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Original article

The benefits of endurance training in cardiomyocyte function in hypertensive rats are reversed within four weeks of detraining

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ABSTRACT

The aim of the present study was to verify the effects of low-intensity endurance training and detraining on the mechanical and molecular properties of cardiomyocytes from spontaneously hypertensive rats (SHRs). Male SHRs and normotensive control Wistar rats at 16-weeks of age were randomly divided into eight groups of eight animals: NC8 and HC8 (normotensive and hypertensive control for 8 weeks); NT8 and HT8 (normotensive and hypertensive trained at 50–60% of maximal exercise capacity for 8 weeks); NC12 and HC12 (normotensive and hypertensive control for 12 weeks); NDT and HDT (normotensive and hypertensive trained for 8 weeks and detrained for 4 weeks). The total exercise time until fatigue (TTF) was determined by a maximal exercise capacity test. Resting heart rate (RHR) and systolic arterial pressure (SAP) were measured. After the treatments, animals were killed by cervical dislocation and left ventricular myocytes were isolated by enzymatic dispersion. Isolated cells were used to determine intracellular global Ca^{2+} ($[Ca^{2+}]_i$) transient and cardiomyocyte contractility (1 Hz; ~25 °C). $[Ca^{2+}]_i$ regulatory proteins were measured by Western blot, and the markers of pathologic cardiac hypertrophy by quantitative real-time polymerase chain reaction (q-RT-PCR). Exercise training augmented the TTF (NC8, 11.4±1.5 min vs. NT8, 22.5±1.4 min; HC8, 11.7±1.4 min vs. HT8, 24.5±1.3 min; $P<0.05$), reduced RHR (NT8initial, 340±8 bpm vs. NT8final, 322±10 bpm; HT8initial, 369±8 bpm vs. HT8final, 344±10 bpm; $P<0.05$), and SBP in SHR animals (HC8, 178±3 mm Hg vs. HT8, 161±4 mm Hg; $P<0.05$). HC8 rats showed a slower $[Ca^{2+}]_i$ transient (Tpeak, 83.7±1.8 ms vs. 71.7±2.4 ms; T50%decay, 284.0±4.3 ms vs. 264.0±4.1 ms; $P<0.05$) and cell contractility (Vshortening, 86.1±6.7 μm/s vs. 118.6±6.7 μm/s; Vrelengthening, 57.5±7.4 μm/s vs. 101.3±7.4 μm/s; $P<0.05$), and higher expression of ANF (300%; $P<0.05$), skeletal α-actin (250%; $P<0.05$) and a decreased α/β-MHC ratio (70%; $P<0.05$) compared to NC8. Exercise training increased $[Ca^{2+}]_i$ transient (NC8, 2.39±0.06 F/F_0 vs. NT8, 2.72±0.06 F/F_0; HC8, 2.28±0.05 F/F_0 vs. HT8, 2.82±0.05 F/F_0; $P<0.05$), and cell contractility (NC8, 7.4±0.3% vs. NT8, 8.4±0.3%; HC8, 6.8±0.3% vs. HT8, 7.8±0.3%; $P<0.05$). Furthermore, exercise normalized the expression of ANF, skeletal α-actin, and the α/β-MHC ratio in HT8 rats, augmented the expression of SERCA2a (NC8, 0.93±0.15 vs. NT8, 1.49±0.14; HC8, 0.83±0.13 vs. HT8, 1.32±0.14; $P<0.05$) and PLB_{ser16} (NC8, 0.89±0.18 vs. NT8, 1.23±0.17; HC8, 0.77±0.17 vs. HT8, 1.32±0.16; $P<0.05$), and reduced PLBt/SERCA2a (NC8, 1.21±0.19 vs. NT8, 0.50±0.21; HC8, 1.38±0.17 vs. HT8, 0.66±0.21; $P<0.05$). However, all these adaptations returned to control values within 4 weeks of detraining in both SHR and normotensive control animals. In conclusion, low-intensity endurance training induces positive benefits to left ventricular myocyte mechanical and molecular properties, which are reversed within 4 weeks of detraining.

Abbreviations: NC8 and HC8, normotensive and hypertensive control for 8 weeks; NT8 and HT8, normotensive and hypertensive trained for 8 weeks; NC12 and HC12, normotensive and hypertensive control for 12 weeks; NDT and HDT, normotensive and hypertensive trained for 8 and detrained for 4 weeks; MRS, maximal running speed; TTF, total exercise time until fatigue; RHR, resting heart rate; SAP, systolic arterial pressure; LIET, low-intensity endurance training; DET, detraining.

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1. Introduction

Hypertension is an independent risk factor for cardiovascular disease and a precursor of heart failure [1]. The hemodynamic overload imposed by hypertension results in a pathological pattern of concentric cardiac hypertrophy, which is commonly associated with upregulation of fetal genes, fibrosis, cardiac dysfunction, and apoptosis [2–4].

The spontaneously hypertensive rat (SHR) is a widely used model of human essential hypertension. The compensated state of the SHR model has been reported as early as three months, while failure is reported at 18–24 months [5,6]. At the cellular level, in the compensated state of hypertension, it has been reported that left ventricular myocyte shortening increases, whereas the action potential duration and the time course of intracellular global Ca^{2+} ($[Ca^{2+}]_i$) transient, cell shortening and relaxation are prolonged in SHR [7–9].

