § $P < 0.05$, §§ $P < 0.01$ vs SHR-S

While ACE and AT1 expression concomitantly increased, ACE2 and Mas expression showed heterogeneous changes in response to hypertension and exercise (Fig. 5). In the myocardium of WKY-E, the ACE2 and Mas mRNA levels were increased at 16 weeks, but the ACE2 protein level remained unchanged. In SHR-S, the ACE2 mRNA level decreased after 8 weeks, but no change was detected in the ACE2 protein level until 16 weeks. By contrast, Mas mRNA expression in SHR-S did not decrease

until 16 weeks. Notably, 16 weeks of exercise increased the ACE2 and Mas mRNA levels in SHR much more than those in WKY-S and restored ACE2 protein to a level comparable to that in WKY-S.

As far as the key effector peptides were concerned, Ang II and Ang(1-7) showed different time-related responses to hypertension and ExT (Fig. 6). In sedentary SHR, the myocardial Ang II content was significantly increased at 8 weeks and was further increased at 16 weeks, whereas

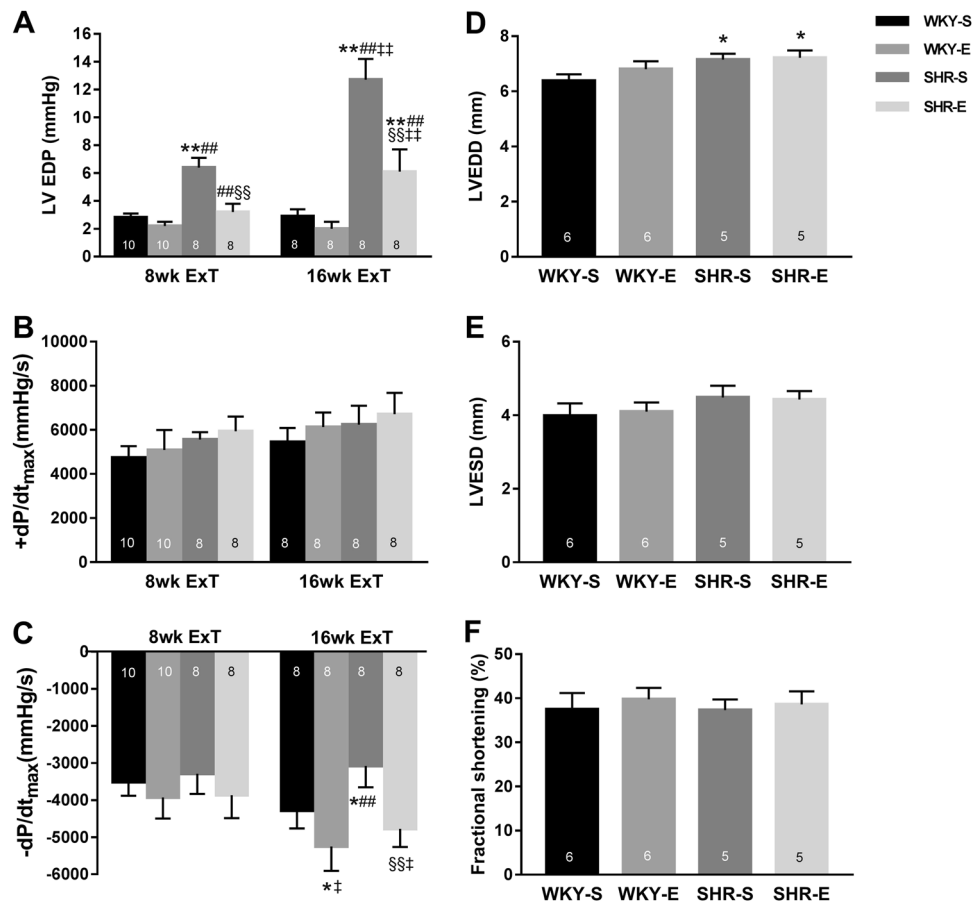


Fig. 3 The indexes of left ventricular function in WKY and SHR after 8 or 16 weeks of sedentariness (-S) or exercise training (-E). **a** LV end-diastolic pressure (LVEDP), **b** maximal rate of LV pressure rise (+dP/dt_{max}) and **c** maximal rate of LV pressure decrease (-dP/dt_{max}) were recorded by cardiac catheterization after 8 or 16 weeks of sedentariness (-S) or exercise (-E). **d** LV end-diastolic diameter (LVEDD),

e LV end-systolic diameter (LVESD), and **f** fractional shortening (FS) were determined by echocardiography after 16 weeks of sedentariness (-S) or exercise training (-E). *n* = 5–10/group. Data are the means ± SD. **P* < 0.05, ***P* < 0.01 vs WKY-S; ###*P* < 0.01 vs WKY-E; §§*P* < 0.01 vs SHR-S; ‡*P* < 0.05, ‡‡*P* < 0.01 vs 8 weeks of the same treatment

