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Exercise training and detraining modify the morphological and mechanical properties of single cardiac myocytes obtained from spontaneously hypertensive rats

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Abstract

We determined the effects of exercise training and detraining on the morphological and mechanical properties of left ventricular myocytes in 4-month-old spontaneously hypertensive rats (SHR) randomly divided into the following groups: sedentary for 8 weeks (SED-8), sedentary for 12 weeks (SED-12), treadmill-running trained for 8 weeks (TRA, 16 m/min, 60 min/day, 5 days/week), and treadmill-running trained for 8 weeks followed by 4 weeks of detraining (DET). At sacrifice, left ventricular myocytes were isolated enzymatically, and resting cell length, width, and cell shortening after stimulation at a frequency of 1 Hz (~25°C) were measured. Cell length was greater in TRA than in SED-8 (161.30 ± 1.01 vs 156.10 ± 1.02 μm , $P < 0.05$, 667 vs 618 cells, respectively) and remained larger after detraining. Cell width and volume were unaffected by either exercise training or detraining. Cell length to width ratio was higher in TRA than in SED-8 (8.50 ± 0.08 vs 8.22 ± 0.10 , $P < 0.05$) and was maintained after detraining. Exercise training did not affect cell shortening, which was unchanged with detraining. TRA cells exhibited higher maximum velocity of shortening than SED-8 (102.01 ± 4.50 vs 82.01 ± 5.30 $\mu\text{m/s}$, $P < 0.05$, 70 cells per group), with almost complete regression after detraining. The maximum velocity of relengthening was higher in TRA cells than in SED-8 (88.20 ± 4.01 vs 70.01 ± 4.80 $\mu\text{m/s}$, $P < 0.05$), returning to sedentary values with detraining. Therefore, exercise training affected left ventricle remodeling in SHR towards eccentric hypertrophy, which remained after detraining. It also improved single left ventricular myocyte contractile function, which was reversed by detraining.

Key words: Physical activity; Inactivity; Hypertension; Cardiomyocytes

Introduction

The compensated state of the spontaneously hypertensive rat (SHR) model is characterized by concentric hypertrophy and increased cardiac function (1,2). At the cellular level it has been reported that the dimensions and shortening of the left ventricular myocyte increase in this state, whereas the action potential duration and the time course of contraction and relaxation are prolonged (1,3). Such changes expose the heart to arrhythmic stimuli (4).

While regular physical exercise is suggested as a non-pharmacological therapeutic approach to treat hypertension (5-7), its effects on the heart are reversible with detraining

(8,9). There is evidence at the whole heart level that exercise training provides beneficial adaptations to cardiac contractile function and morphology in hypertensive rats (5). Nevertheless, to date the effects of exercise training and detraining on the left ventricular single myocyte morphological and mechanical properties of SHR are not known.

Since lifestyle modifications are effective for the prevention, treatment, and control of hypertension with regular exercise being an important component (10), the study of the effect of exercise training and detraining on single cardiac myocytes may provide new insights into the understanding

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of the cellular mechanisms underlying such phenomena. Therefore, the aim of the present study was to determine the effects of exercise training followed by a period of detraining on the morphological and mechanical properties of single left ventricular myocytes obtained from SHR rats in the compensated state of hypertension.

Material and Methods

Four-month-old male SHR rats were randomly divided into the following groups: sedentary for 8 weeks (SED-8, N = 7), sedentary for 12 weeks (SED-12, N = 7), treadmill-running trained for 8 weeks (TRA, N = 7), and exercised followed by 4 weeks of detraining (DET, N = 7). Rats were housed in collective cages under an inverted 10- to 14-h light/dark cycle and had free access to water and standard rodent chow. Blood pressure was measured in all animals at the beginning of the protocol and before sacrifice by the indirect tail-cuff method. All experimentation was conducted in accordance with internationally accepted ethical principles concerning the care and use of laboratory animals and was approved by the Animal Care and Use Committee of the Federal University of Viçosa, MG, Brazil (protocol #42/2008).

Animals in the TRA and DET groups were submitted to a progressive exercise training program on a motor-driven treadmill (Insight Equipamentos Científicos, Brazil) for 8 weeks, 5 days/week (Monday to Friday) (adapted from Ref. 11). Briefly, in the 1st week, animals ran at 10 m/min for 15 min/day, 0% grade. Then, treadmill speed and exercise duration were progressively increased up to a setting of 14 m/min for 30 min/day by the end of the 2nd week. During the 3rd week the exercise protocol reached the speed of 16 m/min and the duration of 60 min/day, 0% grade, a schedule that was maintained until week 8. Rats from the DET group underwent training for 8 weeks as described above and then remained sedentary (i.e., "detraining") for 4 weeks. To detrain, the rats were allowed to roam their cages freely under the same conditions as those for the SED groups.

