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Swim training attenuates the adverse remodeling of LV structural and mechanical properties in the early compensated phase of hypertension☆

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ABSTRACT

Aim: Investigate to what extent low-intensity swim training for six weeks counterbalances the adverse remodeling due to the advance of pathological hypertrophy in the left ventricle (LV) structural and mechanical properties in the early compensated phase of hypertension in male SHR.

Main methods: Four-month-old male SHR and Wistar rats were randomly divided into Sed (sedentary) and Ex (exercised) groups. The exercised rats were submitted to a swimming protocol (1 h/day, 5 times/week, no additional load) for six weeks. LV tissue and isolated myocytes were used to assess structural and mechanical properties. Myocytes were stimulted at frequencies (F) of 1 and 3 Hz at 37 °C.

Key findings: Exercised SHR showed improvement in cardiovascular parameters compared to sedentary SHR (mean arterial pressure: 13.22%; resting HR: 14.28.%). About structural and mechanical properties, swim training induced a decrease in LV myocyte thickness (10.85%), number of inflammatory cells (21.24%); collagen type III (74.23%) and type I (85.6%) fiber areas; amplitude of single myocyte shortening (47% to F1 and 28.46% to F3), timecourses of shortening (16.5% to F1 and 7.55% to F3) and relaxation (15.31% to F3) compared to sedentary SHR.

Significance: Six weeks of swim training attenuates the adverse remodeling of LV structural and mechanical properties in the early compensated phase of hypertension in male SHR.

1. Introduction

The Spontaneously Hypertensive Rats (SHR) strain is used as a model for studying human hypertension [1,2]. In such model, pressure overload is progressive being hypertension evidenced as soon as the rats reach two months of age [3]. While blood pressure peaks at four months, expressional changes, active cardiac hypertrophic growth and enhanced function is observed until about six months of age [3]. Such compensatory adaptations stabilizes and is maintained until the age of 12 to 18 months when the transition phase to heart failure takes place (18 to 24 months), and impaired myocardial performance is evident [3–5].

Parallel to the progression of hypertrophy in response to pressure overload, left ventricle (LV) tissue of SHR faces adverse remodeling. Such phenomenon is characterized by a combination of myocyte hypertrophy and nonmyocyte cell proliferation with increased deposition of collagen, resulting in increased fibrosis disproportionate to myocyte hypertrophy, and hence myocardial stiffness [6–10]. This framework has been observed early in the compensated phase of hypertension (i.e. at the age of 3.5 months) in this model, although cardiac function is preserved [6]. In addition, increases in pro-inflammatory markers (e.g. IL-1β; IL-6; TNFα; ICAM-1; iNOS; PPARγ) have also been found in the LV tissue of 5.5-month-old SHR [11–13].

Aerobic exercise training has been proved efficient to counteract the progression of such designed pathological evolution early in the compensated phase of hypertension in SHR. For example, moderate-intensity aerobic exercise training attenuated the systolic dysfunction by restoring the systolic left ventricular elastance female SHR [10]. In

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addition, male SHR rats exhibited enhanced cardiac pro-survival (i.e. reduced pro-apoptotic) pathways in response to moderate- to vigorous-intensity aerobic exercise training [13,14]. Moreover, low-intensity aerobic exercise training improved LV myocyte contractile function, and normalized the expression of excitation-contraction-coupling regulatory proteins in male SHR [9,15,16]. Nevertheless, little attention has been given to the progression of cardiac inflammation in the compensated phase of hypertension in SHR. Recently, it was reported that low-intensity aerobic exercise training increased the expression of proteins related to cardiac myocyte force generation, as well as those related to cardioprotection (i.e. anti-oxidative stress and anti-inflammation) in hearts of male SHR [13,17].

Therefore, given that inflammation is an important factor contributing to heart failure in the end stage of hypertension, we thought to investigate to what extent a low-intensity swim training for six weeks would counterbalance the adverse remodeling due to the advance of pathological hypertrophy in the LV structural (i.e. extracellular matrix) and mechanical (i.e. myocyte contractility) properties in the early compensated phase of hypertension by assessing collagen deposition, inflammation and single myocyte contractile function in male SHR.

2. Methods

2.1. Animals

Four-month old male SHRs and Wistar rats were kept in polyethylene boxes with 4 rats per box in a room (12-h light/dark cycle) with temperature of 22 °C. The animals from each strain were randomly divided into experimental groups of 6 animals each ($n = 6$): Wistar sedentary (Wistar Sed), Wistar exercised (Wistar Ex), SHR sedentary (SHR Sed) and SHR exercised (SHR Ex). All animals had free access to commercial chow and water ad libitum. The animals were weighed prior to the beginning of the protocol and at the end of the last training session. This study was conducted in accordance with the ethical principles in animal research adopted by the EU Directive 2010/63/EU for animal experiments. The animal care and protocols in this study were reviewed and approved by the Ethical Committee of the University of Ouro Preto (protocol number 2012/54).

