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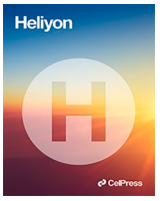

Research article

High-intensity interval training and moderate-intensity continuous training alleviate vascular dysfunction in spontaneously hypertensive rats through the inhibition of pyroptosis

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ABSTRACT

Evidence-based guidelines suggest that High-Intensity Interval Training (HIIT) is more beneficial than aerobic exercise for patients with cardiovascular disease, but the differences in underlying pathophysiological mechanisms require further confirmation. The comparison between HIIT and Moderate-Intensity Continuous Training (MICT) in regulating vascular dysfunction in spontaneously hypertensive rats (SHR), along with their underlying mechanisms, has not been previously reported. The purpose of this study is to provide an experimental basis for exercise prescription therapy in hypertensive patients. In this study, six-week-old male SHR were randomly assigned to a HIIT group, MICT group, or sedentary group. Wistar Kyoto rats (WKY) of the same age were used as the control group. The weight, heart rate, and blood pressure of the rats were monitored weekly throughout twelve weeks of treadmill training. At the end of the protocol, serum and aortic vascular tissues were collected for further vascular function tests and molecular and biochemical analyses. The results show that MICT is more favorable for weight control than HIIT, while both forms of exercise offer equal protection against hypertension. However, MICT demonstrates a greater benefit in preserving vascular morphology, whereas HIIT is more effective in reducing oxidative stress. Both HIIT and MICT ameliorate vascular dysfunction in SHR by suppressing nucleotide-binding domain and leucine-rich repeat pyrin-domain containing protein 3 (NLRP3)-induced pyroptosis. The superior effect of HIIT on vascular dysfunction may be related to the inhibition of oxidative stress injury through AMPKα-SIRT1 activation. This study provides insight into the dose-effect relationship of exercise for cardiovascular health and offers foundational evidence for the development of exercise prescription therapies.

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1. Introduction

Hypertension is a leading cause of premature death worldwide, affecting over one billion people [1,2]. It is a significant risk factor for cardiovascular diseases, including stroke, heart failure, and kidney disease [3,4]. The vascular injury caused by high blood pressure is complex, often manifesting as endothelial dysfunction, vascular inflammation, and arterial remodeling [4–7]. Key features of this damage include reduced nitric oxide (NO) production, an imbalance between oxidative and antioxidant systems, and elevated inflammatory cytokine levels [4,8].

Inflammasomes, multiprotein complexes that regulate the immune response to infections and physiological imbalances, play a critical role in hypertension-related vascular injury. Inflammasome that has been well studied in recent years includes nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 1 (NLRP1), NLRP3, absent in melanoma 2 (AIM2), and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 4 (NLRC4) [5,9–11]. NLRP3 has attracted more attention in recent studies. NLRP3 inflammasome is closely related to cardiovascular diseases and vascular endothelial cell function [12–14]. Previous studies have confirmed that excessive activation of the inflammasome pathway is detected in SHR aorta, and silencing of NLRP3 gene can improve hypertension, vascular inflammation, and aortic remodeling [12,13,15]. Besides, genetic deletion of the NLRP3 inflammasome improves ischemia-reperfusion (I/R) injury, myocardial infarction, and atherosclerosis by inhibiting inflammatory responses [13]. Currently, NLRP3 inflammasome is considered as an important target in the pathogenesis and treatment of cardiovascular diseases.

Pyroptosis, a form of programmed necrosis, is distinguished by its reliance on caspase-mediated formation of plasma membrane pores, leading to the release of pro-inflammatory cytokines and cell death [16,17]. This process is closely tied to NLRP3 activation and is increasingly recognized as a key driver of cardiovascular inflammation [18]. Pyroptosis amplifies the inflammatory response by releasing interleukin-1β (IL-1β) and IL-18, contributing to the pathogenesis of vascular damage in hypertensive conditions [19].

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is also closely linked to cardiovascular diseases [20]. While ROS play a physiological role as signaling molecules [21], their overproduction can damage lipids, proteins, and DNA, exacerbating vascular injury [22]. Physical exercise has been shown to modulate oxidative stress, enhance endothelial function, and interact with pyroptosis and inflammation, providing a potential intervention for cardiovascular dysfunction. Despite the well-established roles of oxidative stress and pyroptosis in these diseases, effective preventive strategies remain limited [23].

AMP-activated protein kinase (AMPK) is a central regulator of cellular energy metabolism [24–26]. It contains three subunits: α, β, and γ, with the Thr-172 phosphorylation site on the α subunit playing a critical role in regulating AMPK function. AMPK stimulates endothelial nitric oxide synthase (eNOS) activity by phosphorylating eNOS, and its protective effects on vascular dysfunction and inflammation-related vascular damage have been extensively studied [25–28]. On the other hand, it has been reported that metabolic disorders associated with hypertension may switch off AMPK signaling, leading to impaired peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) activity and diminished mitochondrial function [27].

Sirtuins, particularly SIRT1 and SIRT3, play vital roles in protecting against vascular inflammation and oxidative stress. These proteins are crucial in maintaining mitochondrial function and regulating the body's response to metabolic stress [29]. Studies suggest that AMPK activation enhances SIRT1 and SIRT3 activity, contributing to improved mitochondrial health and reduced disease progression [30,31]. Exercise training has been shown to activate the AMPK-SIRT1/3 pathway, further underscoring the importance of physical activity in mitigating vascular dysfunction [32,33].

Proper physical exercise can effectively improve hypertension through different molecular mechanisms. An appropriate exercise prescription can promote the bioavailability of NO and upregulate antioxidants to reduce oxidative stress, thereby improving endothelial function and reducing the risk of high blood pressure [34–36]. While high-intensity continuous training (HICT) may increase cardiovascular risk, HIIT has shown promise in reducing complications associated with chronic cardiovascular disease [34,37–39]. HIIT refers to intermittent exercise that involves alternating short bursts of high-intensity activity with lower-intensity activity for recovery. Evidences indicated that traditional moderate-intensity continuous training (MICT) and HIIT can both increase cardiac ejection fraction, which relate to the benefits in cardiovascular disease treatment.

Our previous research has demonstrated that different intensities of continuous training affect oxidative stress and vascular inflammation in SHR [33]. Given that exercise intensity is crucial in designing effective hypertension interventions, it is essential to compare the effects of different exercise modalities. In this study, we aim to compare the impact of HIIT and MICT on vascular function in SHR, with the goal of identifying potential mechanisms of action. These findings will provide valuable insights for developing more effective exercise-based strategies for blood pressure control.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Ethics Committee of Chongqing Medical University (Approval Number: 201729) and adhered to the "Regulations on the Management of Laboratory Animals of China (2017 Revision)" and the ethical guidelines of Chongqing Medical University.

