Regional effects of low-intensity endurance training on structural and mechanical properties of rat ventricular myocytes

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Carneiro-Júnior MA, Prímola-Gomes TN, Quintão-Júnior JF, Drummond LR, Lavorato VN, Drummond FR, Felix LB, Oliveira EM, Cruz JS, Natali AJ, Mill JG. Regional effects of low-intensity endurance training on structural and mechanical properties of rat ventricular myocytes. J Appl Physiol 115: 107–115, 2013. First published May 2, 2013; doi:10.1152/japplphysiol.00041.2013.—We tested the effects of low-intensity endurance training (LIET) on the structural and mechanical properties of right (RV) and left ventricular (LV) myocytes. Male Wistar rats (4 mo old) were randomly divided into control (C, n = 7) and trained (T, n = 7), treadmill running at 50–60% of maximal running speed for 8 wk groups. Isolated ventricular myocyte dimensions, contractility, Ca2+ transients {intracellular Ca2+ concentration ([Ca2+]i)}, and ventricular Ca2+-ATPase [Ca2+-ATPase (SERCA2a)] regulatory proteins were measured. LIET augmented cell length (C, 152.5 ± 2.0 μm vs. T, 162.2 ± 2.1 μm; P < 0.05) and volume (C, 5,162 ± 131 μm3 vs. T, 5,506 ± 132 μm3; P < 0.05) in the LV but not in the RV. LIET increased cell shortening (C, 7.5 ± 0.3% vs. T, 8.6 ± 0.3%; P < 0.05), the [Ca2+]i transient amplitude (C, 2.49 ± 0.06 F/F0 vs. T, 2.82 ± 0.06 F/F0; P < 0.05), the expression of sarcoplasmic reticulum Ca2+-ATPase 2a (C, 1.07 ± 0.13 vs. T, 1.59 ± 0.12; P < 0.05), and the levels of phosphorylated phospholamban at serine 16 (C, 0.99 ± 0.11 vs. T, 1.34 ± 0.10; P < 0.05), and reduced the total phospholamban-to-sarcoplasmic reticulum Ca2+-ATPase 2a ratio (C, 1.19 ± 0.15 vs. T, 0.40 ± 0.16; P < 0.05) in the LV without changing such parameters in the RV. In conclusion, LIET affected the structure and improved the mechanical properties of LV but not of RV myocytes in rats, helping to characterize the functional and morphological changes that accompany the endurance training-induced cardiac remodeling.

Exercise; calcium transient; contraction; excitation-contraction coupling regulatory proteins; right ventricle

The pumping action of the entire heart originates from a coordinated set of contractions that are produced during every beat by the cardiomyocytes (6), which account for the most myocardial mass due to single cell size (4, 7).

Volumetric overload that occurs during exercise training simultaneously involves the right and left cardiac chambers. In contrast, pressure overload mainly occurs in the left ventricle (LV). Therefore, cardiomyocytes from the right ventricle (RV) and LV are subjected to diverse hemodynamic loads during exercise training. Endurance training-induced cardiac remodeling consists of mild to moderate eccentric LV hypertrophy and RV dilatation, whereas strength training causes concentric LV hypertrophy and appears to impact the RV minimally, constituting the concept of athlete’s heart (11, 15, 43, 45). The increased capacity to produce higher values of cardiac output is the central adaptation that is induced in the heart by endurance training, and this capacity is the major determinant for oxygen supply to the peripheral tissues (8, 30). Several chronic changes produced in the heart due to endurance training are secondary to cardiomyocyte adaptations. Because the stroke volume might change in response to variations in exercise intensity and workload, it is conceivable that the force and extent of cardiomyocyte contraction might additionally change in individuals who are subjected to long-term endurance training (30).

Clinical and experimental studies have demonstrated that improvement in aerobic fitness is linked to the development of an enhanced cardiac function, which is mainly due to morphological and functional adaptations of the LV myocytes (10, 17, 19, 20, 25, 27–29, 35, 47). In LV isolated myocytes, our group and others have been shown that endurance training increases the developed force (14, 35), the amplitude of intracellular global Ca2+ [intracellular Ca2+ concentration ([Ca2+]i)] transients (10, 25), and the cellular fractional shortening (10, 27, 28, 47). In addition, endurance training shortens the time course of global [Ca2+]i transients and the duration of contraction and relaxation and increases the length, width, and volume of the LV myocytes (27, 28, 47). The improvement in LV myocyte contractility appears to be associated with an increased expression of key proteins that regulate [Ca2+]i, such as the sarcoplasmic reticulum (SR) Ca2+-ATPase (SERCA2a) and phospholamban (PLB), which is phosphorylated at threonine 17 (PLB thr17) (25, 47) and at serine 16 (PLB ser16) (7, 44). These structural and biochemical adaptations in the LV myocytes in response to endurance training could translate into improved cardiac pump function.

