Original Research

Effects of moderate-continuous and high-intensity interval aerobic training on cardiac function of spontaneously hypertensive rats

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Impact Statement

Non-pharmacological interventions, namely moderate-intensity continuous (MICT) and high-intensity interval (HIIT) aerobic training, have been used to counteract the harmful effects of hypertension. Both have shown positive effects on the heart of spontaneously hypertensive rats (SHR) at the cellular level. However, measuring the contractile function of cardiomyocytes at physiological temperatures (~37°C) and stimulation rates closer to those replicating the heart rate at rest and during exercise is crucial. It is important to analyze the effects of exercise on cardiac function from the whole animal (i.e. *in vivo*) to the organ (i.e. heart) and cellular (i.e. intracellular calcium transient and cell contractility) levels. Here, MICT and HIIT had beneficial effects on the heart of SHR. Thus, these regimes should be tested in humans to verify their effectiveness and safety under clinically controlled conditions.

Abstract

The aim of this study was to verify the effects of moderate-intensity continuous (MICT) and high-intensity interval (HIIT) aerobic training on cardiac morphology and function and the mechanical properties of single cardiomyocytes in spontaneously hypertensive rats (SHR) in the compensated phase of hypertension. Sixteen-week-old male SHR and normotensive Wistar (WIS) rats were allocated to six groups of six animals each: SHR CONT or WIS CONT (control); SHR MICT or WIS MICT (underwent MICT, 30 min/day, five days per week for eight weeks); and SHR HIIT or WIS HIIT (underwent HIIT, 30 min/day, five days per week for eight weeks). Total exercise time until fatigue and maximum running speed were determined using a maximal running test before and after the experimental period. Systolic (SAP), diastolic (DAP), and mean (MAP) blood pressures were measured using tail plethysmography before and after the experimental period. Echocardiographic evaluations were performed at the end of the experimental period. The rats were euthanized after *in vivo* assessments, and left ventricular myocytes were isolated to evaluate global intracellular Ca^{2+} transient ($[Ca^{2+}]_i$) and contractile function. Cellular measurements were performed at basal temperature (~37°C) at 3, 5, and 7 Hz. The results showed that both training programs increased total exercise time until fatigue and, consequently, maximum running speed. In hypertensive rats, MICT decreased SAP, DAP, MAP, interventricular septal thickness during systole and diastole, and the contraction amplitude at 5 Hz. HIIT increased heart weight and left ventricular wall thickness during systole and diastole and reduced SAP, MAP, and the time to peak $[Ca^{2+}]_i$ at all pacing frequencies. In conclusion, both aerobic training protocols promoted beneficial adaptations to cardiac morphology, function, and mechanical properties of single cardiomyocytes in SHR.

Keywords: Cardiomyocytes, cardiac function, calcium transient, hypertension, spontaneously hypertensive rats, aerobic training

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Introduction

Systemic arterial hypertension is characterized by progressive, long-term detrimental adaptations that cause cardiac morphological and mechanical dysfunctions.[1–4] Hypertension is considered the main risk factor for the development of cardiovascular disorders that may lead to heart failure.[5]

The spontaneously hypertensive rat (SHR) is an experimental model widely used to study human hypertension because these animals show a progressive increase in blood pressure from the pre-hypertension state in the early life

period until sustained hypertension that lasts for the rest of their life.[6] Therefore, this model allows the analysis of relevant cardiovascular parameters.[7,8] Compensated cardiac hypertrophy in SHR can usually be detected in the third month of age, whereas overt heart failure is found around the 18th–24th month.[8] In the compensated phase of hypertension, functional and electrical changes such as increased shortening and action potential duration and changes in the intracellular Ca^{2+} transient ($[Ca^{2+}]_i$) are observed in left ventricle (LV) myocytes, culminating in prolonged cell contraction and relaxation time courses.[1,4,9,10]

Aerobic exercise training has been used as a non-pharmacological intervention to counteract the harmful effects of hypertension on the heart. Moderate intensity continuous aerobic training (MICT), mainly walking and/or running, with intensity ranging from 40 to 60% of the maximum oxygen consumption (VO_{2max}), has been the most indicated for hypertensive individuals. MICT causes an eccentric-like type of cardiac hypertrophy by increasing the ventricular chamber internal diameter and improving cardiac function without increasing the ventricular wall thickness.[11]

Studies have shown the effectiveness of high-intensity aerobic interval training (HIIT) against myocardial damage generated by hypertension.[12–14] HIIT leads to the parallel growth of sarcomeres and considerably increases the thickness of the left ventricular wall.[15] This is accompanied by modest enlargement of the ventricular chamber internal diameters, contributing to the maintenance of normal cardiac function.[11] The major concern related to HIIT is the increased risk of cardiovascular accidents, although Weston, Wisløff, and Coombs[14] showed in a meta-analysis that such risks are not greater than those existing when using MICT.