Six-week-old male spontaneously hypertensive rats (SHR) and age-matched male Wistar-Kyoto rats (WKY), weighing approximately 130–150 g, were obtained from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The rats were housed in

the Experimental Animal Center at Chongqing Medical University under controlled conditions: five animals per cage, maintained at a room temperature of 22 °C, with a 12-h light/dark cycle and 50 % humidity. The facility adhered to specific pathogen-free (SPF) standards.

1.WKY (n = 6): WKY sedentary control group;
2.SHR (n = 6): SHR sedentary control group;
3.SHR-MICT (n = 6): SHR moderate-intensity continuous training group;
4.SHR-HIIT (n = 6): SHR high-intensity interval training group.

The weight, heart rate, and blood pressure of the rats were monitored weekly during the twelve-week treadmill training. After the exercise protocol, the animals were sacrificed, and serum and aortic vascular tissues were collected for further vascular function tests and molecular and biochemical investigations.

2.2. Training protocol

Exercise protocols were conducted using a treadmill (SA101C; SANS Biological Technology, Jiangsu, China) designed specifically for research on the physiology and pathology of small experimental animals. The treadmill, operating at 220 V and 50 Hz, featured eight running lanes, speed control ranging from 0 to 100 m/min with a resolution of 0.01 m/min, and was connected to treadmill software for continuous speed monitoring.

The exercise training protocol was based on established guidelines for animal exercise and training [33,40].The exercise speed was determined through a progressive exercise test that measured the maximum aerobic velocity (MAV, vVO2 max), a method adapted for rats. Maximum speed tests were performed on SHR rats to set the treadmill speeds for the MICT group (45–55 % VO_2max) and the HIIT group (70–80 % VO_2max) during the exercise regimen.Following a one-week acclimatization period, the SHR-MICT and SHR-HIIT groups underwent progressively increasing speed training (exercise prescription) over 12 weeks, 5 days per week, with a treadmill incline of 0° for all groups. The detailed exercise protocol is outlined in Table 1.

2.3. Weight, heart rate, and blood pressure measurements

The rats were weighed weekly using an animal scale (Shimadzu, Japan) between 8:00 and 12:00 a.m. every Sunday. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) were measured in conscious rats using a noninvasive tail-cuff system (Softron, Japan). To ensure accuracy, the rats were accustomed to the tail-cuff procedure before the actual measurements. Prior to each measurement, the rats were placed in an incubator set to 37 °C for 10 min. Blood pressure readings were obtained by averaging three measurements per rat.

2.4. Vascular morphometry analysis

The ascending thoracic aortas were carefully dissected from the rats. Paraffin sections of 5 μm thickness were prepared and stained with hematoxylin and eosin [33]. Vascular intima-media thickness was measured using ImageJ 1.80v software (Bethesda, Maryland, USA). This measurement was taken at five different locations across each cross-section of the vessel, from the endothelial surface to the boundary between the medial and adventitial layers. All images were captured using a Leica DM4B upright metallurgical microscope (Leica Inc, Germany).

2.5. Vascular reactivity experiment

The thoracic aortas were promptly dissected and placed in a physiological salt solution buffer (PSS) [41,42]. Adherent tissues were removed, and the aortas were cut into 3-mm rings, which were then transferred to the Multi Myograph System chamber (DMT620, Denmark) to prevent damage to the vascular endothelium. The rings were mounted in chambers containing warmed (37 °C), oxygenated (95 % O2 and 5 % CO2) PSS. Vascular tension was continuously recorded using LabChart software (DMT620).The aortic rings were initially maintained at a basal tension of 2 g for 90 min and were then pre-stimulated twice with KCl (60 mmol/L)-PSS. Endothelial integrity was assessed by evaluating the relaxation response of norepinephrine (NE, 10^{-7} M)-precontracted vessels to acetylcholine (ACh, 10^{-5} M), with an 80 % relaxation indicating intact endothelium. Subsequently, the aortas were precontracted with NE ($10^{\wedge-7}$ M) until maximum contraction was achieved and the tension curve stabilized. Cumulative concentration-response curves (ranging from 10^{-9} M to 10^{-5} M) for ACh were constructed to assess endothelium-dependent relaxation. Average concentration-response curves were then plotted.

2.6. Western blot analysis

Protein expression was assessed using Western blot analysis [43,44]. Tissues were lysed on ice for 1 h with a lysis buffer. Protein concentrations were determined using the Bradford assay (Beyotime, Shanghai, China), and 50 μg of protein was used for each Western blot. Proteins were separated by 10 % SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with 5 % nonfat milk for 1 h at 22 °C and then incubated with primary antibodies overnight at 4 °C. Following this, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at 22 °C. Protein bands were visualized using a chemiluminescence detection reagent (Beyotime). The intensity of the bands was quantified using Image Lab 6.0 software, and the

Table 1
Exercise training prescription.

Week	MICT						HIIT					
	Monday-Friday				Sat	Sun	Monday-Friday				Sat	Sun
	Training				Rest		Training				Rest	
1	preconditioning training regimen : Progressive incresing speed											
	1–14 m/min				Rest		1–45 m/min				Rest	
	Maximum speed	**Time**	**Stage**	**Progressive increasing treadmill speed**	**Rest**		**Maximum speed**	**Time**	**Stage**	**Progressive increasing treadmill speed**	**Rest**	
2–5	14 m/min	60min	1–5min 6–10min 11–55min 56–60min	5 m/min 10 m/min 14 m/min Per minute ↓ 3 m/min	Rest		45 m/min	60min	1–5min 6–10min 11–55min 56–60min	1–35 m/min 35–45 m/min 45 m/min Per minute ↓ 5 m/min	2 min rest after 30 s sprint	Rest	
6–9	15 m/min	60min	1–5min 6–10min 11–55min 56–60min	5 m/min 10 m/min 15 m/min Per minute ↓ 3 m/min			50 m/min	60min	1–5min 6–10min 11–55min 56–60min	1–40 m/min 40–50 m/min 50 m/min Per minute ↓ 5 m/min			
10–12	16 m/min	60min	1–5min 6–10min 11–55min 56–60min	5 m/min 10 m/min 16 m/min Per minute ↓ 3 m/min			55 m/min	60min	1–5min 6–10min 11–55min 56–60min	1–45 m/min 45–55 m/min 55 m/min Per minute ↓ 5 m/min			

expression level of the target protein was determined by the ratio of the target protein's gray value to that of the internal control protein (β-actin or total).