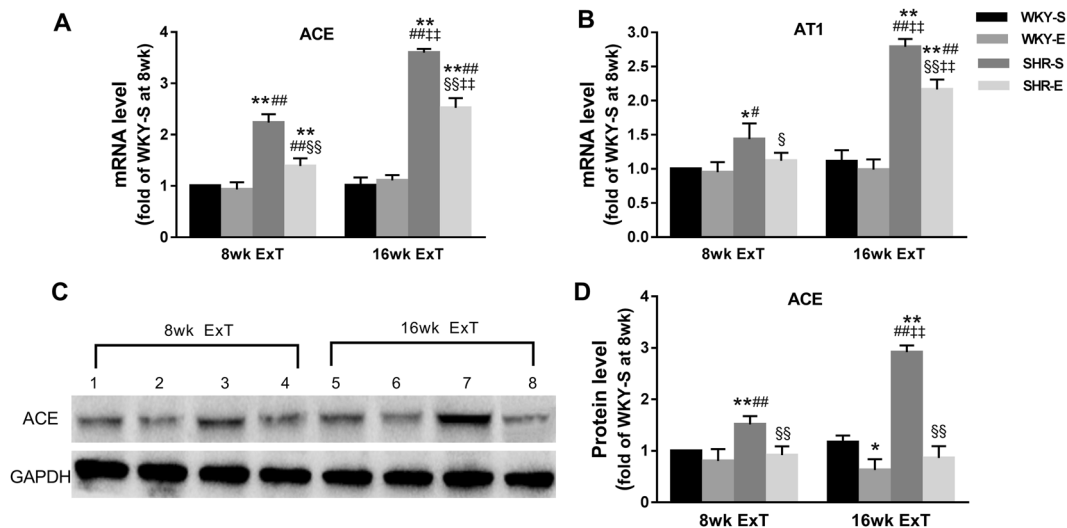


Fig. 4 Expression levels of mRNAs encoding angiotensin converting enzyme (ACE) (**a**) and angiotensin II type 1 receptor (AT1) (**b**) and the protein levels of ACE (**c**, **d**) in the left ventricle in WKY and SHR after 8 or 16 weeks of sedentariness (-S) or exercise training (-E). *n* = 6/

group. Data are the means ± SD. **P* < 0.05, ***P* < 0.01 vs WKY-S; #*P* < 0.05, ##*P* < 0.01 vs WKY-E; §*P* < 0.05, §§*P* < 0.01 vs SHR-S; ‡*P* < 0.05, ‡‡*P* < 0.01 vs 8 weeks of the same treatment

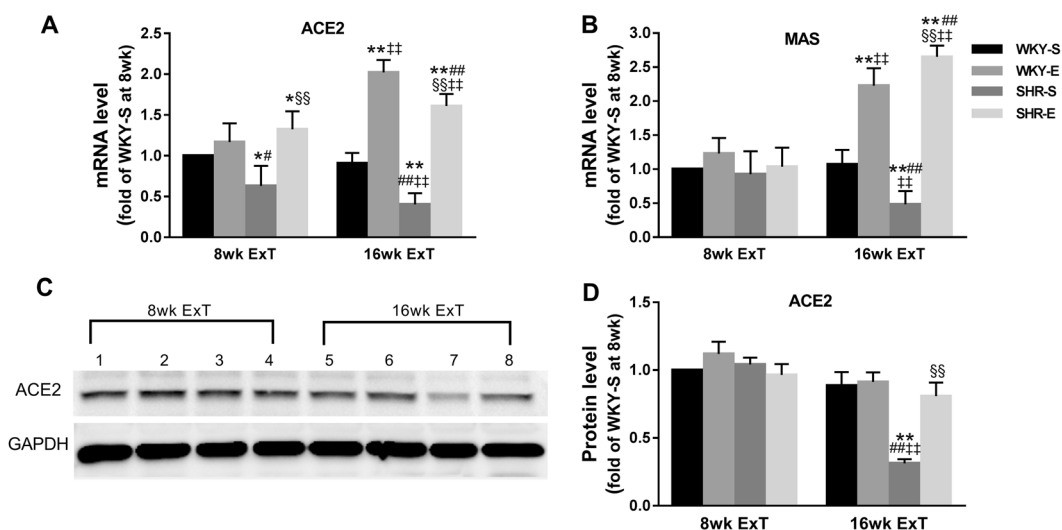


Fig. 5 Expression levels of mRNAs encoding angiotensin converting enzyme 2 (ACE2) (a) and MAS receptor (b) and the protein levels of ACE2 (c, d) in the left ventricle in WKY and SHR after 8 or 16 weeks of sedentariness (-S) or exercise training (-E). $n = 6/\text{group}$. Data are