2.2. Swim training

Animals from Wistar Ex and SHR Ex groups were submitted to a swimming protocol for six weeks as previously described [18]. Briefly, on the first day, the animals swam without load for 20 min; on the second day for 40 min and from the third day for 60 min, 5 times per week (Monday to Friday). The animals swam individually and without human intervention in warm water (30 ± 2 °C). The animals from Wistar Sed and SHR Sed groups were placed in recipients with shallow water (5 cm), 5 times per week (Monday to Friday) for six weeks.

2.3. Determination of cardiovascular parameters in anesthetized rats

Two days after the last exercise session, the animals were anesthetized with ketamine and xylazine (50 mg/kg and 10 mg/kg, ip, respectively) to obtain a direct arterial pressure (AP) signal. The catheter inserted to the femoral artery was coupled to a TruWave® pressure transducer (Edwards Lifescience, Canada) for continuous acquisition of AP signal. To measure the difference of potential relative to lead II of electrocardiogram (ECG), stainless steel hypodermic needles were inserted into the subcutaneous tissue of the animals. AP and ECG records were obtained during 2 min. The signals of ECG and AP were simultaneously sampled at a rate of 1200 cps per channel, with an A/D conversion board of 16 bits resolution (DaqBoard/2001, USA). The digital records of the experiments were converted by Matlab 7.0 software (MathWorks, USA) and the signals were analyzed by visual inspection of the record with support of WinDaq/EX Playback and Analysis software (DATAQ Instruments, USA). Three segments of 2 s were used for subsequent evaluation of cardiovascular parameters. The ECG parameters used were QT interval and QTc index [QTc interval corrected by Fridericia index [19]: QTc = QT / (RR)1/3)], recommended for RR intervals of < 500 ms were extracted from the ECG.

2.4. Record of arterial pressure and heart rate in awake rats

The assessment of AP and heart rate (HR) in awake rats was performed 24 h after ECG and AP records described above from femoral artery. The cannula was directed by subcutaneous tissue and externalized on the animal's back. After this procedure the rats were kept in individual cages, with water and ad libitum feed. Heparinized arterial cannula was coupled to a computerized data acquisition system (Powerlab). A Gould pressure transducer connected to an amplifier (PM-1000, CWE) monitored AP. The pulsatile arterial pressure (PAP) signal was derived for a cardiotachometer (PM-1000, CWE) to obtain HR. PAP and HR were continuously sampled by a 12-bit analog/digital conversion system (Powerlab 4/20) at a 800 Hz and stored on a hard disk. Subsequently, the software (Powerlab 4/20) was used to process the signals and obtain mean arterial pressure (MAP) and HR.

2.5. Structural analysis

One day after the in vivo measurements, the animal was euthanized by cervical dislocation under resting conditions and the heart was quickly removed. Fragments of the left ventricular wall not exceeding 1 cm in thickness were dissected and fixed in 80% of methanol solution and 20% of dimethylsulfoxide (DMSO) [20] and stored in freezer (− 20 °C) until the processing. Paraffin sections of approximately 4 μm thickness were obtained by microtomy techniques and fixed on glass slides. The slides obtained were stained byh Hematoxylin and Eosin (HE) or Picrosirius Red techniques to evaluate the main structural alterations.

Quantitative evaluations were performed using digital morphometric techniques. All tests were performed in a single slide of tissue sections from each animal. Ten random images of the cardiac tissue were obtained [21] with a 10× (1 pixel = 0.912 μm) and a 40× (1 pixel = 0.228 μm) eye lens objective on the Leica DM5000 microscope coupled to the digital camera (Leica DFC 300 FX, C-mount 0.7, 1.4 Megapixel, 1392 × 1040 resolution). All images were captured and analyzed using the image processing and analysis softwares Leica Application Suite v3.6 and Leica Qwin V3, respectively, at Multiuser Laboratory of Núcleo de Pesquisas em Ciências Biológicas of UFOP. In order to evaluate the cardiac inflammatory status, a binary image was created, and the nuclei of all cell types presented in the cardiac tissue were counted. The result obtained in the Wistar Sed group was considered the expected cellular pattern of the cardiac tissue without inflammatory infiltration and the difference ($P < 0.05$) between the number of the cell types nuclei normally present in the cardiac tissue and nuclei of leukocytes recruited in the inflammatory process were counted, allowing a quantitative assessment of inflammation present in the other experimental groups [22]. The thickness of the myocytes was determined through interactive measurements by drawing a line connecting the upper and lower cell borders in the nucleus region [18]. Only myocytes with visible nuclei and cell boundaries were included. One hundred cardiomyocytes were analyzed in each animal.