Cardiac structural and functional adaptations to exercise reflect cellular changes. Both MICT and HIIT have shown positive effects on the hearts of SHR at the cellular level. Notably, the efficiency of cardiomyocyte contractile function results in improved ejection fraction and fractional shortening and, hence, increased exercise capacity. For instance, MICT increases the amplitude and decreases the duration of the systolic and diastolic phases of the $[Ca^{2+}]_i$, as well as increases the contraction amplitude and relaxation velocity of myocytes isolated from the LV of SHR.[10] Krzesiak *et al*.[16] showed that HIIT increases the capacity of contraction and relaxation and decreases the $[Ca^{2+}]_i$ decay time of single LV myocytes. However, in this study, the LV myocytes were evaluated at room temperature at a stimulation rate of 1 Hz. Regarding exercise adaptations, it is essential to measure the contractile function of cardiomyocytes at physiological temperatures (i.e. ~37°C) as well as at stimulation rates closer to those replicating the heart rate at rest (i.e. ~5 Hz) and during aerobic exercise training (i.e. 7–9 Hz) of animals. Moreover, it is important to analyze the effects of MICT and HIIT on cardiac function from the whole animal (i.e. *in vivo*) to the organ (i.e. heart) and cellular (i.e. intracellular calcium transient and cell contractility) levels.

Therefore, the aim of this study was to verify the effects of MICT and HIIT on cardiac morphology and function and the mechanical properties of single cardiomyocytes in SHR in the compensated phase of hypertension.

Materials and methods

Experimental animals and treatments

Twenty-four male SHR (~270 g) and 24 male normotensive Wistar (WIS) rats (~360 g), aged 16 weeks, were housed in collective polycarbonate cages under 12–12 h light/dark cycles, with the light phase beginning at 6:00, in a temperature-controlled room (22 ± 2°C; humidity: 50 ± 10%), with free access to water and standard rodent chow. The rats were weighed weekly during the experimental period using a digital electronic scale (Mars, Brazil; model AS5500C).

Before the experimental procedures, the rats were randomly assigned to six groups of six animals each as follows: SHR CONT, hypertensive control rats; SHR MICT, hypertensive rats that underwent MICT; SHR HIIT, hypertensive rats that underwent HIIT; WIS CONT, normotensive control rats; WIS MICT, normotensive rats that underwent MICT; and WIS HIIT, normotensive rats that underwent HIIT, and the experimental period covered eight weeks.

The Ethics Committee in Animal Use of the Universidade Federal de Viçosa approved the experimental protocol (No. 98/2017) according to the Guide for the Care and Use of Laboratory Animals.

MICT and HIIT protocols and performance assessment

The MICT protocol, adapted from Carneiro-Júnior *et al*.,[10] was performed on a motor-driven treadmill at 60% of the maximum running speed, for 30 min/day and five days per week (Monday–Friday). The HIIT protocol, adapted from Lee *et al*.,[13] was performed on a treadmill with 20 repetitions of 30 s at 90% of the maximum running speed, followed by an active interval of 60 s at 45% of the maximum running speed, totaling 30 min for five days per week (Monday–Friday). The exercise protocols were performed in a softly lit room between 14:00 and 16:00.

Rats were adapted to the treadmill and had the maximum running speed and total exercise time until fatigue, as previously described.[10] Briefly, all rats were placed on the treadmill for adaptation (10 min/day, 0° inclination, 5 m/min for five days).[17] Adaptation was performed between 14:00 and 16:00. After 48 h, the test was carried out at 5 m/min and 0° inclination, with increments of 3 m/min every 3 min until each rat was fatigued. The test was repeated after four weeks of training to readjust the running speed and after eight weeks of training, 24 h after training sessions, to assess the final total exercise time until fatigue and maximum running speed.

Assessment of systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressure

SAP, DAP, and MAP of each rat were assessed before the beginning of the exercise training protocol and 36 h after the last exercise training session. The measurements were obtained using tail plethysmography (LE5001; Panlab®, Spain) without any anesthetic method, as previously described.[10]

First, the rats were adapted to the containment device and heat process for one week. Next, for SAP, DAP, and MAP measurements, each rat was placed in a containment device with constant aeration and heated to 29°C–32°C for 10 min to ensure vasodilation occurred. The pressure cuff and pulse transducers were placed approximately 2–3 cm from the tip of the tail, where the tissue had less muscle mass and greater pulse sensitivity. The gain was adjusted from the pulse transducer precision level to the proper level, and the cuff was activated and inflated until it occluded the blood flow through the tail artery, and the pulse signal disappeared. The cuff then began to deflate, and SAP, DAP, and MAP were determined from the moment of pulse signal re-uptake. During the evaluations, three SAP, DAP, and MAP measurements were performed for each rat, and an intermediate value was considered for registration. All measurements were performed in a softly lit room between 8:00 and 11:00.

Echocardiographic evaluation

Echocardiographic evaluation was performed 48 h after the last physical training session, as previously described by Lavorato *et al*.[18] For analysis, the rats were anesthetized with isoflurane. The examinations were performed between 8:00 and 11:00. The examination included two-dimensional studies with a frame rate of 120 fps and M-mode, using an ultrasound system (MyLabTM30, Esaote, Genoa, Italy) with an 11.0 MHz phased array transducer of nominal frequency. Two-dimensional and M-mode transthoracic echocardiograms were obtained with a sweep speed of 200 mm/s and adjusted according to the heart rate. The thickness of the anterior and posterior walls (end-diastole and end-systole) and the dimensions of the LV were measured using a modified method recommended by the American Society of Echocardiography for three consecutive cardiac cycles.[19] The M-mode records were analyzed offline by a blinded observer using the analysis system of the device. Each variable was measured in three different cardiac cycles, and the average measurement was used for statistical analysis.[20,21]