the means \pm SD. * $P < 0.05$, ** $P < 0.01$ vs WKY-S; # $P < 0.05$, ## $P < 0.01$ vs WKY-E; § $P < 0.05$, §§ $P < 0.01$ vs SHR-S; † $P < 0.05$, ‡ $P < 0.01$ vs 8 weeks of the same treatment

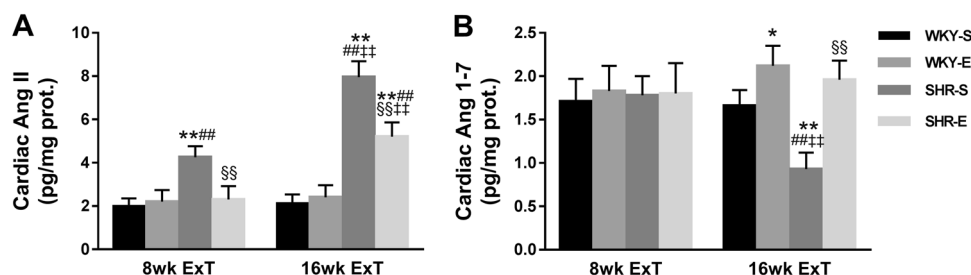


Fig. 6 Amounts of angiotensin II (Ang II) (a) and Ang(1-7) (b) in the left ventricle of WKY and SHR after 8 or 16 weeks of sedentariness (-S) or exercise training (-E). The amounts were determined by ELISA

and normalized to the amount of total protein. $n = 8/\text{group}$. Data are the means \pm SD. * $P < 0.05$, ** $P < 0.01$ vs WKY-S; ### $P < 0.01$ vs WKY-E; §§ $P < 0.01$ vs SHR-S; ‡ $P < 0.01$ vs 8 weeks of the same treatment

Ang(1-7) was not decreased until after 16 weeks. ExT simultaneously attenuated the elevation of Ang II and abolished the reduction of Ang(1-7) in SHR. In WKY, myocardial Ang(1-7) was mildly but significantly increased by ExT at 16 weeks, whereas Ang II remained unaffected throughout the training period.

Discussion

Hypertension is an independent risk factor for cardiac hypertrophy. Proper exercise has been documented to be conducive to controlling BP and alleviating cardiac hypertrophy [10–12]. The present study studied the effects of ExT in SHR started at 6 weeks of age, which is equivalent to the prehypertension stage in humans, and observed that either 8 or 16 weeks of low-intensity exercise significantly slowed the progression of hypertension. Compared to sedentary SHR, exercised SHR exhibited a decrease in SBP

of approximately 12 and 17 mmHg after 8 and 16 weeks, respectively. At 8 weeks, exercised SHR showed almost no sign of myocardial hypertrophy, as their LV mass and mass index were comparable to those of WKY. At 16 weeks, sedentary SHR developed hypertrophy, whereas hypertrophy was remarkably attenuated, although not abolished, in exercised SHR. These results show that hypertension has a predominant contributive effect on hypertrophy, whereas prehypertension ExT slows the progression of hypertension and cardiac hypertrophy.

As far as treadmill exercise is concerned, it could be stressful for rats depending on the exercise intensity. Soya et al. [27] characterized the effects of treadmill running on the activation of the hypothalamo-pituitary-adrenal axis with regard to running intensity. They reported that a speed of no more than 20 m/min did not induce increases in the running stress marker lactate or stress-associated physiological responses. In addition, relatively long training durations (>4 weeks) shifted the lactate threshold (the work rate

at which the steady state of blood lactate accumulation leads to its breakdown), which allows for running at higher speed without apparent stress. The present study set the running speed at 18–20 m/min, which is below the lactate threshold described by Soya et al. [27]. Meanwhile, 8 and 12 weeks were used as the training durations, which were presumably long enough for rats to undergo running habituation and avoid stress. A similar protocol of ExT has been used in multiple studies [15, 19, 20]. Moreover, all the measurements in our study were obtained 24–48 h after the termination of exercise. This time interval is much longer than the 12–16 h recovery period described by Thompson et al. [28]. Overall, the present exercise protocol was unlikely to induce stress, which had a minor influence on the outcome of the study.