For the quantitative analysis of collagen fibers (types I and III), the coloring technique of Picrosirius Red [23] was used and the counting of the fibers under a polarized light optic microscope was performed by digital birefringence analysis. Twenty random images of cardiac tissue with a 20× (1 pixel = 0.456 μm) objective lens were obtained on the Leica DM5000 microscope, coupled to the digital camera (Leica DFC 300 FX, C-mount 0.7, 1.4 Megapixel, 1392 × 1040 resolution). The polarized light allowed to distinguish three colors: the green coloration, characteristic of thin collagen fibers, reticular fibers type III and the

Table 1
Weights, heart rate, mean arterial pressure and QTc Index.

	Wistar Sed	Wistar Ex	SHR Sed	SHR Ex
Initial BW (g)	330.5 ± 7.29	336.1 ± 8.64	286.3 ± 4.98$^{*\#}$	284.1 ± 3.14$^{*\#}$
Final BW (g)	382.7 ± 6.71^{+}	363.5 ± 8.38^{+}	314.2 ± 4.2$^{*\#+}$	303.4 ± 2.89$^{*\#+}$
HW (g)	1.17 ± 0.03	1.18 ± 0.02	1.32 ± 0.04$^{*\#}$	1.39 ± 0.03$^{*\#}$
HW/FBW (mg/g)	3.17 ± 0.13	3.38 ± 0.06	4.34 ± 0.12$^{*\#}$	4.58 ± 0.13$^{*\#}$
HR (bpm)	356 ± 1	324 ± 3*	448 ± 10*	384 ± 3$^{\#\infty}$
MAP (mm Hg)	124 ± 1	112 ± 1	189 ± 4*	164 ± 2$^{\#\infty}$
QTc Index (ms)	125.2 ± 5.39	88.22 ± 3.59*	110.9 ± 3.61*	92.89 ± 3.12$^{\infty}$

Data expressed as mean ± SEM of 6 animals per group. IBW, initial body weight. FBW, final body weight. HW, heart weight. HR, heart rate. MAP, mean arterial pressure.
* Statistically different from Wistar Sed.
$^{\#}$ Statistically different from Wistar Ex.
$^{\infty}$ Statistically different from to SHR Sed.
$^{+}$ Statistically different from the initial value within the same group.

spectrum of yellow to full color, indicating dense collagen type I fibers. All pixels of the collagen type I or III were selected to obtain another binary image to calculate the area in μm^2 [22]. All techniques used the Leica Application Suite software to capture the images. The Leica Qwin V.3.2.1 software (Leica Switzerland) was used to analyze the images.

2.6. Cardiomyocyte isolation

One day after the in vivo measurements, after being weighed, the animal was euthanized by cervical dislocation. The heart was rapidly excised and extraneous tissue was dissected away. The heart was flushed with a modified Hepes-Tyrode solution of the following composition (in mM): 130 Na^+, 5.4 K^+, 1.4 Mg^{2+}, 140 Cl^-, 0.75 Ca^{2+}, 5 Hepes, 10 glucose, 20 taurine and 10 creatine, pH 7.4, at room temperature. The heart was then blotted dry and weighed before being mounted on a Langendorff perfusion apparatus. Left ventricular myocytes were isolated as previously described [24]. Briefly, the heart was perfused with the Hepes-Tyrode solution in a constant stream until the coronary vessels were clean. Soon after, the perfusion was changed to the calcium-free solution containing 0.1 mM tetraethylene glycol acid (EGTA) for 4 to 6 min. The perfusion was then changed to a solution containing 1 mg/ml collagenase type II (Worthington, USA) and 0.1 mg/ml protease (Sigma) for 10–15 min. After perfusion, the left ventricle was separated, weighed and cut into small pieces. The samples were placed in a flask containing 5 ml of enzymatic solution (collagenase and protease). The flasks were shaken for 5 min in a water bath at 37 °C. Next, the contents of the flasks were filtered and centrifuged. The supernatant was removed and the cardiomyocytes suspended in the 750 mM $CaCl_2$ solution. The agitation and filtration procedures were repeated. The isolated myocytes were stored in Petri plates in refrigerator (5 °C) and used within 2–3 h of isolation. All the solutions were oxygenated (O_2 100%) and maintained at a temperature of 37 °C during the isolation process.

2.7. Measurements of cell contractility

Cell contractility was measured as previously described [15]. Briefly, myocytes were placed in a chamber on the stage of an inverted microscope (Nikon Eclipse — TS100, USA) and bathed with the Hepes–Tyrode solution containing (in mM) 137 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 0.5 $MgCl_2$, 5 HEPES, 5.6 glucose, and 1.8 $CaCl_2$, (pH 7.4) with 5 N NaOH. Individual myocytes were selected for study if they had a clear, regular striated (sarcomere) pattern, did not spontaneously contract in the absence of external stimulation, and responded to 1-Hz stimulation with a single twitch at ~37 °C. Steady-state 1 and 3 Hz contractions were elicited via platinum bath electrodes (Myopacer, Field Stimulator, Ionoptix, USA) with 5 ms duration voltage pulses and an intensity of 20 V. Cells were visualized on a monitor with a NTSC camera (Myocam, Ionoptix, USA) in partial scanning mode and was sampled at 240 Hz. This image was used to measure cell shortening in response to electrical stimulation using a video motion edge detector (IonWizard, Ionoptix, USA). Cell shortening was calculated from the output of the edge detector using an IonWizard A/D converter (Ionoptix, Milton, MA, USA). Cell shortening (expressed as a percentage of resting cell length), time to the peak of contraction and time to 50% of the relaxation were calculated.