Primary antibodies used in this study included those against endothelial nitric oxide synthase (eNOS), Phospho-eNOS (Ser1177), inducible nitric oxide synthase (iNOS), Phospho-AMPKα (Thr172), SIRT1, SIRT3, and GSDMD, which were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against NLRP3 were obtained from AIFang Biological (AIFang, Hunan, China), while antibodies against ASC and NLRP1 were sourced from Santa Cruz Biotechnology (Dallas, Texas, USA). Antibodies targeting Superoxide Dismutase 2 (MnSOD), NADPH oxidase 4(NOX4), AIM2, Caspase-1, and β-actin, along with secondary antibodies, were acquired from Proteintech Group (Chicago, IL, USA). All other chemicals used in the study were procured from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified.

2.7. Biochemical analyses

Oxidative stress indicators in serum were assessed using reagent kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China)) [43]. Superoxide Dismutase (SOD) activity was measured by the hydroxylamine method. Glutathione Peroxidase (GSH-Px) activity was determined using a colorimetric method. Catalase (CAT) activity was quantified using the ammonium molybdate spectrometric method.

2.8. DHE staining

Superoxide production in aortic tissue was assessed using the fluorescent dye Dihydroethidium (DHE; Beyotime). Frozen aortic sections were stained with DHE (10-5 M) for 30 min at 37 °C. Following staining, tissues were washed three times with phosphate-buffered saline (PBS). Images were captured using a confocal microscope with an excitation wavelength of 535 nm and an emission wavelength of 610 nm. All sections were processed under identical conditions. DHE fluorescence intensity was quantified using

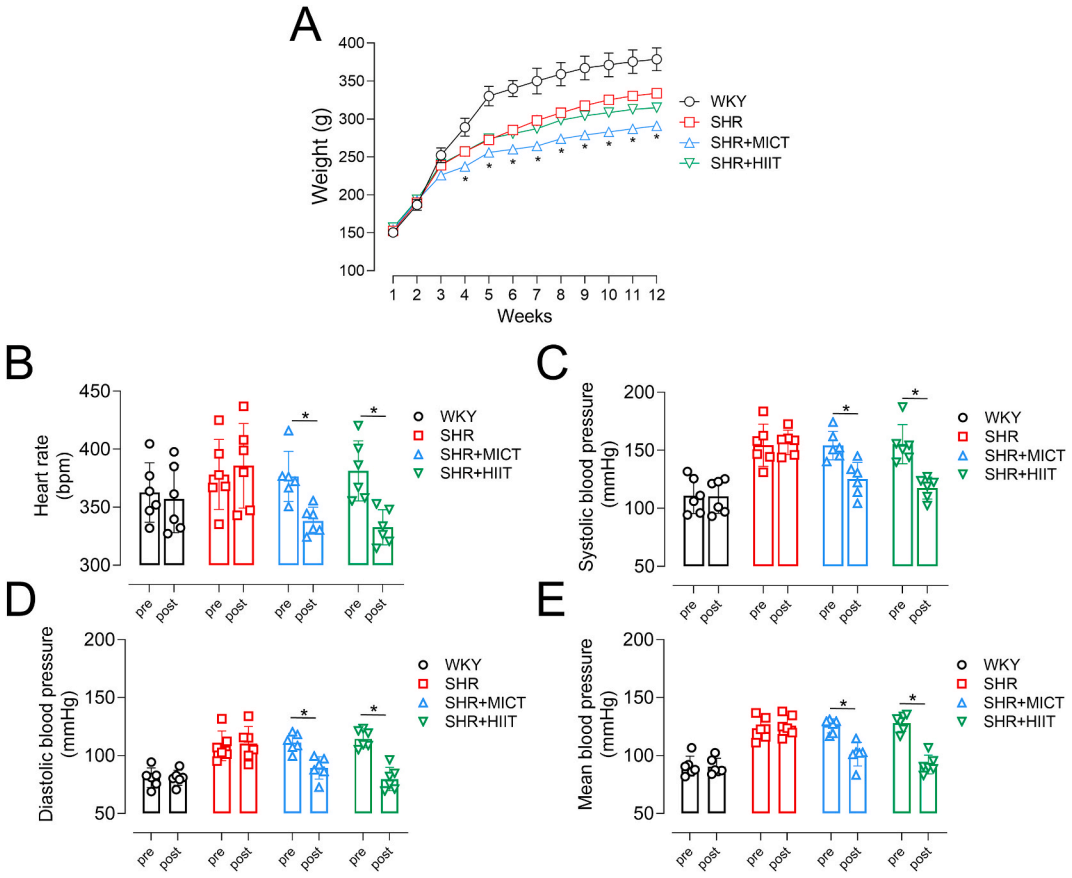

Fig. 1. Effects of MICT and HIIT on body weight, heart rate, and blood pressure in SHR. The WKY group, SHR group, SHR-MICT group, and SHR-HIIT group underwent treadmill training with varying intensities and methods for 12 weeks. Changes in body weight were recorded (A). *P < 0.05 indicates a significant difference between SHR-MICT and SHR-HIIT. (B) Heart rate, (C) systolic blood pressure, (D) diastolic blood pressure, and (E) mean arterial pressure were measured using the tail-cuff method before exercise (1st week) and after 12 weeks of exercise. *P < 0.05 denotes a significant difference between pre-exercise and post-exercise measurements.

ImageJ software.

2.9. Immunofluorescence staining

Aorta sections were permeabilized with 0.1 % Triton X-100 in PBS for 20 min, blocked with 5 % goat serum for 1 h, and incubated overnight at 4 °C with primary antibodies. Following this, the sections were stained with 4′,6-diamidino-2-phenylindole (DAPI) for 5 min and then with fluorescence-conjugated secondary antibodies (Beyotime) for 1 h at 37 °C in the dark. Fluorescence microscopy (Leica Microsystems, Germany) was used for observation. The primary antibodies used were 8-OHdG (1:200, Bioss) and NLRP3 (1:200, AIFang). Fluorescence intensity was analyzed with ImageJ 1.8 software.