It has been documented that SHR experience a cardiac compensatory period at the age of 12–24 weeks. During this period, the heart is prone to compensatory hypertrophy, conserved systolic function [13, 29], and impaired diastolic function [30]. Hypertrophy of cardiomyocytes initially acts as a compensatory mechanism to preserve systolic function, though it eventually becomes a major risk factor for congestive heart failure and overall mortality [29]. Diastolic dysfunction is an early event in SHR that may result from impaired calcium processing, T-tubule disorganization, and structural disturbances, such as cardiac fibrosis [30, 31]. Our observation period fell within the compensatory period of SHR. In accordance with the literature, we observed substantial increases in LVEDP and LVEDD and decreases in $-dP/dt_{\max}$ but no changes in LVEDS, $+dP/dt_{\max}$ and FS for SHR. These results indicated that SHR suffered from diastolic dysfunction, while systolic function was compensated for within our observation period.

An accumulation of data shows that regular exercise has extensive benefits for human health. Certain traditional forms of physical activity, such as Tai Chi and Tuina, have recently been shown to be associated with protective biomedical mechanisms [32, 33]. The meta-analysis performed by Schlüter et al. [11] shows that exercise, independent of duration and intensity, reduces blood pressure in young and prehypertensive SHRs but not in older SHRs with established hypertension. For cardiac morphology and function, low- to moderate-intensity exercise has been documented to be beneficial [13, 19]. Low- to moderate-intensity exercise has also been shown to benefit the hypertensive heart, independent of age [34]. Although certain exercises, such as resistance exercise and moderate- to high-intensity exercise, may induce eccentric LV hypertrophy [35], worsening cardiac function has rarely been observed, even in untreated severe hypertensive rats [36]. The present study employed low-intensity ExT, which did not induce physiological hypertrophy in control WKY rats. There is evidence that

ExT can attenuate or even reverse compensated hypertrophy and diastolic dysfunction in hypertensive rats [10, 12, 13]. A number of clinical trials show that ExT independently lowers BP [37]. For young prehypertensive subjects, Beck et al. [38, 39] observed that 8 weeks of resistance or endurance exercise effectively reduced BP, which was associated with reduced peripheral arterial stiffness and improved endothelial function in resistance arteries [20]. The mechanisms underlying the benefits of ExT involve the inhibition of oxidative stress [29], the reduction of extracellular matrix [40], the prevention of myofibroblast activation [41], and the transformation between pathological to physiological hypertrophy [13]. In a population-based study of middle-aged normotensive subjects, Oh et al. [42] reported that an exaggerated blood pressure response to acute exercise significantly predicted the future development of hypertension or cardiovascular risk. However, early ExT can be utilized to prevent future hypertension. Indeed, we observed that either 8 or 16 weeks of low-intensity ExT lowered SBP, relieved cardiac hypertrophy and fibrosis, and alleviated diastolic dysfunction. The structural and functional improvements in the hypertensive heart induced by ExT were associated with decreases in interstitial and perivascular collagen deposition, which may be at least partially explained by the suppression of collagen mRNA expression.

Ang II is a well-established contributor to the development of hypertension and cardiac hypertrophy. The binding of Ang II to AT1 receptors in the vasculature is involved in multiple signaling pathways, including those involving phospholipase A2 (PLA2), PLC, PLD, and NADPH oxidase, all of which lead to potent vasoconstriction and vascular proliferation and fibrosis [43]. The activation of AT1 in cardiomyocytes activates several intracellular pathways, such as the MAP kinase and JAK/STAT pathways, leading to myocyte hypertrophy [44]. Meanwhile, Ang II stimulates collagen synthesis via AT1 in cardiac fibroblasts, resulting in cardiac fibrosis, which underlies the development of cardiac hypertrophy [44]. Pharmaceutical inhibitors of the ACE–Ang II–AT1 axis, such as ACE inhibitors and AT1 antagonists, have been used to treat hypertension and heart failure in humans [1–3]. Intriguingly, ExT may serve as an alternative or supplementary inhibitor of the ACE–Ang II–AT1 axis. ExT has been associated with reduced Ang II levels in a variety of organs, including the heart [15], blood vessels [45], brain [46], and plasma [15]. This association was further demonstrated by the present study.