Isolation of cardiomyocytes

The rats were euthanized by decapitation after *in vivo* measurements, and LV cardiomyocytes were enzymatically isolated as previously described.[22,23] Briefly, hearts were removed and placed in a Langendorff system and perfused for approximately 5–7 min with a HEPES-Tyrode's solution (pH 7.3 at 37 °C) having the following composition (in mM): 130 NaCl, 1.43 $MgCl_2$, 5.4 KCl, 0.75 $CaCl_2$, 5.0 HEPES, 10.0 glucose, 20.0 taurine, and 10.0 creatine. Next, the solution was switched with a calcium-free solution containing EGTA (0.1 mM) for 6 min. Subsequently, the hearts were perfused for ~15 min with a solution of 1 mg/mL collagenase type II (Worthington, USA) and 0.2 mg/mL protease (Sigma-Aldrich, USA). Next, the LV was removed and cut into small pieces. Finally, the cells were dispersed by shaking the cardiac tissues in glass flasks at 37°C for 3 min. The cells were then centrifuged (30 *g*) for 20 s and resuspended in HEPES-Tyrode's solution. Only calcium-tolerant cardiomyocytes at rest and with clear streaks were recorded. Cells were used for 2–3 h.

$[Ca^{2+}]_i$ transient measurements

Measurements of the $[Ca^{2+}]_i$ transient of LV myocytes were performed as described by Kondo *et al*.,[24] using an inverted microscope (Nikon Eclipse TS100, USA) equipped with an objective oil immersion lens (S Fluor, 40×, Nikon, USA). Myocytes were electrically stimulated (MyoPacer, Field Stimulator; IonOptix, Milton, MA, USA) using a pair of platinum electrodes with a 0.2 ms supra-threshold pulse (20 V) at frequencies of 3, 5, and 7 Hz and a temperature of 37°C. The $[Ca^{2+}]_i$ transient properties were analyzed using the IonWizard software (IonWizard, 6.3; IonOptix). The parameters analyzed were the amplitude and the time to peak and to 50% decay of the $[Ca^{2+}]_i$ transient.

Cardiomyocyte contraction measurement

Single-cell contractions were assessed as previously described.[22,23] The contractions (3, 5, and 7 Hz) were evoked using platinum electrodes (MyoPacer, Field Stimulator), with voltage pulses of 5 ms in duration and intensity of 20 V. Cell shortening elicited by electric pulses was obtained using an edge detection system (IonWizard) and analyzed using the IonWizard software (IonWizard, 6.3). The contraction amplitude and the time to peak contraction and to 50% cell relaxation were evaluated.

Statistical analysis

Data distribution was assessed using the Shapiro–Wilk normality test. Comparisons of the variables between the six experimental groups were performed using the analysis of variance (ANOVA) factorial 2 (normotensive versus hypertensive) by 3 (control versus MICT versus HIIT), followed by Tukey's post hoc test. For temporal comparisons, the paired *t*-test (parametric data) or Wilcoxon test (non-parametric data) was used. Data are presented as mean ± standard error of the mean (SEM). The statistical program SigmaPlot v.12.0® was used, and $P < 0.05$ was considered significant.

Results

Hypertensive rats had significantly lower initial and final weights than normotensive rats ($P < 0.001$) (Table 1). HIIT significantly reduced the final weight of normotensive rats compared with that in the control. The SHR HIIT group showed significantly higher values of total heart weight than the SHR CONT, SHR MICT ($P < 0.01$), and WIS HIIT ($P < 0.001$) groups. Heart weight and LV weight to body weight ratios and LV weight were significantly higher in hypertensive rats than those in normotensive rats ($P < 0.001$). No effects of either MICT or HIIT were observed.

Hypertensive rats had significantly higher SAP values than normotensive ones ($P < 0.001$) (Table 2). The SHR HIIT group showed a significant reduction final SAP ($P < 0.05$) compared with its initial value. Furthermore, hypertensive rats showed significantly higher DAP values than normotensive rats ($P < 0.001$). The SHR MICT group showed a significant reduction in DAP ($P < 0.05$) compared with its initial value and its respective control. Hypertensive rats had significantly higher MAP values than normotensive ones

Table 1. Body weight, heart weight, left ventricle weight, and ratios.

	WIS CONT	WIS MICT	WIS HIIT	SHR CONT	SHR MICT	SHR HIIT
IBW (g)	357 ± 10	357 ± 10	359 ± 10	271 ± 11[a]	263 ± 10[b]	257 ± 12[c]
FBW (g)	446 ± 13*	409 ± 13*	399 ± 13[a]*	278 ± 14[a]	286 ± 13[b]*	307 ± 15[c]*
HW (g)	1.44 ± 0.1	1.42 ± 0.2	1.35 ± 0.1	1.51 ± 0.2	1.45 ± 0.2	1.70 ± 0.3[c,d,e]
HW/BW (mg/g)	3.26 ± 0.2	3.47 ± 0.2	3.42 ± 0.2	5.71 ± 0.3[a]	5.11 ± 0.2[b]	5.55 ± 0.3[c]
LVW (g)	1.24 ± 0.1	1.18 ± 0.1	1.12 ± 0.1	1.47 ± 0.2[a]	1.35 ± 0.2[b]	1.59 ± 0.2[c]
LVW/BW (mg/g)	2.78 ± 0.4	2.88 ± 0.4	2.81 ± 0.4	5.63 ± 0.4[a]	4.75 ± 0.4[b]	5.18 ± 0.4[c]

WIS CONT: normotensive control rats; WIS MICT: normotensive rats that performed MICT; WIS HIIT: normotensive rats that performed HIIT; SHR CONT: hypertensive control rats; SHR MICT: hypertensive rats that performed MICT; SHR HIIT: hypertensive rats that performed HIIT; IBW: initial body weight; FBW: final body weight; HW: heart weight; LVW: left ventricular weight.
Data are mean ± SEM of six animals in each group.
ANOVA ($P < 0.05$):
[a]versus WIS CONT.
[b]versus WIS MICT.
[c]versus WIS HIIT.
[d]versus SHR CONT.
[e]versus SHR MICT in the same row.
Paired *t* test ($P < 0.05$): *versus IBW in the same column. Wilcoxon test ($P < 0.05$): *versus IBW in the same column for SHR HIIT.