However, global cardiac performance depends on simultaneous and synchronous functioning of both ventricles. Regional myocardial function in response to exercise training has only recently been studied (1, 2, 12, 46, 48). Baggish et al. (1) showed that endurance athletes developed eccentric LV hypertrophy and biventricular dilatation with improved diastolic func-
tion, while strength athletes develop isolated, concentric LV hypertrophy with diminished diastolic relaxation, without alterations in the RV parameters. Nevertheless, the structural and mechanical adaptations of the RV myocytes in response to endurance training have not been studied in experimental models, and this could contribute to a better characterization of regional cellular phenotypes of exercise-induce cardiac remodeling.

At rest and/or during exercise training, the RV pumps blood into the relatively low-pressure pulmonary vasculature. Accordingly, RV chamber pressures are much lower, and the workload of RV differs significantly from that of the LV (42). Therefore, in the present study, we hypothesized that these differences between the pressure levels on the LV and RV chambers may determine different patterns of structural and mechanical adaptations of LV and RV myocytes in response to low-intensity endurance training (LIET).

MATERIALS AND METHODS

Experimental animals. Four-month-old male Wistar rats were housed together in cages under a 12:12-h light-dark cycle in a temperature-controlled room (22°C), and they had free access to water and standard rodent diet. The animals were randomly assigned to the T group (trained for 8 wk, n = 7) or C group (controls, n = 7). The experimental protocols were approved by the Ethics Committee for Animal Use at the Federal University of Viçosa (protocol no. 48/2011), in accordance with the Guide for the Care and Use of Laboratory Animals/2011.

Exercise training protocol. Exercise training was performed on a motor-driven treadmill (Insight Equipamentos Científicos), 60 min/day, 5 days/wk for 8 wk. Before beginning the exercise training program, all animals from the C and T groups were placed on the treadmill for adaptation (10 min/day, 0% grade, 0.3 km/h) during 5 days. Two days after the fifth exercise session, all animals were subjected to a running test to determine the maximal running speed (MRS). Each animal began running at 0.3 km/h at 0% grade, and the treadmill speed was increased by 0.18 km/h every 3 min until the animals were fatigued (defined as the treadmill speed at which the animals could no longer maintain pace). The exercise training was performed 5 days/wk (Monday to Friday) for 8 wk. 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 (10, 31). Briefly, the animals ran at 50% of the MRS (~0.5–0.6 km/h), 0° grade, for 10 min in the first training day. In the first week, exercise duration was augmented in 5 min/day, and the treadmill speed was kept constant. During the second week, exercise duration was increased in 5 min/day and the treadmill speed in 2% of the MRS per day so that, from the first day of the third week until the end of the fourth week, the rats ran at 50–60% of the MRS (~0.6–0.7 km/h), 0° grade, during 1 h/day. In addition, the MRS test was performed at the end of the fourth week of training for the animals from the T group to update the training intensity. Thus, from the first day of the fifth week until the end of the eighth week, the rats ran at 50–60% of the MRS, which corresponded to ~0.8–0.9 km/h, 0° grade, during 1 h/day. Two days after the last training session, the MRS test was repeated on animals from the C and T groups to evaluate their total exercise time until fatigue (TTF). During the training period, animals from the C group were handled every day and subjected to a short period of mild exercise (5–10 min, 0% grade, 0.3 km/h, 3 days/wk). This exercise intensity and duration were below the levels required to produce training adaptations (3).

The rats’ body weight (BW) was measured every week, and the resting heart rate (RHR) was recorded before beginning the training protocol and 2 days after the last exercise session using a tail-cuff method (PowerLab 4/30, ADInstruments).