Some researchers have reported changes in multiple RAS components that are induced by exercise in SHR. Filho et al. [14] used four-month-old SHR, which were older than the rats used in our study, and observed that swimming training for 8 weeks decreased plasma Ang II levels and

increased ventricular Ang(1-7) and Mas expression but did not change ventricular Ang II or plasma Ang(1-7) levels. Silva et al. [15] monitored the effects of low-to-moderate intensity treadmill training in 11- to 12-week-old SHR for 8 weeks and observed that ventricular Ang II in SHR decreased promptly after exercise, followed by plasma Ang II, while ventricular and plasma Ang(1-7) increased in parallel. Ventricular and plasma Ang II were also decreased in WKY after 4 weeks of exercise. Compared with these studies, the present study observed younger SHR over a longer exercise period. Although we consistently observed that exercise had blood pressure-lowering effects in SHR, our results for RAS components are different in many ways from those reported in previous studies. We observed sustained increases in the activity of the ACE–Ang II–AT1 axis in SHRs but significant decreases in ventricular Ang(1-7) after only 16 weeks. For exercised WKY, we observed no decrease in ventricular Ang II but an increase in ventricular Ang(1-7) after 16 weeks. These discrepancies may result from the differences in the rat ages and the training protocol used. Nevertheless, the consistent inhibition of the ACE–Ang II–AT1 axis in response to ExT presumably contributed to the alleviation of hypertension and cardiac hypertrophy in SHR.

While overactivation of the ACE–Ang II–AT1 axis is generally believed to be deleterious, the ACE2–Ang(1-7)–Mas axis has been shown to be protective against hypertension and cardiac hypertrophy [4–7]. Ang(1-7) is a newly identified contributor to the RAS, in which it counterbalances the actions of Ang II [8]. Available data indicate that most if not all of the effects of Ang(1-7) are mediated by Mas. Enhancement of Ang(1-7) and Mas activity have been observed to have extensive effects on the cardiovascular system, including vasodilatory, antihypertrophy, and antifibrosis effects [8, 9]. The present study observed the inactivation of the ACE2–Ang(1-7)–Mas axis in SHR myocardium, while ExT led to the recovery of ACE2 and Mas expression in SHR. Moreover, the mRNA levels of Mas in SHR were much higher than those in WKY-S after 16 weeks of exercise. ExT also markedly upregulated ACE2 mRNA and Mas mRNA in WKY rats, but the level of ACE2 protein remained unaffected. A limitation of the present study is the lack of data for AT1 and Mas proteins due to the unavailability of specific antibodies against AT1 and Mas from commercial sources [47–50]. Therefore, to obtain conclusive data for Mas and AT1 expression, further investigation is needed.

A notable finding of this study is that the two key effectors, Ang II and Ang(1-7), showed temporal responses to hypertension and ExT. As described in the results, the elevation of myocardial Ang II in SHR occurred relatively early and increased over time. ExT suppressed the Ang II levels in SHR but had no effect on the Ang II levels in

WKY. By contrast, the decrease in Ang(1-7) appeared late. ExT for 16 weeks not only restored the Ang(1-7) levels in SHR but also increased the Ang(1-7) levels in WKY, while relatively shorter-term training showed no effect. These results indicate that the increase in Ang II was fast and persistent and could be blunted by ExT. By contrast, the decrease in Ang(1-7) in SHR appeared relatively late and could be reversed by ExT. Long-term ExT seems to increase Ang(1-7) in normotensive rats, which may help to explain why normotensive individuals can benefit from exercise.

Exercise likely has beneficial effects on RAS components in the heart as a result of multiple mechanisms that underlie the beneficial effects of ExT on the RAS system. There is plenty of evidence indicating that ExT reduces cardiac oxidative stress in SHR [29, 51, 52], while oxidative stress has been shown to cause an imbalance in RAS components [53, 54]. Furthermore, antioxidant reagents are capable of reversing this imbalance [53–55]. Another involved mechanism is the reduction of proinflammatory cytokines by ExT [46, 56]. It has been reported that the inhibition of cytokines in the brain reverses the alterations in RAS components and attenuates hypertension and cardiac hypertrophy [56, 57]. Furthermore, there is an established positive feedback loop for Ang II, in which an increase in Ang II itself can increase the levels of its pathway components and act as the primary driver of the imbalance between AT1R-ACE and AT2R-ACE2 [58, 59]. That is, a decrease in Ang II by ExT would facilitate the reversal of the RAS imbalance. Nevertheless, novel molecules, such as microRNAs [45, 60], and other unknown mechanisms may also play a role in the protection conferred by ExT on the RAS system.

In summary, the present study observed that prehypertension ExT delayed the onset and progression of hypertension and attenuated cardiac hypertrophy. A novel finding of our study is the temporal regulation of the ACE and ACE2 axes. Suppression of the Ang II level was indicated to play a major role in the early protection induced by ExT, while upregulation of the Ang(1-7) level was implicated only after a relatively long period of ExT.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No. 81372111, 81670295) and the Natural Science Foundation of Fujian Province of China (No. 2014J01339).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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