2.8. Statistical analysis

Two-way ANOVA with post-hoc testing by Bonferroni were used to compare the effects of training and hypertension. For comparisons between the means of initial and final body mass, the paired Student's *t*-test was used. All analyzes were performed by GraphPad Prism version 5.0 software. A statistical significance level of 5% was adopted.

3. Results

3.1. General characteristics, cardiovascular parameters and QTc index

All animals increased the body weight by the end of the protocol. SHR presented lower body weight at the end of 6th week compared to that of Wistar group (Table 1).

There was main effect for both exercise and hypertension factors for HR in awake rats ($P < 0.05$). In addition, interaction between the factors for the HR data was found ($P < 0.05$). We observed a reduction in HR values from Wistar Ex group compared to those from Wistar Sed. Likewise, the SHR Ex group presented lower values of HR compared to SHR group Sed. In contrast, even after exercise, the SHR Ex group exhibited values higher HR than Wistar Ex group.

There was interaction between factors exercise and hypertension ($P < 0.05$) for MAP in awake rats. SHR Sed animals presented higher MAP values compared to Wistar Sed rats. At the end of 6 weeks of swimming protocol, the SHR Ex group showed lower MAP compared to that of SHR Sed group.

There was no interaction between factors hypertension and exercise for QT values ($P > 0.05$). There was main effect of exercise for QTc values ($P < 0.05$). In addition, there was interaction between the factors ($P < 0.05$). The exercised groups (Wistar Ex and SHR Ex) had significantly lower values for the QTc index compared to their respective controls.

3.2. Structural analysis of cardiac tissue

Fig. 1 shows histological sections of the left ventricular tissue. Tissue from Wistar Sed (a) shows appearance compatible with normality with absence of the inflammatory process and myocytes of thickness varying between 7 and 11 μm. In the Wistar Ex tissue (b), there is a diffuse inflammatory process (arrow) and mild degree of hypertrophy (arrowhead). In SHR Sed tissue (c) there is a moderate multifocal inflammatory process (arrow) and a moderate degree of

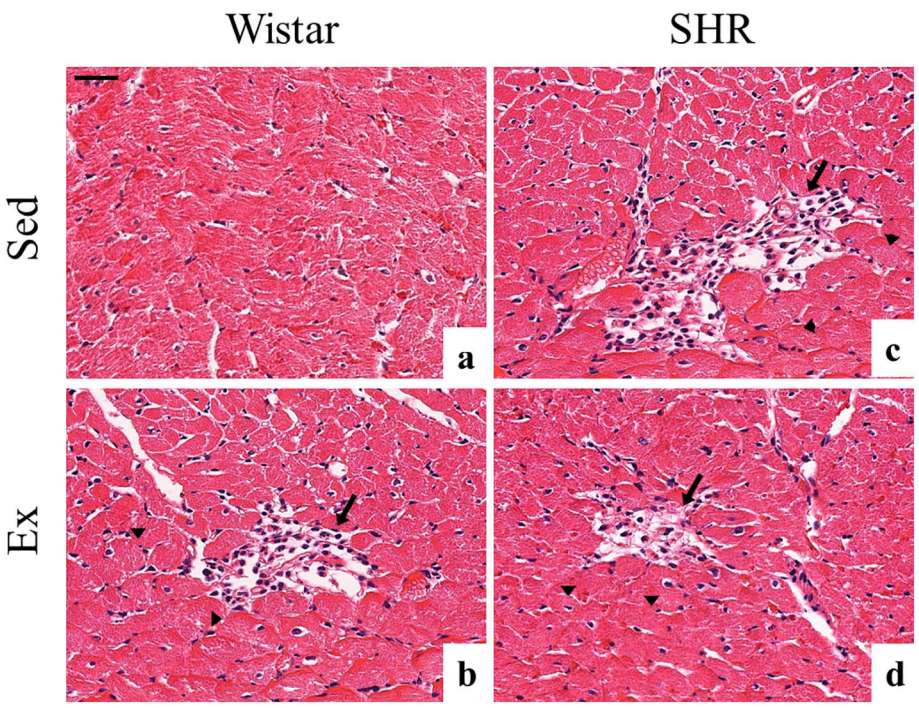

Fig. 1. Representative photomicrographs of left ventricular tissue exhibiting inflammatory cells (arrow) and myocytes (arrowhead) (a): Wistar Sed; (b) Wistar Ex; (c) SHR Sed; (d) SHR Ex. Hematoxylin-Eosin staining. Bar = 50 μm.

hypertrophy (arrowhead). In SHR Ex (d) is observed a discrete multifocal inflammatory process (arrow), with mild hypertrophy (arrowhead).