Two days after the last training session, all animals were submitted to a progressive treadmill running test to measure running capacity until fatigue, when the exercise time to fatigue (TTF) was determined as previously described (12). Briefly, 1 week before the exercise tests until fatigue, the sedentary rats were familiarized with the treadmill by running 5 min for 5 consecutive days (5° slope) at incremental speeds (10, 10, 11, 13, 15 m/min). Then, in the subsequent week every rat was evaluated for running capacity until fatigue. Fatigue was defined and the test was stopped when the rat could no longer keep pace with the treadmill speed.

Two days after the exercise test, animals were sacrificed and left ventricular myocytes were isolated enzymatically as described previously (13). Briefly, the heart was removed,

weighed and mounted on a custom-designed Langendorff system, perfused for 3-5 min with calcium-free solution following perfusion with 1 mg/mL collagenase type II (USA). After 15-20 min of tissue digestion, fragments of the left ventricle were obtained and single cells were isolated by mechanical dispersion and stored at 5°C until use.

Cellular contractility was measured as described previously (13). Briefly, isolated cells were placed in an experimental chamber with a glass coverslip base mounted on the stage of an inverted microscope. Cells were perfused with HEPES Tyrode's solution containing 1 mM CaCl₂ and field stimulated at the frequency of 1 Hz (20 V, 5 ms duration square pulses). Cells were imaged using an NTSC camera in a partial scanning mode. Cell shortening in response to electrical stimulation was measured with a video-edge detection system at a 240-Hz frame rate (Ionoptix, USA). All parameters were evaluated using customized software developed in the MatLab® platform (13). Experiments were performed at room temperature (~25°C). Only calcium-tolerant, quiescent, rod-shaped myocytes showing clear cross striations were studied. The cell image was also used to determine cell length and width, which were used to calculate the cell volume (14) and length-to-width ratio. Cell shortening, maximal velocity of shortening and maximal velocity of relengthening were measured in 10 cells per animal in each experimental group. Cell length and width were measured in 497 to 671 cells in the experimental groups.

Data were analyzed by ANOVA according to the following one-way fixed effects model: $Y_{ij} = \mu + G_i + E_{ij}$, where Y_{ij} denotes the j th observation ($j = 1, 2, \dots, n_i$, with n_i = number of observations for groups 1 to 4, respectively) of the response variables from the i th ($i = 1, 2, 3, 4$) group. When the group effect was significant ($P < 0.05$), means were compared by the Tukey-Kramer test. Data are reported as means \pm SEM.

Results

The TTF of TRA animals was longer than that of SED-8 (18.30 \pm 1.80 vs 9.70 \pm 2.20 min, respectively, $P < 0.01$). This exercise training adaptation returned to control levels after 4 weeks of detraining.

Blood pressure, body weight (BW), heart weight (HW), and HW to BW ratio, were not statistically different among groups after either exercise training or detraining (Table 1). However, exercise training increased the length of left ventricular myocytes (3.33%) when compared to sedentary control animals (TRA vs SED-8). Within 4 weeks of detraining the length of myocytes remained larger in exercised animals (DET vs SED-12). Cell width and volume were not affected by either exercise training or detraining. Cell length to width ratio was higher in TRA than in SED-8 animals and was maintained after the detraining period (Table 1).

Exercise training did not affect cell shortening (reported as % resting cell length), which was unchanged after the

detraining period (Figure 1A). Nevertheless, TRA animal cells exhibited higher maximum velocity of shortening than SED-8 animals (24%; Figure 1B). This adaptation was reversed almost completely within 4 weeks of detraining.

In addition, the maximum velocity of relengthening was higher in TRA than in SED-8 animal cells (26%; Figure 1C). This change was reversed to sedentary values after 4 weeks of detraining.

Table 1. Blood pressure, body mass, cardiac weight, and single left ventricular myocyte dimensions of the animals studied.