2.10. Statistical analysis

Data are presented as means ± standard deviation. Normality was assessed using the Shapiro-Wilk test. Comparisons between two groups were made with unpaired two-tailed Student's t-tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data. For multiple groups (≥3), one-way ANOVA with Bonferroni post hoc test was used for normally distributed data, and Kruskal-Wallis test with Dunn's post hoc test for non-normally distributed data. All analyses were performed using GraphPad Prism 9.0 (GraphPad Software, California, USA), with *P*-values <0.05 considered significant.

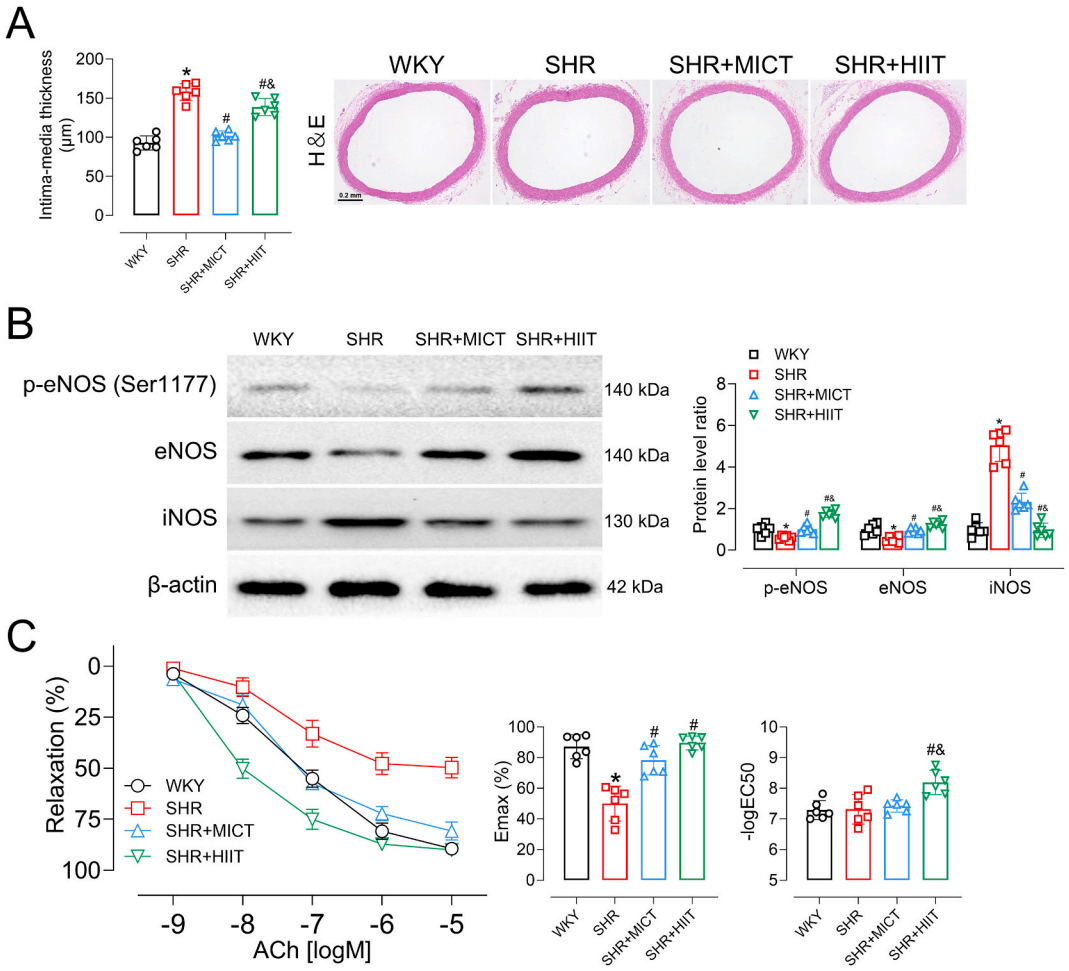

Fig. 2. Effects of MICT and HIIT on vascular structure, p-eNOS, eNOS, iNOS levels, and ACh-induced relaxation of aortas in SHR. WKY, SHR, SHR-MICT, and SHR-HIIT rats underwent 12 weeks of treadmill training with varying intensities and methods. Representative hematoxylin and eosin-stained (HE) images of the aortas in each group were presented (A). The intima media thickness values were quantified using ImageJ software. The protein expression of p-eNOS, eNOS, iNOS in aortas were determined by Western blot (B), ACh-induced relaxation of aortas was measured by vascular reactivity experiment (C), vascular function was represented with Emax and -logEC50. *P < 0.05 (WKY vs. SHR), #P < 0.05 (SHR-MICT, SHR-HIIT vs. SHR), &P < 0.05 (SHR-MICT vs. SHR-HIIT).