Table 2. Blood pressure, total exercise time until fatigue, and maximum running speed.

	WIS CONT	WIS MICT	WIS HIIT	SHR CONT	SHR MICT	SHR HIIT
Initial SAP (mmHg)	126 ± 5	138 ± 5	129 ± 5	207 ± 5[a]	211 ± 4[b]	215 ± 5[c]
Final SAP (mmHg)	125 ± 5	133 ± 5	137 ± 5	199 ± 5[a]	188 ± 5*[b]	191 ± 5*[c]
Initial DAP (mmHg)	76 ± 9	85 ± 9	86 ± 9	151 ± 9[a]	157 ± 8[b]	154 ± 9[c]
Final DAP (mmHg)	74 ± 7	87 ± 7	91 ± 7	147 ± 7*[a]	120 ± 7*[b,d]	139 ± 8[c]
Initial MAP (mmHg)	93 ± 7	103 ± 6	101 ± 6	167 ± 6[a]	173 ± 6[b]	174 ± 6[c]
Final MAP (mmHg)	90 ± 5	102 ± 5	107 ± 5	165 ± 5[a]	142 ± 5[b, d]	149 ± 5[c]
Initial TTF (min)	31 ± 1	28 ± 1[a]	25 ± 1[a,i]	32 ± 1	29 ± 1[d]	26 ± 1[d,e]
Final TTF (min)	27 ± 1*	39 ± 1*[a]	43 ± 1*[a,b]	30 ± 1	39 ± 1*[d]	43 ± 1*[d,e]
Initial MRS (m/min)	34.7 ± 1	31.6 ± 1[a]	28.3 ± 1[a,b]	35 ± 1	32.8 ± 1[d]	28.5 ± 1[d,e]
Final MRS (m/min)	31.3 ± 1*	41 ± 1*[a]	46.3 ± 1*[a,b]	34.1 ± 1	42.5 ± 1*[d]	47 ± 1*[d,e]

WIS CONT: normotensive control rats; WIS MICT: normotensive rats that performed MICT; WIS HIIT: normotensive rats that performed HIIT; SHR CONT: hypertensive control rats; SHR MICT: hypertensive rats that performed MICT; SHR HIIT: hypertensive rats that performed HIIT; SAP: Systolic arterial pressure; DAP: Diastolic arterial pressure; MAP: Mean arterial pressure; TTF: Total exercise time until fatigue: MRS: maximum running speed.
Data are mean ± SEM of six animals in each group.
ANOVA ($P < 0.05$):
[a]versus WIS CONT.
[b]versus WIS MICT.
[c]versus WIS HIIT.
[d]versus SHR CONT.
[e]versus SHR MICT in the same row.
Paired *t* test ($P < 0.05$): *versus initial parameters in the same column.

($P < 0.001$). The SHR MICT group exhibited a significant reduction in MAP compared with the control ($P < 0.05$) and tended to decrease in relation to its initial value ($P = 0.07$).

Both training protocols significantly increased the total time until fatigue and maximum running speed compared with the initial values and respective controls ($P < 0.001$). The WIS HIIT group had significantly higher total time until fatigue and maximum running speed values than the WIS MICT group after training ($P < 0.05$). In contrast, the WIS CONT group showed significantly decreased total time until fatigue ($P < 0.05$) compared with its initial value, and it tended to decrease the maximum running speed ($P = 0.09$).

Table 3 shows the results of the echocardiographic evaluation. The thicknesses of the interventricular septum during diastole (IVS-D) and systole (IVS-S) in the SHR CONT and SHR HIIT groups were significantly higher than those in the WIS CONT and WIS HIIT groups, respectively ($P < 0.05$). The SHR HIIT group had significantly higher IVS-S thickness than the SHR MICT group ($P < 0.05$). Furthermore, the SHR MICT group showed that the IVS-D and IVS-S thicknesses did not differ compared with the WIS MICT group. The SHR groups showed significantly higher values ($P < 0.001$) of LV wall thickness in diastole (LVFW-D) than the normotensive controls, and the SHR HIIT group showed a significantly higher LVFW-D value than the SHR CONT group ($P < 0.05$). For LV wall thickness in systole (LVFW-S), all SHR groups showed significantly higher values than their respective normotensive controls ($P < 0.001$). The LVFW-S values of the SHR HIIT group were significantly higher than those of the SHR CONT and SHR MICT groups ($P < 0.05$). No differences in ejection fraction or fractional shortening were identified between the groups.

Table 3. Echocardiographic parameters.