Along with anti-hypertensive therapy, an active lifestyle is recommended for the management of high blood pressure (BP) in hypertensive individuals, and exercise training is an important hypotensive non-pharmacological therapeutic strategy [1,10–13]. Although high-intensity exercise training may lead to adverse remodeling and produce a substrate for cardiac arrhythmias [14], the recommended low-intensity exercise training improves whole heart inotropic performance, myocardial β-adrenergic responsiveness, phosphorylation of key $[Ca^{2+}]_i$ regulatory proteins (e.g., ryanodine receptor and phospholamban), and attenuates systolic dysfunction in the compensatory phase of hypertension in female SHRs [15–18]. Moreover, low-to-moderate exercise training improved midventricular shortening, myocardial capillary density, and decreased fibrosis and calcineurin activity in the myocardium of male SHRs [19].

Although previous studies have demonstrated that aerobic exercise training enhances single cardiomyocyte contractile function in normotensive rats [20–24] and that detraining reverses the improved cardiomyocyte contractile function to control levels in these animals [22], to date little is known about the effects of aerobic exercise training and detraining on the cardiomyocyte contractile function in hypertensive rats. Our group demonstrated that low-intensity exercise training induces beneficial effects to left ventricular myocytes' mechanical [25] and electrical [9] properties and that the mechanical adaptations are partially reversed within 4 weeks of detraining; however, the underlying mechanisms are not known.

This study was designed to investigate the effects of low-intensity endurance training (LIET) and detraining (DET) on the mechanical and molecular properties of cardiomyocytes from SHR animals in the compensated state of hypertension.

2. Materials and methods

2.1. Experimental animals

Four-month old male SHRs and normotensive Wistar rats were housed in collective cages under 12–12 h light/dark cycles in a temperature-controlled room (22 °C) and had free access to water and standard rodent chow. Eight experimental groups were allocated as follows: normotensive and hypertensive control for 8 weeks (NC8 and HC8); normotensive and hypertensive trained for 8 weeks (NT8 and HT8); normotensive and hypertensive control for 12 weeks (NC12 and HC12); and normotensive and hypertensive trained for 8 and detrained for 4 weeks (NDT and HDT).

Experimental protocols were approved by the Ethic Committee in Animal Use from Federal University of Viçosa (Protocol #48/2011) in accordance with the Guide for the Care and Use of Laboratory Animals/2011.

2.2. Exercise training protocol and detraining

The exercise training protocol was carried out on a motor-driven treadmill (Insight Equipamentos Científicos, Brazil), 5 days per week (Monday to Friday), 60 min/day, for 8 weeks. Before the beginning of the exercise-training program, the animals were placed on the treadmill for adaptation (10 min/day, 0% grade, 0.3 km/h) for 5 days. Forty-eight hours after the adaptation period, a test was performed to determine the maximal running speed (MRS) of each animal. The animals started running at 0.3 km/h, 0% grade, and the treadmill speed was increase by 0.18 km/h every 3 min until fatigue, which was defined as when the test was interrupted because the animals could no longer keep pace with the treadmill speed. The training intensity throughout the training period was monitored using the progressive increase of time and running speed, which reached 1 h/day, 0% grade, at 50–60% of MRS on the third week (adapted from Melo [26]).The MRS test was also performed at the end of the 4th week of training in animals from NT8, HT8, NDT, and HDT groups in order to update the training intensity. Forty-eight hours after the last training session, the MRS test was repeated in all animals, and after 4 weeks of detraining in animals from NC12, NDT, HC12, and HDT groups to evaluate their total exercise time until fatigue (TTF).

During the experimental period, the animals in control groups (NC8, NC12, HC12, and HC8) were handled every day and submitted to a short period of mild exercise (5–10 min, 0% grade, 0.3 km/h, 3 days/week). This exercise intensity and duration were below the levels required to evoke training adaptation [27]. The body weight (BW) from all rats was measured every week. The systolic arterial pressure (SAP) and resting heart rate (RHR) were recorded at the beginning and after 48 h of each experimental period by the tail-cuff methods previously described [19].

2.3. Cardiomyocyte isolation

Two days after the last MRS test, the rats were weighed and killed by cervical dislocation under resting conditions, and their hearts were quickly removed. Left ventricular myocytes were enzymatically isolated as previously described [28]. Briefly, the hearts were mounted on a Langendorff system and perfused for ~5 min with a modified Hepes–Tyrode solution of 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, pH 7.3 at 37 °C. The perfusion solution was changed for the calcium-free solution with EGTA (0.1 mM) for 6 min. Afterwards, the hearts were perfused for 15–20 min with a solution containing 1 mg/ml collagenase type II (Worthington, USA). The digested heart was then removed from the cannula, and the ventricles were removed and weighed. The left ventricle was separated, weighed, and cut into small pieces. The left ventricle tissues were placed into small conical flasks with collagenase-containing solution supplemented with 1% bovine serum albumin. The cells were dispersed by agitating the flasks at 37 °C for periods of 5 min. Then, single cells were separated from the non-dispersed tissue by filtration. The resulting cell suspension was centrifuged and resuspended in Hepes–Tyrode solution. Non-dispersed tissue was subjected to further enzyme treatment. The isolated cells were stored at 5 °C until use. Only calcium-tolerant, quiescent, rod-shaped cardiomyocytes showing clear cross-striations were studied. The isolated cardiomyocytes were used within 2–3 h of isolation.

2.4. Intracellular Ca^{2+} measurements

Intracellular calcium ($[Ca^{2+}]_i$) transients were evaluated as previously described [29]. Briefly, freshly isolated cardiomyocytes obtained from resting left ventricles were loaded with 5 μM fluo-4 AM (Molecular Probes, Eugene, OR, USA) for 20 min at room temperature and then washed with an extracellular Hepes–Tyrode solution to remove excess dye. $[Ca^{2+}]_i$ transients were elicited by field-stimulating cardiomyocytes through a pair of platinum electrodes with a 0.2 ms supra-threshold voltage square pulse. Cells were stimulated at 1 Hz to produce steady-state conditions. A Meta LSM 510 scanning system (Carl Zeiss GmbH,