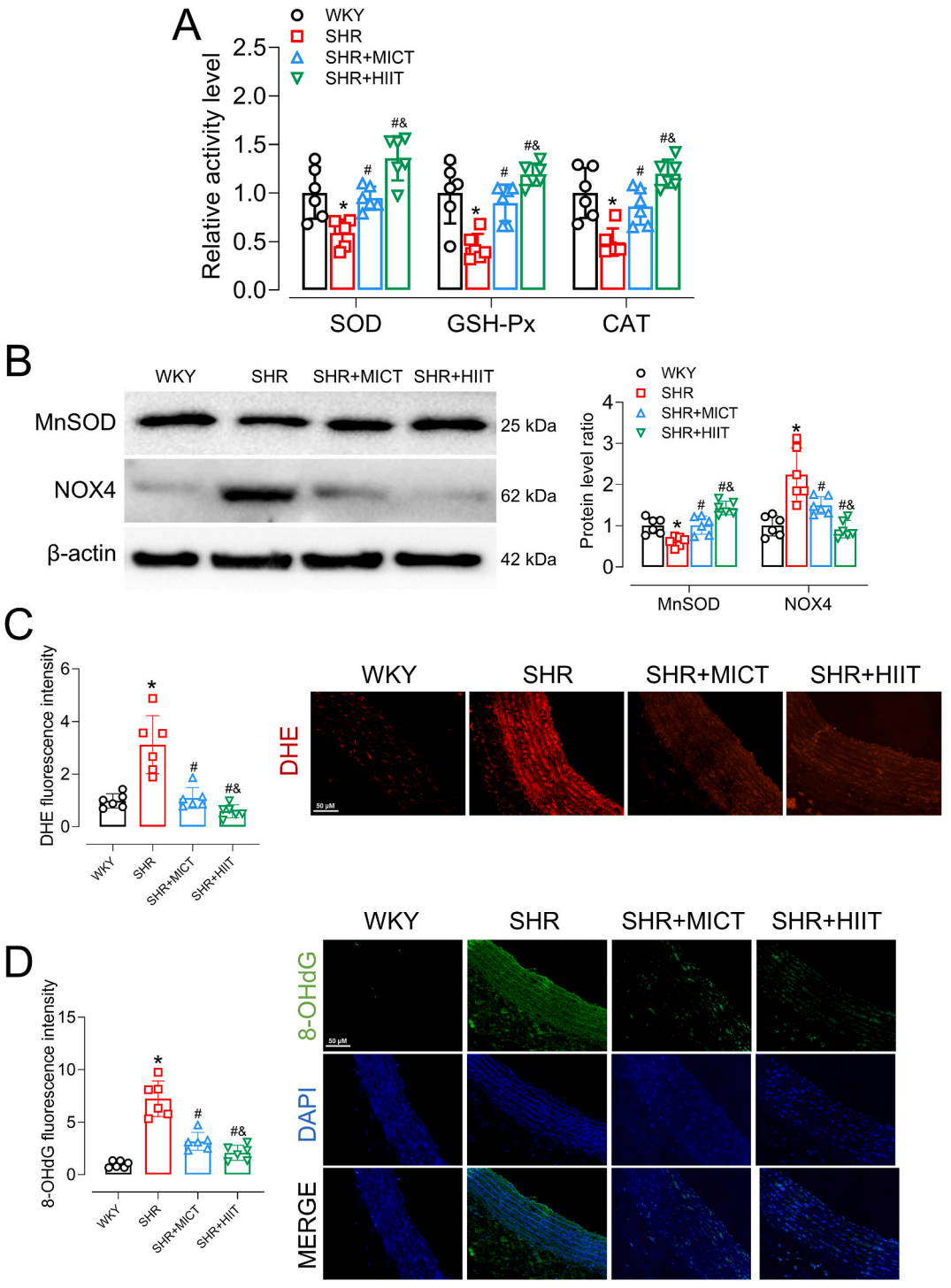

Fig. 3. Effects of MICT and HIIT on Oxidative Stress in SHR. WKY, SHR, SHR-MICT, and SHR-HIIT rats underwent 12 weeks of treadmill training at varying intensities. (A) Serum levels of SOD, GSH-Px, and CAT were measured across all groups. (B) Western blot analysis was used to assess MnSOD and NOX4 protein levels in the aorta. (C) DHE fluorescence intensity and (D) 8-OHdG expression levels in aortas were evaluated using immunofluorescence. *P < 0.05 (WKY vs. SHR), #P < 0.05 (SHR-MICT, SHR-HIIT vs. SHR), &P < 0.05 (SHR-MICT vs. SHR-HIIT).

3. Results

3.1. Effects of MICT and HIIT on body weight, heart rate, and blood pressure in SHR

We measured the rats' weight weekly and observed varying degrees of weight change across the different groups (Fig. 1A). Notably, significant differences in weight were found between the MICT and HIIT groups ($P < 0.05$). Heart rate and blood pressure were assessed using the tail-cuff method (Fig. 1B–E). Significant differences were observed between pre-exercise (1st week) and post-exercise (12th week) for both MICT and HIIT. Both MICT and HIIT significantly reduced heart rate, SBP, DBP, and mean blood pressure (MBP) in SHR ($P < 0.05$).

3.2. Effects of MICT and HIIT on vascular structure and function of aortas in SHR

To explore the potential impact of MICT and HIIT on the vascular structure of SHR, we examined the vascular morphology of the thoracic aorta using slice staining technology (Fig. 2A). Analysis with ImageJ revealed that the intima-media layer of SHR arteries was