The mean data for left ventricular tissue is presented in Fig. 2. There was main factor effect of hypertension on the number of inflammatory cells ($P < 0.05$) as it was augmented in SHR animals. Interaction between the factors was found for both inflammatory cell number and myocyte thickness ($P < 0.05$). Animals from Wistar Ex group showed a higher number of inflammatory cells compared to Wistar Sed group (Fig. 2A). Moreover, SHR Sed group exhibited a higher number of inflammatory cells than Wistar Sed group (Fig. 2A). In contrast, the SHR Ex group presented a lower number of inflammatory cells than that of the SHR Sed group (Fig. 2A). The inflammatory infiltrate observed in the trained groups is not chronic, with predominance of mononuclear cells, mainly macrophages.

The myocyte thickness was augmented in Wistar Ex and SHR Sed compared to Wistar Sed group. In contrast, the myocyte thickness was diminished in SHR Ex group, compared to that of SHR Sed (Fig. 2B).

Fig. 3 shows photomicrographs of left ventricular tissue presenting collagen types I and III. In Wistar Sed (a) reticular fibers (type III collagen, arrow) are present. In Wistar Ex (b) there is histological aspect compatible with the normality and absence of collagen deposition. In SHR Sed (c), collagen deposition with expressive increase of reticular fibers (type III collagen, arrow) and type I collagen fibers (arrowhead) are observed. In SHR Ex (d), there is histological aspect compatible with normality, demonstrating absence of collagen deposition.

The mean data for left ventricular collagen fibers are presented in Fig. 4. There was main effect of exercise and hypertension factors for the area of type I and type III collagen fibers ($P < 0.05$). There was interaction between the factors for both type I and type III fibers ($P < 0.05$). SHR Sed animals presented a higher area of type I and type III fibers compared to Wistar Sed animals (Fig. 4). However, the swim training decreased the area of both type I and type III fibers in SHR animals.

3.3. Left ventricular myocyte contractile function

The contractile function of cardiomyocytes stimulated at 1 Hz is presented in Fig. 5. There was exercise and hypertension factors main effects for time to peak of shortening ($P < 0.05$). Myocytes from SHR Sed animals had higher time to peak of shortening compared to those from Wistar Sed (Fig. 5A). Myocytes from SHR Ex animals exhibited lower time to peak of shortening than those from SHR Sed. The time to half relaxation showed main effect only for the hypertension factor as it increased this parameter ($P < 0.05$) (278.8 ± 12.2 vs 195.3 ± 10.1 ms). However, there was no interaction between the factors hypertension and exercise.

Regarding cell shortening (Fig. 5B), we observed that there was main exercise factor effect ($P < 0.05$), as it reduced this parameter. We also found interaction between factors ($P < 0.05$). Myocytes from SHR Sed had higher shortening compared to those from Wistar Sed and SHR Ex group exhibited lower cell shortening compared to SHR Sed.

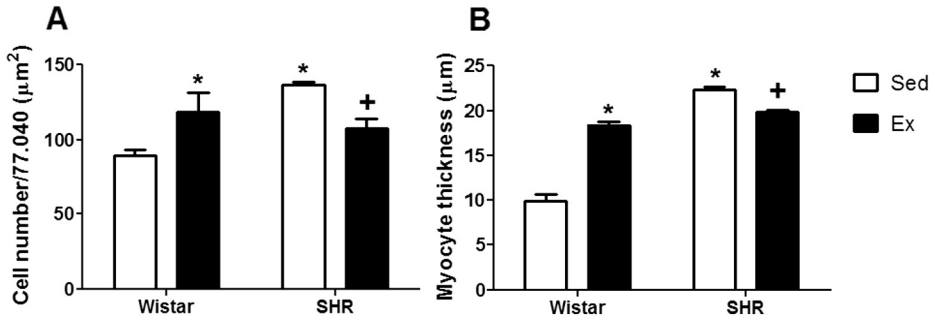

Fig. 2. Structure of cardiac tissue. (A) Number of inflammatory cells in the left ventricle tissue. (B) Myocyte thickness. Data expressed as mean ± SEM of 6 animals each group. *Statistically different from Wistar Sed. + Statistically different from SHR Sed.