	WIS CONT	WIS MICT	WIS HIIT	SHR CONT	SHR MICT	SHR HIIT
IVS-D (mm)	1.71 ± 0.12	1.86 ± 0.12	1.60 ± 0.12	2.19 ± 0.12[a]	1.84 ± 0.12	1.98 ± 0.13[c]
IVS-S (mm)	2.04 ± 0.12	2.34 ± 0.11	2.11 ± 0.11	2.44 ± 0.12[a]	2.19 ± 0.11	2.60 ± 0.13[c,e]
LVDI-D (mm)	7.97 ± 0.28	7.79 ± 0.26	7.72 ± 0.26	7.27 ± 0.28	7.20 ± 0.26	8.15 ± 0.31
LVID-S (mm)	5.23 ± 0.27	4.82 ± 0.25	4.85 ± 0.25	5.10 ± 0.27	4.82 ± 0.25	5.48 ± 0.29
LVFW-D (mm)	1.60 ± 0.15	1.42 ± 0.14	1.52 ± 0.14	1.98 ± 0.15[a]	2.09 ± 0.14[b]	2.49 ± 0.16[c,d]
LVFW-S (mm)	2.30 ± 0.16	2.32 ± 0.15	2.34 ± 0.15	2.69 ± 0.16[a]	2.75 ± 0.15[b]	3.25 ± 0.18[c,d]
Ejection Fraction (%)	68.7 ± 2.9	73.4 ± 2.8	73.4 ± 2.8	63.0 ± 2.9	66.5 ± 2.8	66.5 ± 3.2
Fractional Shortening (%)	34.4 ± 2.2	38.0 ± 2.1	38.4 ± 2.1	30.1 ± 2.2	32.6 ± 2.1	32.7 ± 2.4

WIS MICT: normotensive rats that performed MICT; WIS HIIT: normotensive rats that performed HIIT; SHR CONT: hypertensive control rats; SHR MICT: hypertensive rats that performed MICT; SHR HIIT: hypertensive rats that performed HIIT; IVS-D: thickness of the interventricular septum in diastole; IVS-S: thickness of the interventricular septum in systole; LVID-D: left ventricular internal dimension in diastole; LVID-S: left ventricular internal dimension in systole; LVFW-D: left ventricular free wall dimension in diastole; LVFW-S: left ventricular free wall dimension in systole
Data are mean ± SEM of six animals in each group. WIS CONT, normotensive control rats.
ANOVA ($P < 0.05$):
[a]versus WIS CONT.
[b]versus WIS MICT.
[c]versus WIS HIIT.
[d]versus SHR CONT.
[e]versus SHR MICT in the same row.

The amplitude of the $[Ca^{2+}]_i$ transient (Figure 1(A)) was significantly higher in cardiomyocytes from hypertensive rats at all the stimulation frequencies ($P < 0.001$). When stimulated at 5 and 7 Hz, cardiomyocytes from the SHR HIIT group showed values similar to those of the WIS HIIT group. Concerning the time to peak $[Ca^{2+}]_i$ transient (Figure 1(B)), cardiomyocytes from hypertensive rats showed significantly higher values than those from normotensive rats at all stimulation frequencies ($P < 0.001$). Furthermore, HIIT significantly reduced these values at all frequencies ($P < 0.05$). The time to 50% decay of the $[Ca^{2+}]_i$ transient (Figure 1(C)) was significantly longer in cardiomyocytes from the SHR CONT group at 5 Hz stimulation ($P < 0.05$) compared with that from the WIS CONT group. No effect of the MICT and HIIT protocols on this parameter was found.

The amplitude of shortening (Figure 2(A)) was significantly higher in cardiomyocytes from hypertensive rats than that from the WIS CON and WIS MICT groups at 5 Hz ($P < 0.01$). The SHR MICT group showed a significantly lower ($P < 0.01$) amplitude of cardiomyocyte shortening than the SHR CONT group at 5 Hz. However, the WIS MICT and HIIT groups showed significantly higher amplitudes of cardiomyocyte shortening than their controls ($P < 0.05$). Cardiomyocytes from the WIS MICT group had significantly high amplitudes of shortening ($P < 0.05$) at 5 and 7 Hz. Regarding the time to peak shortening (Figure 2(B)), cardiomyocytes from hypertensive rats showed significantly higher values than those from normotensive ones at 3 Hz ($P < 0.001$). Furthermore, cardiomyocytes from the WIS MICT group showed a significantly lower time to peak shortening than those of the WIS CONT group ($P < 0.05$). When stimulated at 5 and 7 Hz, there were no significant differences in this parameter between the groups. The time to 50% relaxation (Figure 2(C)) was significantly longer in cardiomyocytes from hypertensive rats compared with those of normotensive ones at 3 Hz ($P < 0.001$) and 7 Hz ($P < 0.01$). The values of time to 50% relaxation of cardiomyocytes from the SHR MICT group were similar to those of the WIS MICT group when stimulated at 7 Hz. When stimulated at 3 Hz, the cardiomyocytes from the WIS MICT group showed a significantly lower time to 50% relaxation compared with those from the control ($P < 0.001$), and myocytes from the WIS HIIT group had reduced values of time to 50% relaxation compared with their control ($P = 0.09$).

Discussion

This study reveals for the first time the effects of MICT and HIIT on cardiac morphology and function, and the mechanical properties of single cardiomyocytes in SHR, under conditions like those *in vivo* (i.e. physiological stimulation rates and temperature). Furthermore, we demonstrated that both training protocols promoted beneficial adaptations to heart morphology, function, and mechanical properties of single cardiomyocytes in SHR.