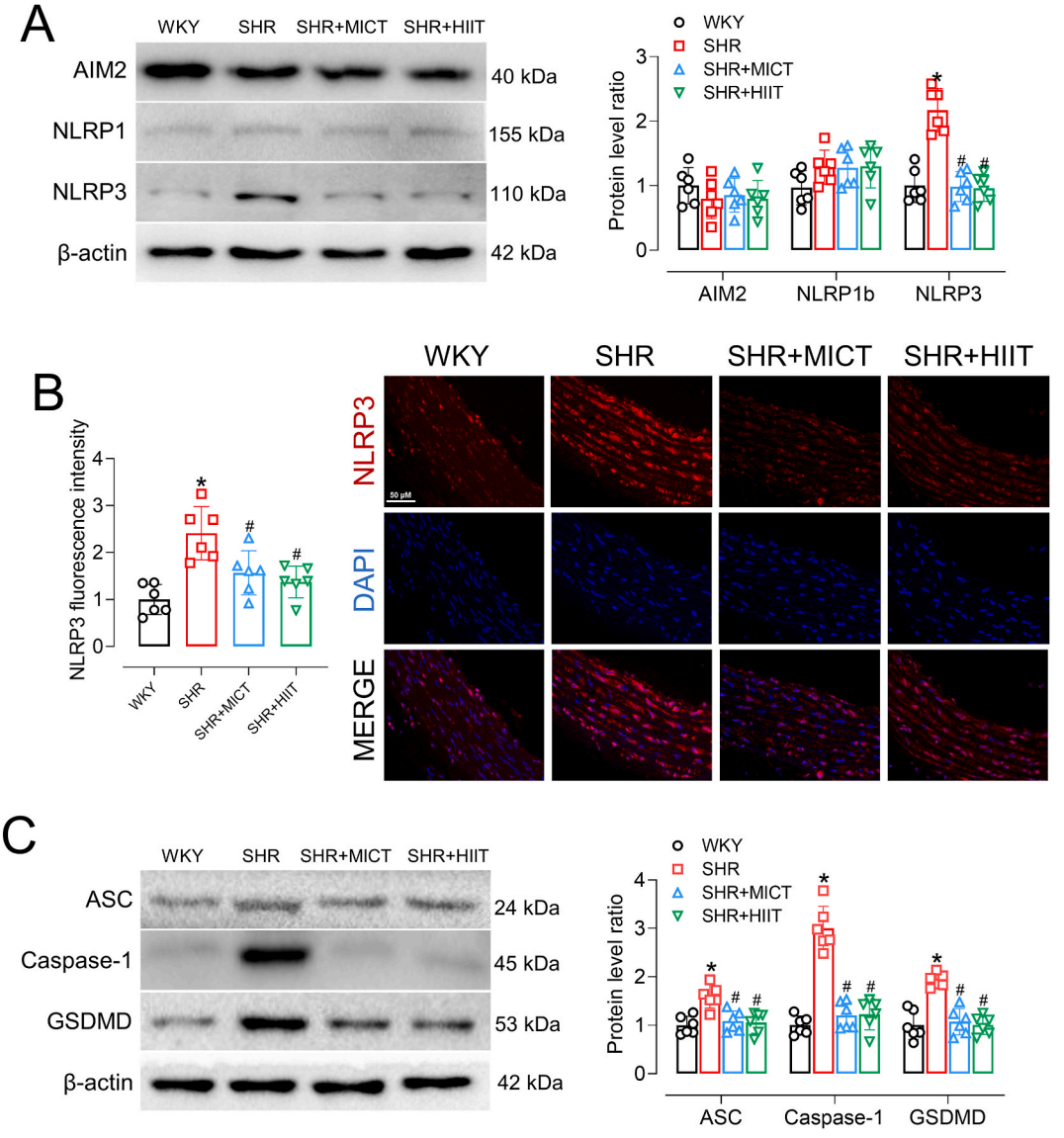

Fig. 4. Effects of MICT and HIIT on Inflammasome and Pyroptosis-Related Proteins in the Aortas of SHR. WKY, SHR, SHR-MICT, and SHR-HIIT rats underwent 12 weeks of treadmill training with varying intensities and methods. (A) Western blot analysis was used to measure the protein expression of AIM2, NLRP1, NLRP3, ASC, Caspase-1, and GSDMD in aortas. (B) Immunofluorescence was employed to assess the expression and localization of NLRP3 in aortas. *$P < 0.05$ (WKY vs. SHR), #$P < 0.05$ (SHR-MICT, SHR-HIIT vs SHR).

significantly thicker compared to that of WKY rats ($P < 0.05$). Additionally, the intima-media layer was thinner in the MICT and HIIT groups compared to the SHR group ($P < 0.05$), with MICT showing a more pronounced effect ($P < 0.05$).

Vascular dysfunction is characterized by disrupted NO production due to alterations in eNOS and iNOS activities. Phosphorylation of eNOS at serine 1177 is crucial for NO production in vascular endothelial cells. Western blot analysis of aortic tissue revealed that SHR rats exhibited reduced levels of p-eNOS (Ser1177) and eNOS compared to WKY rats ($P < 0.05$), while iNOS levels were elevated ($P < 0.05$). Both MICT and HIIT interventions increased the expression of p-eNOS and eNOS and decreased iNOS levels in SHR rats ($P < 0.05$). Notably, there were significant differences in the expression of these proteins between the MICT and HIIT groups ($P < 0.05$) (Fig. 2B).

Impaired ACh-induced relaxation of the aorta is a key indicator of endothelium-dependent dysfunction in hypertensive conditions. We assessed the effects of different exercise interventions on ACh-induced aortic relaxation in SHR rats (Fig. 2C). SHR rats displayed a decreased Emax for ACh-induced relaxation compared to WKY rats. Both MICT and HIIT significantly enhanced ACh-induced relaxation in SHR ($P < 0.05$), with HIIT showing a more pronounced increase in -logEC50 compared to the other groups ($P < 0.05$). These findings suggest that both MICT and HIIT can improve ACh-induced aortic vasodilatory function in SHR, with HIIT demonstrating a superior effect.

3.3. Effects of MICT and HIIT on oxidative stress indicators in serum in SHR

Oxidative stress is a major contributor to endothelial dysfunction [45]. To evaluate the impact of MICT and HIIT on oxidative stress in SHR, we measured serum activities of SOD, GSH-Px, and CAT, key enzymes in the defense against free radicals (Fig. 3A). Compared to WKY rats, SHR exhibited significantly lower levels of SOD, GSH-Px, and CAT ($P < 0.05$). Both MICT and HIIT increased these enzyme activities compared to SHR ($P < 0.05$), with HIIT showing significantly higher levels than MICT ($P < 0.05$).

3.4. Effects of MICT and HIIT on oxidative stress of aortas in SHR

MnSOD is a key antioxidant enzyme that helps maintain the balance between oxidation and antioxidation in the body. NOX4, a major source of reactive oxygen species in the cardiovascular system, plays a crucial role in electron transfer within cell membranes [46]. Western blot analysis revealed that both MICT and HIIT increased MnSOD expression and decreased NOX4 levels in the aorta of SHR ($P < 0.05$). Notably, HIIT and MICT differed significantly in their effects on MnSOD and NOX4 levels ($P < 0.05$).

DHE staining, as shown in Fig. 3C, demonstrated that high blood pressure significantly elevated superoxide production in the aorta, which was reduced following MICT and HIIT. SHR exhibited higher DHE fluorescence compared to other groups, with HIIT showing the lowest fluorescence intensity, significantly different from MICT ($P < 0.05$).

To further assess oxidative stress, we used immunofluorescence to measure 7,8-dihydro-8-oxo-2-deoxyguanosine (8-OHdG) levels in aortic tissue. 8-OHdG, a marker of DNA oxidation and cellular oxidative stress, was elevated in SHR compared to the exercise groups, with HIIT showing superior results compared to MICT (Fig. 3D).

3.5. Effects of MICT and HIIT on inflammasome and pyroptosis related proteins of aortas in SHR

The involvement of the NLRP3 inflammasome in hypertensive vascular dysfunction has been established. To explore how different training methods affect vascular dysfunction in SHR, we assessed the expression of inflammasome core proteins (AIM2, NLRP1, NLRP3), inflammasome assembly proteins (ASC, Caspase-1), and pyroptosis-related proteins (GSDMD) in the aortas using Western blot and immunofluorescence.

Fig. 4A and C shows that the expression levels of NLRP3, ASC, Caspase-1, and GSDMD were significantly higher in the aortas of SHR compared to WKY ($P < 0.05$). Both MICT and HIIT significantly reduced these elevated levels in SHR ($P < 0.05$), with no significant difference between MICT and HIIT ($P > 0.05$). Additionally, there were no significant differences in AIM2 and NLRP1 expression across all groups ($P > 0.05$). Immunofluorescence analysis (Fig. 4B) revealed that NLRP3 expression was lower in the MICT and HIIT groups compared to SHR ($P < 0.05$), and NLRP3 was present throughout all vessel layers.