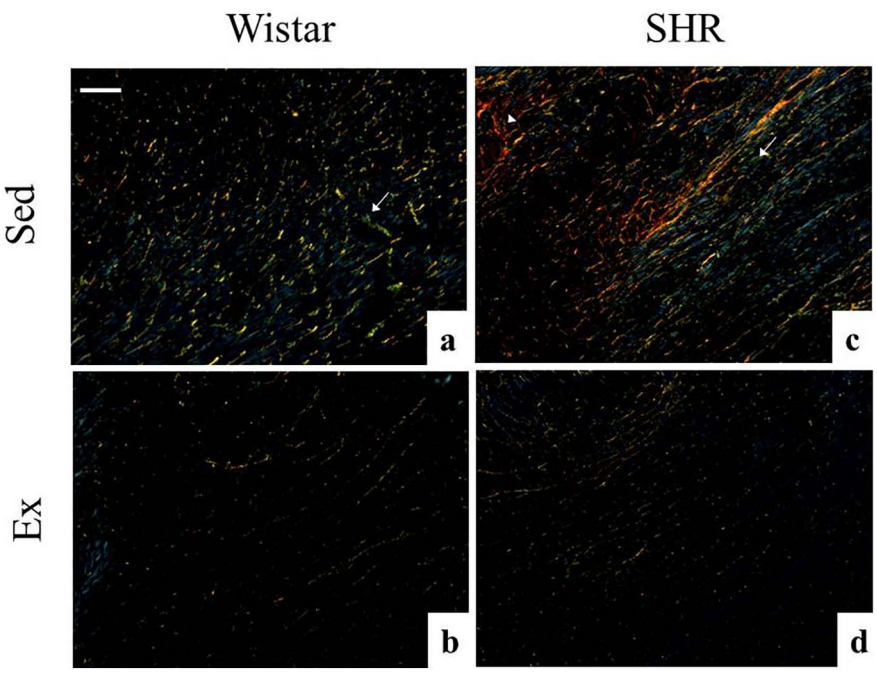

Fig. 3. Representative photomicrographs of left ventricular tissue exhibiting collagen fibers type I and III. (a): Wistar Sed; (b) Wistar Ex; (c) SHR Sed; (d) SHR Ex. Picrosirius Red staining. Bar = 50 μm. Arrow: type III collagen fibers; arrowhead: type I collagen fibers.

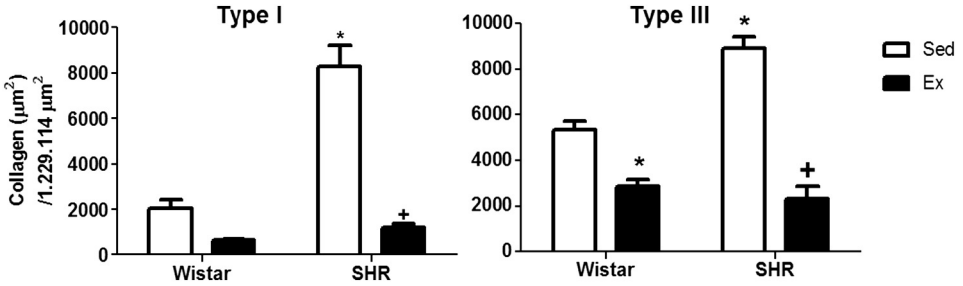

Fig. 4. Area of collagen fibers. Data expressed as mean ± SEM of 6 animals each group. *Statistically different from Wistar Sed. + Statistically different from SHR Sed.

The contractile function of cardiomyocytes stimulated at 3 Hz is presented in Fig. 6. There was main effect of exercise factors and hypertension for the time to peak of shortening ($P < 0.05$). We also observed interaction between the factors ($P < 0.05$). The time to peak of the contraction was higher in SHR Sed compared to Wistar Sed (Fig. 6A). However, swim training decreased these values in the SHR animals.

The time to half relaxation has the main effect of exercise and hypertension factors ($P < 0.05$), as hypertension increased this parameter and exercise counteracted this effect. There was also interaction between the factors ($P < 0.05$). Myocytes from SHR Sed group presented higher values than Wistar Sed (Fig. 6B). Nevertheless, swim training reduced these values in the hypertensive rats.

The myocyte shortening had no main effect of either exercise or hypertension factor ($P > 0.05$). However, there was interaction between the factors ($P > 0.05$). Myocytes from SHR Ex animals presented a lower shortening compared to those from SHR Sed (Fig. 6C).