Cardiomyocyte isolation. Two days after the last MRS test, the rats were weighed and then killed by cervical dislocation, and their hearts were quickly removed. The RV and LV myocytes were enzymatically isolated, as previously described (34). Briefly, the hearts were mounted on a Langendorff system and perfused for ~5 min with a modified HEPES-Tyrode solution with the following composition (in mM): 130 NaCl, 1.43 MgCl2, 5.4 KCl, 0.75 CaCl2, 5 HEPES, 10 glucose, 20 taunine, and 10 creatine, pH 7.3, at 37°C. The perfusion solution was changed to a calcium-free solution containing EGTA (0.1 mM) for 6 min, and, after that, hearts were perfused for 15–20 min with a solution containing 1 mg/ml collagenase type II ( Worthington). The digested heart was subsequently removed from the perfusion apparatus, and the RV and the LV were carefully isolated and weighed. The interventricular septum was considered to be part of the LV. The RV and the LV were cut into small pieces and placed separately in small conical flasks with collagenase-containing solution supplemented with 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO). The cells were dispersed by agitation the flask for periods of 5 min at 37°C. Then the single cells were separated from the nondigested tissue by filtration. The resulting cell suspension was centrifuged and resuspended in the HEPES-Tyrode solution. The nondigested tissue was subjected to further enzyme treatment, and the isolated cells were stored at ~5°C until needed. Only the calcium-tolerant, quiescent, rod-shaped cardiomyocytes, which demonstrated clear cross striations, were studied. The isolated cardiomyocytes were used within 2–3 h after isolation.

$[Ca^{2+}]_i$, measurements. $[Ca^{2+}]_i$ transients were evaluated, as previously described (37). Briefly, the freshly isolated cardiomyocytes from the RV and LV were loaded with fluo 4-AM, membrane-permeant form of the $Ca^{2+}$ indicator at 5 μM (Molecular Probes, Eugene, OR) for 20 min at room temperature and subsequently washed with the 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 suprathreshold voltage square pulses. Cells were stimulated at 1 Hz to produce steady-state conditions. A Meta LSM 510 scanning system (Zeiss) with a ×63 oil immersion objective was used for confocal fluorescence imaging. Fluoo 4 was excited at 488-nm Argon laser, and the emission intensity was measured at >510 nm. For recording $[Ca^{2+}]_i$ transients, the cardiomyocytes were scanned with a 512-pixel line, which was positioned randomly along the longitudinal axis of the cell; however, care was taken to avoid crossing the nuclei. The cells were scanned every 1.54 ms, and the sequential scans were stacked to create two-dimensional images with time on the x-axis. The digital image was processed using custom-written routines in the Matlab platform. The $Ca^{2+}$ levels were reported as F/F0, where F is the maximal fluorescence intensity average measured during the systolic phase of the $[Ca^{2+}]_i$ transients, and F0 is the minimal fluorescence intensity average measured between 1-Hz contractions during the diastolic phase of the $[Ca^{2+}]_i$ transients. In addition, the time to peak of the $[Ca^{2+}]_i$ transient and time from peak transient to one-half resting level of $[Ca^{2+}]_i$ were determined.

Measurement of cellular contractility and dimensions. Cellular contractility was evaluated as previously described (38). Briefly, the isolated cardiomyocytes were placed in a chamber with a glass coverslip base mounted on the stage of an inverted microscope (Nikon Eclipse, TS100). The chamber was perfused with HEPES-Tyrode solution at room temperature. Steady-state 1-Hz contractions were elicited via platinum bath electrodes (Myopacer, Field Stimulator, Ionoptix) with 5-ms voltage pulses and an intensity of 20 V. The cells were visualized on a PC monitor with a NTSC camera (Myocam, Ionoptix) in a partial scanning mode. This image was used to measure cell shortening (our index of contractility) in response to electrical stimulation using a video motion edge detector (IonWizard, Ionoptix). The cell image was sampled at 240 Hz. Cell shortening was calculated from
the output of the edge detector using an A/D converter (Ionoptix, Milton, MA). Cell shortening (expressed as a percentage of resting cell length) and maximal velocity of contraction and relaxation were calculated. In addition, the cell image was used to determine the cell dimensions. The measurements of resting cell length and midpoint width were used to calculate the length-to-width ratio (length/width), as previously described (9), and volume was calculated considering the cardiomyocytes as rod shaped.

Western blotting. Western blotting was performed as previously described (18) with minor modifications. Briefly, the RV and LV samples harvested from the myocardium after enzymatic perfusion, which were previously frozen in liquid nitrogen, were homogenized in a buffer containing 50 mM potassium phosphate (pH 7.0), 0.3 M sucrose, 0.5 mM DTT, 1 mM EDTA (pH 8.0), 0.3 mM PMSF, 10 mM NaF, and phosphatase inhibitors cocktail (1:100, Sigma-Aldrich, St. Louis, MO). Samples were subjected to SDS-PAGE in polyacrylamide gels (either 6 or 8%, depending on the molecular weight) and uniform transfer efficiency were monitored by staining the membrane with 0.3% Ponceau. Then the membrane was blocked [5% nonfat dry milk, 10 mM Tris·HCl (pH 7.6), 150 mM NaCl, and 0.1% Tween 20] for 2 h at room temperature and incubated with specific antibodies overnight at 4°C. The following primary antibodies (Abcam) were used: polyclonal antibodies for total PLB (PLBt, 1:1,000), PLBser16 (1:1,000), and SERCA2a (1:2,500); monoclonal antibodies for Na+/K+ ATPase (Abcam, 1:30 h at room temperature. Immunocomplexes were detected by using the chemiluminescence reaction (ECL kit; Amersham Biosciences) and subsequent densitometric analyses using the ImageJ software. Expression levels of GAPDH were used to normalize the data.