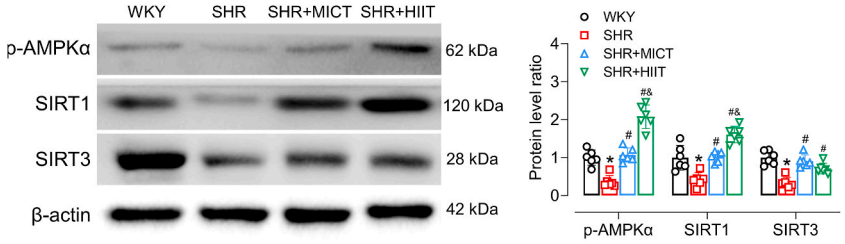

Fig. 5. Effects of MICT and HIIT on p-AMPKα, SIRT1, and SIRT3 Expression in the Aortas of SHR. WKY, SHR, SHR-MICT, and SHR-HIIT rats underwent 12 weeks of treadmill training with varying intensities. Western blot analysis was used to determine the protein expression of p-AMPKα, SIRT1, and SIRT3 in aortas. *$P < 0.05$ (WKY vs. SHR), #$P < 0.05$ (SHR-MICT, SHR-HIIT vs. SHR), &$P < 0.05$ (SHR-MICT vs. SHR-HIIT).

3.6. Effects of MICT and HIIT on p-AMPKα, SIRT1, and SIRT3 expression of aortas in SHR

To elucidate the mechanisms through which exercise training improves vascular dysfunction and to explore the differences between MICT and HIIT, we assessed the protein expression of p-AMPKα, SIRT1, and SIRT3 in the aortas using Western blot analysis. As shown in Fig. 5, SHR exhibited significantly lower levels of p-AMPKα, SIRT1, and SIRT3 compared to WKY rats ($P < 0.05$). Both MICT and HIIT significantly elevated the expression of these proteins in SHR ($P < 0.05$). Notably, HIIT markedly enhanced the levels of p-AMPKα and SIRT1 compared to MICT ($P < 0.05$), while the expression of SIRT3 was similar between HIIT and MICT ($P > 0.05$).

4. Discussion

This study demonstrates several key findings regarding the impact of MICT and HIIT on SHR. First, both MICT and HIIT positively influenced blood pressure and vascular dysfunction in SHR. Second, MICT was more effective than HIIT in managing body weight in SHR. Third, SHR aortic vessels exhibited activation of NLRP3 inflammasome-induced pyroptosis, which was associated with vascular inflammation and endothelial dysfunction. Fourth, both MICT and HIIT mitigated NLRP3 inflammasome activation, reduced pyroptosis, and improved vascular inflammation and oxidative stress levels. Fifth, HIIT had a more pronounced effect on the antioxidant system compared to MICT. Finally, HIIT enhanced endothelial-dependent relaxation and nitric oxide utilization more effectively than MICT, likely due to the activation of the AMPKα/SIRT1 pathway.

This study provides novel insights into the effects of Moderate-Intensity Continuous Training (MICT) and High-Intensity Interval Training (HIIT) on vascular morphology and function, as well as inflammatory and oxidative stress responses in the aortas of SHR. Both training protocols were shown to inhibit pyroptosis activation in the aorta, with HIIT resulting in superior vascular function. This improvement in vascular function with HIIT may be linked to enhanced nitric oxide production and reduced inflammasome activation, potentially due to improvements in mitochondrial function.

Our exercise regimen resulted in increased exercise capacity in the trained rats. Although previous studies have reported that both MICT and HIIT can improve cardiovascular dysfunction [3,33,43,47–49], their comparative effectiveness has been inconsistent. Some studies indicate that HIIT produces more significant improvements in cardiac or vascular function compared to MICT, while others report no significant differences between the two methods [50–54]. HIIT is known to improve sympathetic activity and increase vagal tone, which can reduce heart rate. In contrast, MICT typically enhances aerobic respiratory function and impacts cardiovascular metabolism [52,53,55,56]. This study aimed to quantify and compare the effects of HIIT and MICT on vascular function in hypertensive conditions.

We observed that hypertension led to an increase in vascular intima media thickness and a decrease in nitric oxide production and utilization. Continuous moderate-intensity exercise is known to promote beneficial physiological changes in vascular morphology. Interestingly, MICT demonstrated a greater improvement in these aspects compared to HIIT. HIIT, characterized by short bursts of high-intensity exercise followed by low-intensity periods, induces vascular adaptations that resemble those of resistance training. This intense stimulus may lead to increased vascular smooth muscle layer thickness, which could exacerbate pathological thickening in hypertensive vessels. These findings highlight the need for further investigation into the potential pathological effects of HIIT and the mechanisms underlying these adaptations [33,43,48,49].

Previous systematic reviews and meta-analyses have indicated that both MICT and HIIT effectively reduce total body fat in overweight adults [50,53]. Our study extends these findings to animal models, demonstrating for the first time that both MICT and HIIT reduce body weight in SHR. Notably, MICT was more effective than HIIT in promoting weight loss, a result consistent with clinical studies in adults with metabolic syndrome. Thus, MICT may be a more advantageous exercise prescription for hypertensive patients with overweight or obesity, potentially leading to greater reductions in body fat percentage and overall fat mass.

Endothelial dysfunction in hypertension can result from reduced NO production or impaired NO supply. As an endogenous vasodilator, NO plays a crucial role in regulating blood vessel morphology and reducing reactive oxygen species production. Abnormal eNOS expression disrupts NO production and impairs endothelial function. Our findings align with previous studies indicating that vascular function in spontaneously SHR is compromised compared to normotensive controls (WKY). Exercise positively impacted endothelial function, particularly in aortic endothelium-dependent relaxation induced by ACh, which is linked to NO production by eNOS [57,58].

Our data revealed that eNOS and phospho-eNOS (Ser1177) levels were significantly lower in SHR compared to other groups, suggesting impaired NO production in hypertensive rats. Notably, SHR subjected to HIIT had higher eNOS and phospho-eNOS levels compared to those subjected to MICT. This contrasts with previous studies where increased eNOS levels in SHR subjected to HICT did not improve NO production. It has been suggested that HICT may lead to eNOS uncoupling, resulting in abnormal eNOS function, while HIIT appeared to have a beneficial effect [59]. Additionally, increased NO production due to elevated iNOS in HICT might be converted into peroxynitrite (ONOO-), contributing to oxidative stress.