After the intervention period, the trained rats had increased exercise capacity. Hypertensive rats that underwent MICT showed lower DAP and MAP values and tended to decrease SAP. These results may be related to a possible increase in nitric oxide production and a decrease in endothelin-1 expression and sympathetic activity.[24–30] HIIT decreased SAP and tended to decrease MAP values. It has been reported that HIIT leads to better control of sympathetic activity and greater vagal tone, thus reducing heart rate.[14,31]

We observed that hypertension increased IVS-D and IVS-S thicknesses as well as LVFW-D and LVFW-S values in the LV. Hypertension is known to increase afterload, and in the compensated stage, this adaptation occurs to maintain normal cardiac function. In this case, the main change is increased cross-sectional area of cardiomyocytes, an adaptive response to the great pressure overload, and increased extracellular collagen matrix and sympathetic activity.[32,33] More importantly, our MICT protocol reduced IVS-D and IVS-S values. It is well documented that continuous aerobic exercise of light to moderate intensity promotes physiological adaptations in

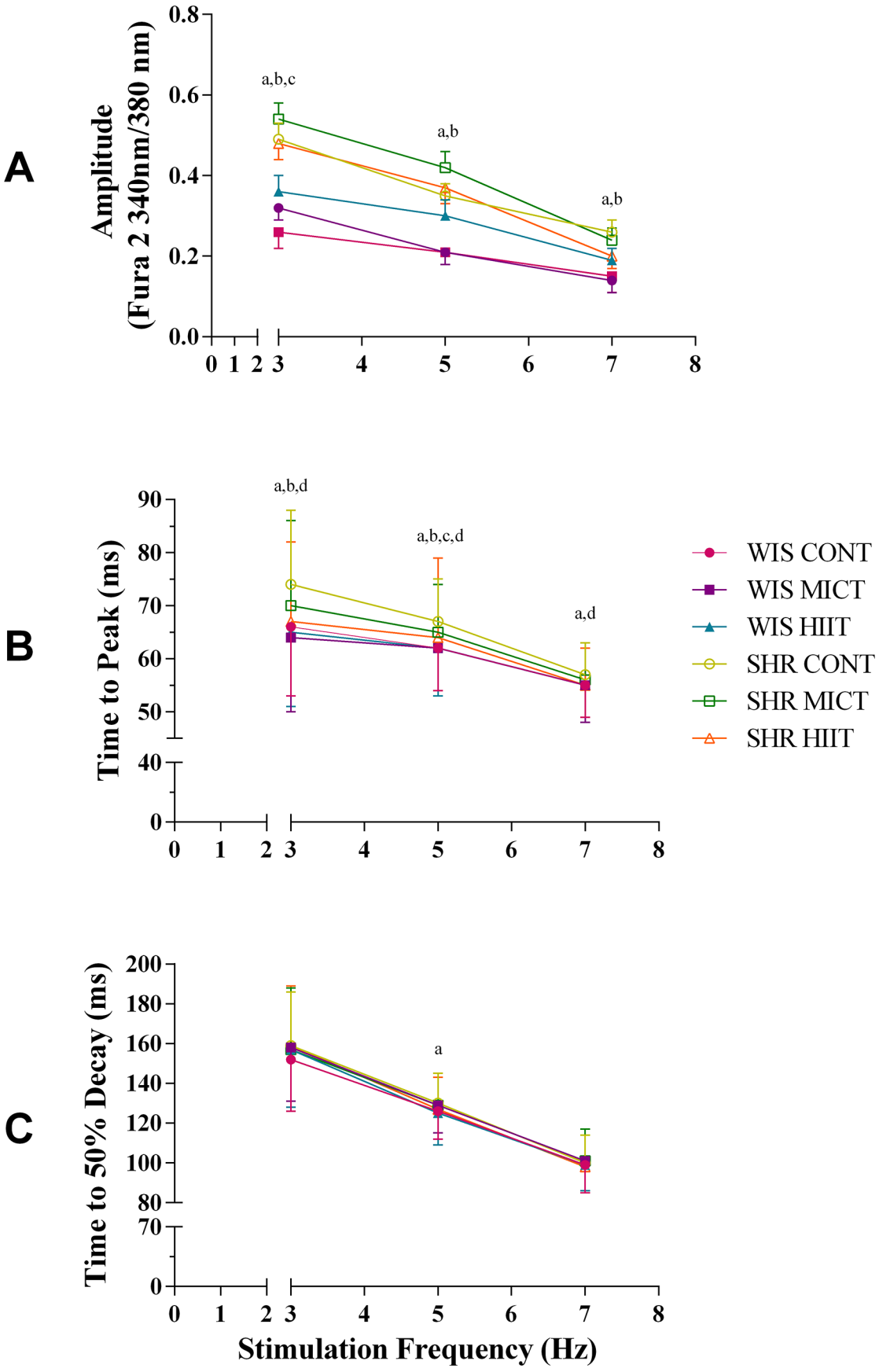

Figure 1. Intracellular calcium transient in single left ventricular myocytes. (A) Amplitude. (B) Time to peak (ms). (C) Time to 50% decay (ms). Data are mean ± SEM of six animals in each group. WIS CONT, normotensive control rats. WIS MICT, normotensive rats that performed MICT. WIS HIIT, normotensive rats that performed HIIT. SHR CONT, hypertensive control rats. SHR MICT, hypertensive rats that performed MICT. SHR HIIT, hypertensive rats that performed HIIT. ANOVA ($P < 0.05$): [a]versus WIS CONT; [b]versus WIS MICT; [c]versus WIS HIIT; [d]versus SHR CONT. (A color version of this figure is available in the online journal.)