Statistical analysis. The Kolmogorov-Smirnov test for normality was initially performed, and data were normally distributed. Paired Student’s t-test was used to compare the effects of training on initial and final values of BW, RHR, and TTF between C and T groups. Unpaired Student’s t-test was used to compare ventricular weight (VW), initial and final values of BW, RHR, and TTF between groups. Two-way ANOVA with post hoc testing by Tukey were used to compare the effects of training and region on [Ca2+]i transients, and cell contractility.

RESULTS

A statistical significance level of 5% was adopted. Values are means ± SE of 7 animals in each group. C, control; T, trained; BW, body weight; VW, ventricular weight; LVW, left ventricular weight; RVW, right ventricular weight; RHR, resting heart rate; TTF, total exercise time until fatigue. *P < 0.05 vs. baseline within the same group. †P < 0.05 vs. C group.

Whole cell [Ca2+]i transients and cell contractility. Figure 2 demonstrates typical line-scan images recorded from field-stimulated cardiomyocytes that were loaded with the fluorescent Ca2+ indicator fluo 4-AM, along with representative records of cardiomyocytes contractions. As shown in Figs. 3A and 4A, compared with the C group, LV myocytes obtained from the T group exhibited ~13% (n = 90 cells) and ~14% (n = 90 cells) increase in the [Ca2+]i transient amplitude and cell shortening (P < 0.05), respectively. LIET increased the cell shortening from LV myocytes compared with RV myocytes (Fig. 4A). No significant differences in the [Ca2+]i transient amplitude and cell shortening in the RV myocytes from the C and T groups were observed. Notably, no differences (P > 0.05) were observed in the basal fluorescence intensity average that was measured between contractions at the diastolic phase of [Ca2+]i, transients between the two groups.

In LV myocytes, LIET decreased time to peak of [Ca2+]i transient (Fig. 3B) and time from peak to one-half resting level of [Ca2+]i (Fig. 3C) of cells from T group compared with those from C group. LV myocytes from C group showed longer time/width than respective RV myocytes group (Fig. 1D). No significant differences were observed in cell length, width, volume, and length/width in the RV myocytes obtained from either group (Fig. 1).

Table 1. Body and ventricular weights, resting heart rate, and physical capacity

<table>
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<th>C</th>
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<tr>
<td>Initial BW, g</td>
<td>406 ± 14</td>
<td>389 ± 13</td>
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<tr>
<td>Final BW, g</td>
<td>439 ± 15**</td>
<td>429 ± 12*</td>
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<tr>
<td>VW, g</td>
<td>1.81 ± 0.13</td>
<td>2.01 ± 0.12</td>
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<td>LVW/BW, mg/g</td>
<td>4.10 ± 0.26</td>
<td>4.80 ± 0.13†</td>
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<tr>
<td>LVW, g</td>
<td>1.42 ± 0.11</td>
<td>1.56 ± 0.10</td>
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<tr>
<td>LVW/BW, mg/g</td>
<td>3.27 ± 0.20</td>
<td>3.67 ± 0.19†</td>
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<tr>
<td>RVW, g</td>
<td>3.07 ± 0.03</td>
<td>3.06 ± 0.03</td>
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<tr>
<td>RVW/BW, mg/g</td>
<td>0.714 ± 0.06</td>
<td>0.860 ± 0.05</td>
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<td>Initial RHR, beats/min</td>
<td>333 ± 10</td>
<td>344 ± 10</td>
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<tr>
<td>Final RHR, beats/min</td>
<td>333 ± 9</td>
<td>320 ± 12†</td>
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<tr>
<td>Initial TTF, min</td>
<td>9.95 ± 1.52</td>
<td>11.52 ± 1.36</td>
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<tr>
<td>Final TTF, min</td>
<td>11.49 ± 1.74</td>
<td>22.67 ± 1.62†</td>
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Values are means ± SE of 7 animals in each group. C, control; T, trained; BW, body weight; VW, ventricular weight; LVW, left ventricular weight; RVW, right ventricular weight; RHR, resting heart rate; TTF, total exercise time until fatigue. *P < 0.05 vs. baseline within the same group. †P < 0.05 vs. C group.
with C group and to RV T group. However, the expression of these proteins was not affected in the RV (Fig. 5). The PLBser16-to-PLBt ratio (LV: C, 0.99/0.22 and T, 1.18/0.20; RV: C, 1.02/0.27 and T, 1.07/0.27), and the expression levels of PLBt (Fig. 5A) and NCX (Fig. 5D) in the RV and LV were not significantly affected by LIET ($P > 0.05$).