4. Discussion

In this study, we investigated to what extent low-intensity swim training for six weeks counterbalances the adverse remodeling due to the advance of pathological hypertrophy in the left ventricle (LV) structural and mechanical properties in the early compensated phase of hypertension in male SHR. Our results showed that alongside the MAP (13.22%) and resting HR (14.28%) reductions compared to sedentary SHR, swim training attenuated myocyte hypertrophic growth (10.85%), collagen deposition (85.6% to Type I and 74.23 to Type III) and inflammation (21.24%) compared to sedentary SHR. Furthermore, exercise training brought LV myocyte contraction to control levels and

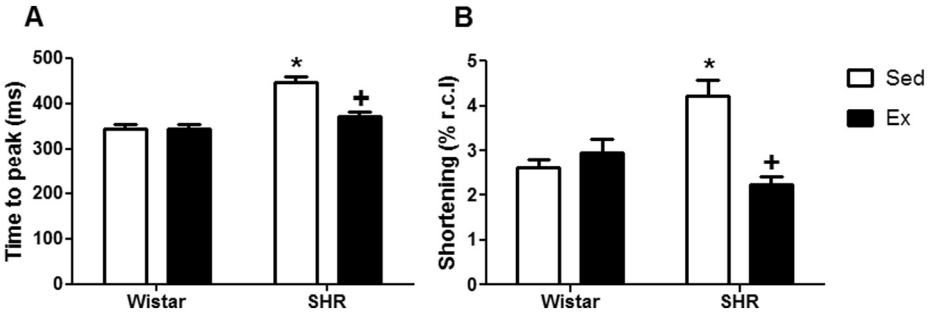

Fig. 5. Contractile function of left ventricular myocytes stimulated at 1 Hz. (A) Time to peak to the shortening. (B) Shortening. Data expressed as mean ± SEM of 50 cells in each group. *Statistically different from Wistar Sed. + Statistically different from SHR Sed.

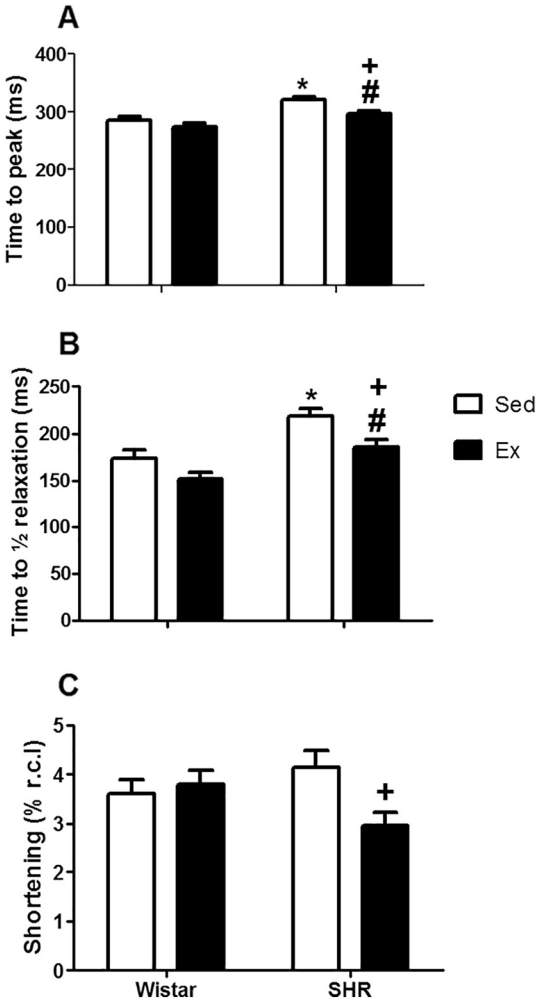

Fig. 6. Contractile function of left ventricular myocytes stimulated at 3 Hz. (A) Time to peak of the shortening (ms). (B) Time to ½ relaxation (ms). (C) Shortening. Data expressed as mean ± SEM of 50 cells in each group. % r.c.l. percentage of resting cell length. *Statistically different from Wistar Sed. #Statistically different from Wistar Ex. + Statistically different from SHR Sed.

mitigated its impaired timecourse.

The observed reduction in the MAP and HR of SHR in response to swim training is believed to be associated with a decrease in the activity of the sympathetic nervous system and the renin-angiotensin-aldosterone system inasmuch as the latter modulates the progression of hypertension in SHR [25]. The angiotensin-converting enzyme inhibitors and Ang II receptor blockers are known to reduce MAP levels, suggesting that the model studied is dependent on this system to maintain high blood pressure [26,27]. The hypotensive effect of swim training on male SHR at the compensated phase was demonstrated elsewhere [28], which was associated with a reduced activity of the renin-angiotensin-aldosterone system in the heart. In addition, although not assessed here, other adaptations to exercise training such as improved muscle vascularization, reduced peripheral resistance and increased endothelial vasodilation can help explain the observed diminution of MAP in exercised SHR [9,29,30].

Resting bradycardia is an important marker of endurance exercise training adaptation in hypertensive rats [10,15]. Such adaptation is associated with the decrease in the sympathetic activity and with the increase in the vagal tone to the heart, and to improvements in the reflex control of the baroreceptors [29,31,32].

We also observed a decrease in the QTc index after swim training in SHR. It indicates that the exercise may have contributed to reduce the cardiac repolarization time, which is prolonged in hypertensive rats [33], thus improving the ventricular electrical activity in these animals. In fact, action potential duration was reduced in LV myocytes of four- to six-month-old male SHR submitted to low-intensity endurance training on a treadmill for eight weeks [34].