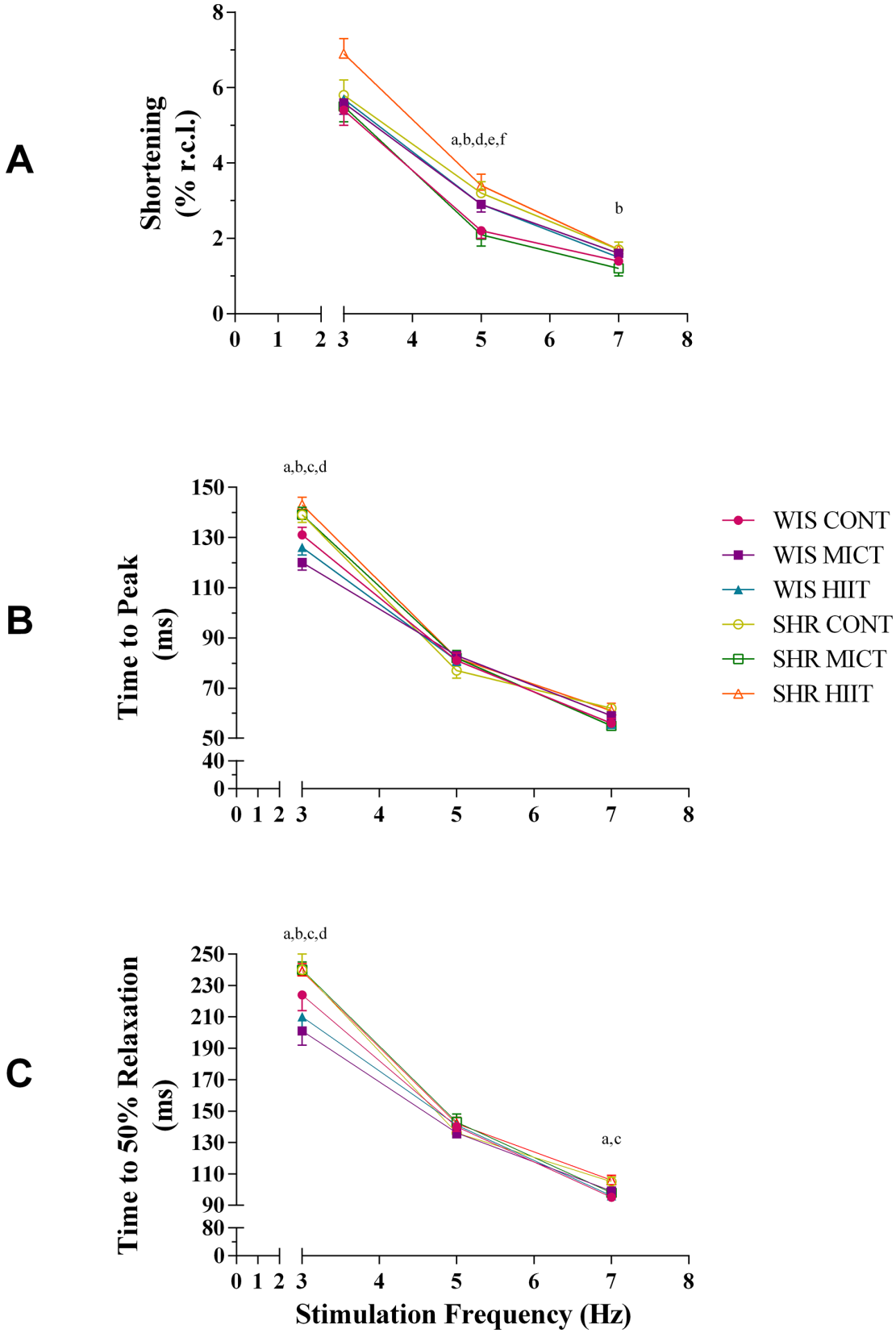

Figure 2. Contractility in single left ventricular myocytes. (A) Shortening (% r.c.l.: percentage of resting cell length). (B) Time to peak (ms). (C) Time to 50% relaxation (ms). Data are mean ± SEM of 6 animals in each group. WIS CONT, normotensive control rats. WIS MICT, normotensive rats that performed MICT. WIS HIIT, normotensive rats that performed HIIT. SHR CONT, hypertensive control rats. SHR MICT, hypertensive rats that performed MICT. SHR HIIT, hypertensive rats that performed HIIT. ANOVA ($P < 0.05$): [a]SHR CONT versus WIS CONT; [b]SHR MICT versus WIS MICT; [c]SHR HIIT versus WIS HIIT; [d]WIS CONT versus WIS MICT; [e]SHR MICT versus SHR CONT and SHR HIIT; [f]WIS CON versus WIS HIIT. (A color version of this figure is available in the online journal.)

cardiac morphology.[4,11,34,35] Eccentric physiological hypertrophy is caused by an increase in blood volume that reaches the heart due to increased venous return provided by skeletal muscle contraction, thus increasing the preload. These factors increase the number of sarcomeres in series, enlarging the cardiac chamber without a large increase in myocardial mass.