	SED-8 (N = 7)	TRA (N = 7)	SED-12 (N = 7)	DET (N = 7)
Initial BP (mmHg)	176.00 ± 6.00	171.00 ± 9.00	175.00 ± 7.00	176.00 ± 6.00
Final BP (mmHg)	176.00 ± 4.00	170.00 ± 8.00	174.00 ± 9.00	175.00 ± 4.00
Final BW (g)	352.70 ± 10.51	371.70 ± 12.49	389.70 ± 10.43	372.30 ± 9.43
HW (g)	2.08 ± 0.08	2.18 ± 0.09	2.02 ± 0.08	2.14 ± 0.08
HW:BW ratio (mg/g)	5.92 ± 0.26	5.91 ± 0.25	5.17 ± 0.09	5.76 ± 0.23
Cell length (µm)	156.10 ± 1.02	161.30 ± 1.01*	156.10 ± 1.00	160.11 ± 1.01**
Cell width (µm)	20.30 ± 0.22	20.20 ± 0.23	21.00 ± 0.20	20.50 ± 0.21
Length/width	8.22 ± 0.10	8.50 ± 0.08*	8.00 ± 0.11	8.33 ± 0.09**
Cell volume (pL)	24.10 ± 0.30	25.12 ± 0.21	25.00 ± 0.30	25.01 ± 0.20

Data are reported as means ± SEM. N = number of animals; SED-8 = animals maintained sedentary for 8 weeks (618 cells); TRA = treadmill-running animals trained for 8 weeks (667 cells); SED-12 = animals maintained sedentary for 12 weeks (497 cells); DET = animals exercised for 8 weeks and then detrained (671 cells); BP = blood pressure; HW = heart weight; HW:BW = HW to BW ratio. *P < 0.05 compared to SED-8; **P < 0.05 compared to SED-12 (one-way ANOVA followed by the Tukey-Kramer *post hoc* test).