MICT and HIIT have gained popularity for managing hypertension, with some suggesting that HIIT offers greater cardiovascular benefits than MICT. Despite this, there is limited research directly comparing the effects of MICT and HIIT on vascular endothelial function. Our study used 6-week-old male SHR rats to model spontaneous hypertension and conducted 12 weeks of MICT and HIIT treadmill training. The results confirmed that both training methods effectively inhibit pyroptosis progression in SHR vascular tissue, with high blood pressure-induced pyroptosis linked to NLRP3 inflammasome activation rather than other inflammasomes.

Our findings indicate that training intensity significantly impacts oxidative stress and vascular function in SHR rats. Low- and moderate-intensity continuous training positively affects endothelial-dependent relaxation, while high-intensity continuous training may impair it. The divergent effects of HIIT and HICT on vascular function warrant further investigation. The impact of exercise on

oxidative stress is influenced by intensity, duration, and level of training. Both prolonged sedentary behavior and intense exercise can exacerbate oxidative stress. Prolonged high-intensity exercise may damage mitochondrial function, altering mitochondrial morphology, reducing their numbers, and inhibiting oxidative phosphorylation. Conversely, appropriate long-term exercise can enhance mitochondrial function. Both low- and moderate-intensity aerobic exercise and high-intensity interval training have been shown to increase MnSOD activity, boost ATP production, improve mitochondrial morphology, and enhance oxygen uptake and respiratory enzyme production.

The NLRP3 inflammasome is activated by pathogen-related molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). This activation leads to the activation of caspase-1, which processes pro-inflammatory cytokines IL-1β and IL-18. Activated caspase-1 also cleaves Gasdermin D (GSDMD) into its N-terminal (NT-GSDMD) and C-terminal (CT-GSDMD) fragments. NT-GSDMD binds to lipids and forms pores in the cell membrane, initiating pyroptosis, a form of inflammatory programmed cell death [60]. GSDMD-mediated pyroptosis contributes to various forms of cardiovascular inflammatory damage. Although the link between exercise and pyroptosis is not fully established, current research highlights the importance of exercise in mitigating pyroptosis-induced vascular inflammation, a key factor in hypertensive vascular dysfunction. Notably, our immunoblotting results for AIM2 and NLRP1 indicate that pyroptosis in SHR aortic tissue is primarily driven by NLRP3 inflammasome activation rather than other inflammasomes.

Absent in melanoma 2 (AIM2) is known as a cytosolic DNA sensor that triggers inflammasome activation in response to pathogens such as Listeria monocytogenes, Streptococcus pneumoniae, and murine cytomegalovirus. Similarly, NLRP1 activates inflammasomes during infections with pathogens like Bacillus anthraciss [61]. NLRP3 activation mechanisms are varied, including the production of mitochondrial reactive oxygen species and the release of oxidized mitochondrial DNA, particularly in hypertension. Exercise is recognized for its anti-inflammatory benefits, with chronic moderate-intensity continuous training shown to inhibit NLRP3 inflammasome activation and reduce inflammasome markers. However, there is limited research on whether exercise improves vascular endothelial dysfunction in SHR by modulating the outcomes of inflammatory injury, pyroptosis, and related upstream and downstream mechanisms.

The impact of various physical training modalities on oxidative stress and vascular function remains a subject of debate [59,62]. Understanding which behaviors and factors are protective or detrimental to the endothelium is still evolving. Given the critical roles of the endothelium and oxidative stress in cardiovascular and metabolic diseases, selecting the appropriate exercise regimen could have significant implications for the prevention and management of these conditions.

Metabolic stress, such as that induced by exercise, influences energy production by stimulating ATP synthesis and activating AMPKα. Our study observed AMPKα activation in the aorta of SHR subjected to both MICT and HIIT, suggesting its potential role in NO synthesis and vascular endothelial function. The activation of p-AMPKα-Thr172 may vary with different exercise intensities. Additionally, AMPKα activation likely enhances MnSOD expression, inhibits NOX4, and helps stabilize vascular oxidative stress levels. Notable changes in MnSOD, NOX4, and p-AMPKα were observed in both SHR-MICT and SHR-HIIT groups. Although the study did not directly measure ROS production in the aorta, the assessment of MnSOD, NOX4, DHE, and 8-OHdG provides indirect evidence of how different exercise regimens impact vascular oxidative stress in SHR.

Both SIRT1 and SIRT3 are promising targets for treating cardiovascular disorders, yet their distinct roles and efficacy in this context remain underexplored. Our findings indicate that exercise training increases SIRT1 and SIRT3 expression in rats, potentially enhancing mitochondrial biogenesis and ROS management. Notably, HIIT resulted in a more pronounced increase in SIRT1 compared to MICT [63,64]. We hypothesize that the greater activation of AMPKα/SIRT1 in HIIT may account for its superior benefits on nitric oxide bioavailability and oxidative stress in SHR.

We hypothesize that intense muscle stimulation during HIIT may produce and release specific metabolites or myokines into the bloodstream. As training intensity and methods change, these metabolites or myokines might activate metabolic functions, influencing AMPKα activation and altering energy supply and oxidative balance. The threshold of these metabolites may explain the differing vascular effects of MICT and HIIT. Additionally, exploring variations in myokine levels at different exercise intensities and their impacts could provide valuable insights into the relationship between exercise and cardiovascular function. This will be a focus of our future research.

Both types of exercise training, MICT and HIIT, demonstrated significant benefits for cardiovascular health in hypertensive rats. Both interventions effectively reduced pathological vessel thickening, decreased heart rate, and lowered systolic, diastolic, and mean blood pressure, while also enhancing endothelium-dependent relaxation. Additionally, the activation of AMPKα/SIRT1 appeared to play a crucial role in mitigating vascular dysfunction in SHR. These findings highlight the potential of incorporating MICT and HIIT into therapeutic strategies for the prevention and management of hypertension.

CRediT authorship contribution statement

Minghao Luo: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Qing Chang:** Project administration, Methodology. **Shuyuan Cao:** Resources, Project administration. **Yang Wang:** Software, Formal analysis. **Zhi Chen:** Resources, Data curation. **Jitang Yang:** Visualization, Validation. **Guochun Liu:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The data generated for this study are available upon reasonable request from the corresponding author.

Ethics approval

All research procedures were approved by the Ethics Committee of Chongqing Medical University and complied with the regulations of the People's Republic of China on the Management of Laboratory Animals.

Informed consent statement

All authors read and approved the final version of the manuscript and agreed to the order of authorship.

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Declaration of competing interest

All authors read and approved the final version of the manuscript and agreed to the author's order of statement. The authors declare no conflict of interest.

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