Regarding the HIIT effects, we observed a significant increase in LVFW-D and LVFW-S values in hypertensive rats. The stimulus generated by HIIT differs from that generated by MICT because HIIT is characterized by short- and high-intensity stimuli interspersed with moments of low intensity. This type of stimulus causes cardiac adaptations closer to those in response to resistance exercise training (i.e. concentric hypertrophy). Such cardiac hypertrophy is a result of increased intraventricular pressure during the systolic phase (i.e. afterload). This increase generates a great parietal tension, which causes an increase in sarcomeres in parallel, resulting in increased myocardial thickness without changing the internal dimensions of the chamber.[11,36]

LV function did not differ in hypertensive rats and was not altered by MICT or HIIT. This can be explained by the fact that the rats used in this study were in the compensated phase of hypertension, where there is cardiac remodeling to counteract the pressure overload, although it does not significantly affect heart function. As the rats were in this phase, the levels of ejection and shortening fractions were not affected. Furthermore, there was no increase in the volume of blood ejection as MICT and HIIT did not generate an increase in the ventricular chamber.[10,37]

At the cellular level, our results showed that hypertension increased the amplitude of the $[Ca^{2+}]_i$ transient in LV myocytes, and HIIT attenuated this value to the levels of cardiomyocytes from normotensive rats. This result was found only at a stimulation frequency of 3 Hz, which reflects cellular behavior at rest. Thus, it is reasonable that HIIT promoted chronic adaptations to cardiomyocytes from the SHR HIIT group.[38] The results reported by Krzesiak *et al*.[16] were divergent as HIIT increased the amplitude of the $[Ca^{2+}]_i$ transient in LV myocytes from hypertensive animals. The HIIT effect observed in this study may be explained by the positive cardiac adaptations caused by HIIT, such as a decrease in pressure overload and, consequently, a decrease in cell overload.

Our results showed that hypertension significantly increased the time to the peak $(Ca^{2+})_i$ transient in single LV myocytes. This phenomenon has been demonstrated previously.[10,16] Hypertension promotes deleterious effects on the cardiac structure and function of T-tubules that may slow calcium entry into the cells and, hence, increase the time to the $[Ca^{2+}]_i$ transient peak.[39] As demonstrated by Krzesiak *et al*.,[16] we showed that the applied HIIT program decreased the time to the peak $[Ca^{2+}]_i$ transient. Although not measured in this study, which we consider a limitation, HIIT has been reported to reorganize the T-tubules structurally and recover their functionality.[16] Another possibility is that HIIT augments the expression of type 2 ryanodine receptors (RYR2), which would accelerate calcium release from the sarcoplasmic reticulum.[4,10,16,40] These mechanisms should be investigated in future studies.

We also observed that the amplitude of LV myocyte contraction in hypertensive rats was higher than that in normotensive ones at 5 Hz. This result differs from those of other studies,[4,10,16] in which hypertensive rats showed lower values than normotensive ones. Such divergences may be due to the difference in cardiomyocyte electrical stimulation frequency and the temperature of the experiments because in some studies, myocytes were stimulated at 1 Hz at room temperature. Moreover, as the rats in this study were in the compensated phase of hypertension, the heart worked harder to maintain its function, thus generating a positive compensatory response.[8,41]

In this study, cardiomyocytes of hypertensive rats showed a longer time to peak contraction when stimulated at 3 Hz. This finding is in line with those reported previously.[4,9,10,16] Hypertension affects the cell membrane, both morphologically and functionally, generating modifications specifically in the T-tubules. This means that the L-type calcium channels are unaligned with the RYR2, which reduces the subspace, reducing Ca^{2+} entry into the intracellular environment and its interaction with RYR2.[4,16]

Left ventricular myocytes from hypertensive rats showed a prolonged time to 50% cell relaxation when stimulated at 3 and 7 Hz. However, myocytes from the SHR MICT group showed the time to 50% relaxation like that of myocytes from the WIS MICT group, at 7 Hz. It has been demonstrated that MICT can increase the expression of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) and Na^+/Ca^{2+} exchangers (NCX) in LV myocytes, which facilitates the re-uptake of Ca^{2+} into the sarcoplasmic reticulum and extrusion of Ca^{2+} through the cell membrane.[10] Although there was no significant difference in time to 50% decay of the $[Ca^{2+}]_i$ transient between the SHR MICT and WIS MICT groups, it is worth noting that the amplitude of the $[Ca^{2+}]_i$ transient was higher in hypertensive animals, and there may be more $[Ca^{2+}]_i$ and a greater uptake by SERCA2a.[10]

However, this study has some limitations. First, echocardiography was performed at the end of the experiment. Despite this, the control groups allowed us to compare the parameters of cardiac function between groups to verify the effects of hypertension and exercise training. Second, we were unable to measure the involvement of either the sympathetic nervous system (i.e. Nitric oxide and Endotelin-1) or calcium regulatory proteins (i.e. RYR2, SERCA2a, PLB, and NCX) to elucidate the underlying molecular mechanisms, which warrant further experiments.

Conclusions

In summary, both types of aerobic exercise training promoted benefits to the heart of hypertensive rats. MICT increased physical performance and decreased DAP, MAP, thicknesses of IVS-D and IVS-S, and contraction amplitude at 5 Hz. Furthermore, HIIT increased physical performance and LVFW-D and LVFW-S values as well as decreased SAP and the time to peak $[Ca^{2+}]_i$ transient at all stimulation frequencies.

Our findings suggest that these training regimes should be tested in humans to verify their effectiveness and safety under clinically controlled conditions. Therefore, adjuvant

therapeutic strategies based on MICT and HIIT are clinically relevant in the prevention and treatment of hypertension-induced cardiomyopathy.

AUTHORS' CONTRIBUTIONS

Authors PZS, AJN and MACJ have given substantial contributions to the conception or the design of the manuscript, authors PZS, MACJ, LMTR, LLS, FRD, LCCC and ECCR to acquisition, analysis, and interpretation of the data. All authors have participated to drafting the manuscript, authors JGM and VNL revised it critically. All authors read and approved the final version of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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