Regarding structural properties, the increase in cardiac fiber thickness in the LV of sedentary SHR associated with the increased number of inflammatory cells and collagen types I and III fibers observed in the present study supports that the pathological profile of hypertension, which culminates in ventricular dysfunction latter on, starts early in the compensated phase in this model. In fact, alongside the compensatory hypertrophic growth in SHR, adverse remodeling and stiffness have been observed in LV cardiac tissue [4,6,9–13].

More important, our data show that swim training counteracted the above mentioned negative cardiac adaptations to hypertension. We observed a reduction in the cardiomyocyte thickness after swim training in the LV of SHR. The attenuated myocyte hypertrophic growth in response to exercise is probably due to increases and decreases in angiotensin (1–7) and Ang II plasma concentrations, respectively, as indicated by Filho et al. [35], which would result in lower pressure load over the LV.

The swimming protocol used here reduced the number of inflammatory cells in the LV tissue in SHR. Although not evaluated here, we believe that our swimming protocol has promoted an anti-inflammatory profile in the LV of hypertensive rats (i.e. decreased TNFα; increased DJ-1 protein) as demonstrated elsewhere [13,17]. The down regulation of the renin-angiotensin system by low-intensity exercise training [35] leads to a lower pressure overload on the LV, resulting in a less stressful pro-inflammatory environment. On the other hand, in normotensive animals there is no inflammation, because there is no stimulus for such condition, and the exercise increased the levels of inflammatory cells. It is well described that physical exercise causes an inflammatory response in normotensive individuals (e.g. increase in proinflammatory cytokines and inflammatory cells) [36,37]. The number of inflammatory cells in the SHR Ex group is similar to that in Wistar Ex group, showing that exercise training in the group with hypertension caused a similar condition to the normotensive animal practicing exercise.

Our results showed that swim training also decreased collagen types I and III deposition in the LV of SHR. Such finding is in line with improved systolic elastance in response to aerobic exercise training in male SHR in the early compensatory phase of hypertrophy [10]. The synthesis of collagen is known to be inhibited by Ang(1–7) [38], which was demonstrated increased by low-intensity exercise training [35]. As mentioned above, such increase Ang(1–7) is consistent with the reduced MAP in our exercised SHR. As for the reduced collagen type III deposition in exercised Wistar rats, there was a methodological limitation due to the increase in the myocyte thickness, which occupied more space in the image in relation to the area filled by interstitial connective tissue. Thus, there was no detection of type III collagen, which gives us the false impression that its content has been reduced. Considering that type III matures for type I, we have not yet observed this response for type I (older collagen).

Concerning cardiac mechanical properties, we observed that the timecourse (i.e. times to peak and to half relaxation) of LV myocyte contraction was prolonged in SHR. Such finding is consistent with the prolonged APD in cardiomyocytes [34] and deceleration time of fractional shortening, as well as lowered E/A ratio [39] observed in SHR at the early compensated phase. Such cellular adaptations have been reported previously [16,40], and is believed to be mainly due to hypertension-induced dysfunctions in the calcium regulatory proteins (i.e. RyR2; SERCA2a; PLB) in the myocardium [15,16]. More important, our swimming protocol counteracted such hypertension effects. The time to peak of shortening reflects the systolic function and is regulated by L-type Ca^{2+} and RyR2 channels. The time to half relaxation of cardiomyocytes reflects diastolic function and is regulated by SERCA2a, PLB and Na^{+}/Ca^{2+} exchanger. Although not measured in the present study,

we believe that our swim training has increased the content or the activity of such proteins. In fact, beneficial adaptations of these proteins to exercise training were reported previously in the heart of both SHR [15,16] and normotensive rats [41,42] Taken together, these exercise benefits to the timecourse of LV myocyte contraction, associated with the morphological adaptations of the LV tissue, contribute to the improvement of both systolic and diastolic function in trained hypertensive rats.

With reference to single myocyte contraction, our results showed that hypertension increased the amplitude of shortening in SHR LV myocytes stimulated at 1 Hz. Such hypertension-induced adaptation in this compensated phase was observed previously [3,35]. The swimming protocol used in the present study counteracted this adaptation bringing the amplitude of shortening to the normotensive control level. It is possible that the positive adaptations observed in the timecourse of contraction have compensated the inotropic-lusitropic synchronism of contraction, thus improving the contraction efficiency. Moreover, such normalization of cell contractile function is in line with the reduction in the myocyte thickness observed in swimming trained SHR, as mentioned above.

5. Conclusion

In conclusion, six weeks of low-intensity swim training improved cardiovascular parameters and attenuated the adverse remodeling of left ventricular structural and mechanical properties in the early compensated phase of hypertension in male SHR compared to sedentary SHR. These findings reinforce the importance and the clinical relevance of endurance exercise training to counteract the hypertension-induced cardiac adverse remodeling.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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