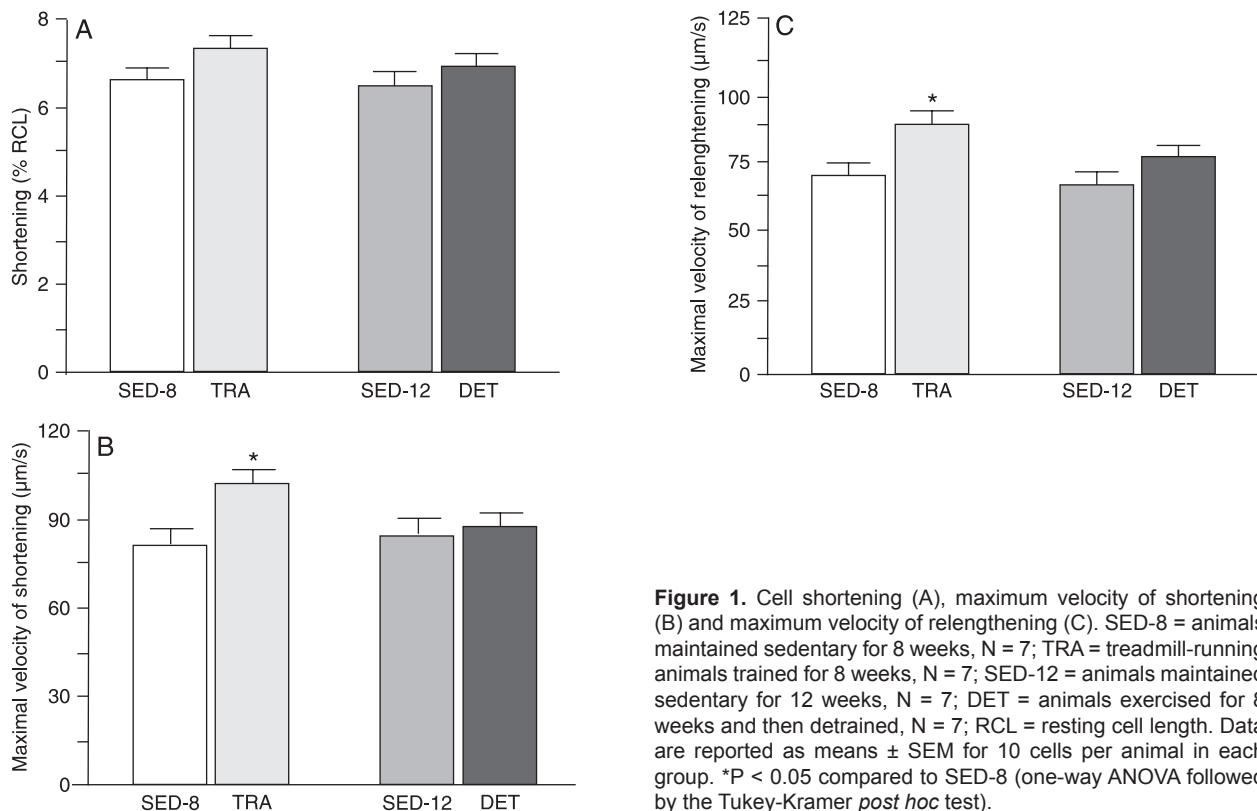


Figure 1. Cell shortening (A), maximum velocity of shortening (B) and maximum velocity of relengthening (C). SED-8 = animals maintained sedentary for 8 weeks, N = 7; TRA = treadmill-running animals trained for 8 weeks, N = 7; SED-12 = animals maintained sedentary for 12 weeks, N = 7; DET = animals exercised for 8 weeks and then detrained, N = 7; RCL = resting cell length. Data are reported as means ± SEM for 10 cells per animal in each group. *P < 0.05 compared to SED-8 (one-way ANOVA followed by the Tukey-Kramer *post hoc* test).

Discussion

Our chronic treadmill running protocol increased the running capacity (TTF) of SHR, which returned to control levels after detraining. Despite no effect of exercise training at the whole heart level (unchanged HW:BW ratio), at the cellular level our data showed longer myocytes in the myocardium of SHR animals that exercised on a treadmill for 8 weeks and that 4 weeks of detraining did not abolish such exercise adaptation. This training-induced elongation of left ventricular myocytes together with the increased cell length-to-width ratio indicates that in the compensated phase of the SHR model myocardium remodeling in response to exercise training occurs towards eccentric hypertrophy. An increase (~3.5%) in the circumferential dimension of an elliptical chamber resulting from a 3.5% increase in myocyte length would result in an increase in chamber volume of at least 7% (15). This type of geometric alteration would have important consequences for the cardiac function of these animals. For example, it would be accompanied by a smaller increase in end-diastolic myocardial wall stress and by an increased stroke volume inasmuch as a larger chamber would result in a greater end-diastolic volume. Nevertheless, the increase in cell length did not affect the HW:BW ratio of TRA. It is possible that a 3.5% increase in cell length does not significantly affect the heart weight of the exercised animals.

We also observed that exercised SHR animals exhibited faster cell shortening and relaxation than sedentary rats and that this adaptation regressed to control values within 4 weeks of detraining. Cardiac myocyte shortening kinetics is known to be regulated by calcium-regulatory proteins, contractile protein isoforms and action potential waveform and duration (16). Although we did not test these mechanisms, the increase in the expression of calcium-regulatory proteins such as sarcoplasmic reticulum ATPase, phospholamban and ryanodine receptors in response to exercise training has been reported in hypertensive rat cardiac muscle (17,18). Exercise training has also been shown to induce a shift in ventricular isomyosin towards V1 (higher ATPase and contractile activity compared to V3 isomyosin) in hypertensive rats (19). The effects of exercise training on the action potential waveform and duration of left ventricular myocytes in hypertensive rats remain to

be investigated. These cell time course adaptations to exercise training would also have an important effect on cardiac function. For example, a faster cell relengthening increases end-diastolic volume as a result of a prolonged diastolic filling time. Whether these exercise training effects on single cardiac myocyte time course of contraction and relaxation will affect the susceptibility of SHR heart to arrhythmic stimuli warrants further investigations.

The present study provides the first observations regarding the effects of aerobic exercise training and detraining on morphological and mechanical properties of single left ventricular myocytes obtained from hypertensive rats. Our findings give support to the improvement of left ventricular performance demonstrated in exercised SHR isolated heart (5,17,19). Although we did not measure cardiac function in the present study, the morphological and mechanical cellular adaptations reported here may contribute to our understanding of the mechanisms underlying the benefits of exercise training on the cardiac function of SHR (i.e., improved left ventricular performance).

We also showed that some cardiac myocyte adaptations to exercise training are lost within 4 weeks of detraining, suggesting that lifestyle modifications towards an active life (i.e., regular exercise) help maintain the benefits of exercise for hypertensive subjects.

Finally, our exercise training protocol did not reduce the blood pressure of TRA animals. While some studies reported reduced blood pressure of SHR in response to exercise training (e.g., Ref. 12), no change was observed by others (e.g., Ref. 20). Perhaps 8 weeks of exercise training was not a long enough period of time to promote a statistically significant reduction in resting blood pressure.

We showed here that exercise training affects left ventricular remodeling in the SHR model towards eccentric hypertrophy, which remain persisted for a 4-week detraining period. Exercise training also improves single left ventricular myocyte contractile function, which was reversed after detraining.

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References

1. Brooksby P, Levi AJ, Jones JV. Contractile properties of ventricular myocytes isolated from spontaneously hypertensive rat. *J Hypertens* 1992; 10: 521-527.
2. Hart G. Cellular electrophysiology in cardiac hypertrophy and failure. *Cardiovasc Res* 1994; 28: 933-946.
3. McCrossan ZA, Billeter R, White E. Transmural changes in size, contractile and electrical properties of SHR left ventricular myocytes during compensated hypertrophy. *Cardiovasc Res* 2004; 63: 283-292.
4. Evans SJ, Levi AJ, Jones JV. Wall stress induced arrhythmia is enhanced by low potassium and early left ventricular hypertrophy in the working rat heart. *Cardiovasc Res* 1995; 29: 555-562.
5. Schaible T, Malhotra A, Ciambone G, Buttrick P, Scheuer J. Combined effects of hypertension and chronic running program on rat heart. *J Appl Physiol* 1987; 63: 322-327.

6. Nho H, Tanaka K, Kim HS, Watanabe Y, Hiyama T. Exercise training in female patients with a family history of hypertension. *Eur J Appl Physiol Occup Physiol* 1998; 78: 1-6.
7. Lee IM, Sesso HD, Oguma Y, Paffenbarger RS Jr. Relative intensity of physical activity and risk of coronary heart disease. *Circulation* 2003; 107: 1110-1116.
8. Bocalini DS, Carvalho EV, de Sousa AF, Levy RF, Tucci PJ. Exercise training-induced enhancement in myocardial mechanics is lost after 2 weeks of detraining in rats. *Eur J Appl Physiol* 2010; 109: 909-914.
9. Kemi OJ, Haram PM, Wisloff U, Ellingsen O. Aerobic fitness is associated with cardiomyocyte contractile capacity and endothelial function in exercise training and detraining. *Circulation* 2004; 109: 2897-2904.
10. Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA. American College of Sports Medicine position stand. Exercise and hypertension. *Med Sci Sports Exerc* 2004; 36: 533-553.
11. Veras-Silva AS, Mattos KC, Gava NS, Brum PC, Negrao CE, Krieger EM. Low-intensity exercise training decreases cardiac output and hypertension in spontaneously hypertensive rats. *Am J Physiol* 1997; 273: H2627-H2631.
12. Lacerda AC, Marubayashi U, Balthazar CH, Leite LH, Coimbra CC. Central nitric oxide inhibition modifies metabolic adjustments induced by exercise in rats. *Neurosci Lett* 2006; 410: 152-156.
13. Roman-Campos D, Duarte HL, Sales PA Jr, Natali AJ, Ropert C, Gazzinelli RT, et al. Changes in cellular contractility and cytokines profile during *Trypanosoma cruzi* infection in mice. *Basic Res Cardiol* 2009; 104: 238-246.
14. Satoh H, Delbridge LM, Blatter LA, Bers DM. Surface:volume relationship in cardiac myocytes studied with confocal microscopy and membrane capacitance measurements: species-dependence and developmental effects. *Biophys J* 1996; 70: 1494-1504.
15. Moore RL, Musch TI, Yelamarty RV, Scaduto RC Jr, Semanchick AM, Elensky M, et al. Chronic exercise alters contractility and morphology of isolated rat cardiac myocytes. *Am J Physiol* 1993; 264: C1180-C1189.
16. Sah R, Ramirez RJ, Oudit GY, Gidrewicz D, Trivieri MG, Zobel C, et al. Regulation of cardiac excitation-contraction coupling by action potential repolarization: role of the transient outward potassium current (I_{to}). *J Physiol* 2003; 546: 5-18.
17. Collins HL, Loka AM, Dicarlo SE. Daily exercise-induced cardioprotection is associated with changes in calcium regulatory proteins in hypertensive rats. *Am J Physiol Heart Circ Physiol* 2005; 288: H532-H540.
18. Garcarena CD, Pinilla OA, Nolly MB, Laguens RP, Escudero EM, Cingolani HE, et al. Endurance training in the spontaneously hypertensive rat: conversion of pathological into physiological cardiac hypertrophy. *Hypertension* 2009; 53: 708-714.
19. Schaible TF, Malhotra A, Ciambone GJ, Scheuer J. Chronic swimming reverses cardiac dysfunction and myosin abnormalities in hypertensive rats. *J Appl Physiol* 1986; 60: 1435-1441.
20. Lajoie C, Calderone A, Beliveau L. Exercise training enhanced the expression of myocardial proteins related to cell protection in spontaneously hypertensive rats. *Pflugers Arch* 2004; 449: 26-32.