**DISCUSSION**

The aim of the present study was to compare the effects of LIET on the structural and mechanical properties of the RV and LV myocytes isolated from adult rats. Our main finding was that LIET produced changes in the morphological, molecular, and mechanical properties of the myocytes isolated from the LV. Confirming our hypothesis, all of these changes were not extensive to the RV myocytes, suggesting that the different hemodynamic overload occurring in the two ventricular chambers during exercise, and not the sympathetic discharge, may produce different adaptations at the tissue, cellular, and molecular levels.

*Whole animal data.* As expected, LIET improved the exercise capacity (measured as TTF) and decreased the RHR. This result suggests that the exercise protocol was efficient in producing cardiac adaptations, which are typical under such conditions. The resting bradycardia has been considered to be a hallmark of exercise training adaptation in experimental animals (10, 13, 16) and humans (39). Lower heart rate during resting appears to be associated with lower myocardial oxygen consumption, increased stroke volume (47), and a decrease in the occurrences of cardiovascular diseases (23, 33).

We observed that LIET increased the VW/BW and LVW/BW, along with LV myocyte length, volume, and length/width. The increase in the RVW/BW of 17% did not reach statistical significance at the level we have adopted (5%). Dispersion of the results related to the RVW was higher than in the LV. It seems, therefore, that the development of hypertrophy in the RV chamber is less uniform in relation to the LV. It is also possible that the time course of exercise-induced hypertrophy in the two ventricular chambers is different, which contributes to higher dispersion in one chamber than in the other. Literature data, however, describe that aerobic exercise-induced cardiac hypertrophy is more pronounced in the LV, while ventricular dilation is common in both ventricles (45). Studies have shown that endurance athletes developed eccentric LV hypertrophy and biventricular dilation with enhanced diastolic function (1, 12).

*Cardiomyocyte hypertrophy.* In our study, an eccentric-like hypertrophy type was observed (i.e., increased cell length/width) in the LV that contributes to chamber enlargement, suggesting that the different hemodynamic overload occurring in the two ventricular chambers during exercise, and not the sympathetic discharge, may produce different adaptations at the tissue, cellular, and molecular levels.

Regulation of the cardiomyocyte size contributes to the cellular involvement in the regulation of cardiac pump function (30). The adaptive growth of the cardiomyocytes in response to training is considered a physiological response to the hemodynamic overload produced by exercise. Cardiac hypertrophy can
be broadly divided into two different patterns, named as concentric or eccentric (32, 36). Eccentric cardiac hypertrophy is induced by the volumetric overload that occurs during dynamic endurance training, such as running. This type of cardiac hypertrophy is characterized by an augmented cardiomyocyte length, which leads to enlarged ventricle chamber that may enhance cardiac output, thereby enabling the heart to meet the demands for increased blood volume during exercise (36, 43). This cellular adaptation correlates with increased VW and chamber volume (the concept of athlete’s heart) and constitutes the underlying mechanism to the organ effect (15, 30).

**Cardiomyocyte inotropism and lusitropism.** Our results demonstrate that LIET induced lusitropic and inotropic benefits to the LV but not RV myocyte mechanical functions. An enhanced systolic function was observed as LIET promoted increases in the amplitude of LV myocyte [Ca$^{2+}$], transient and shortening, as well as faster rates of [Ca$^{2+}$] release and cell shortening. Additionally, we observed an improvement in the diastolic function due to faster LV myocyte [Ca$^{2+}$] transient decay and relengthening. Because [Ca$^{2+}$] transient controls cardiomyocyte contractile activity, exercise training-induced changes in the contractility are mediated by changes in the [Ca$^{2+}$] transient. Indeed, the related changes in the rates of rise and decay of [Ca$^{2+}$] transient and the rates of contraction and relaxation indicate that changes in contractility and in the [Ca$^{2+}$] transient are closely linked (26, 30). The changes in the rate of [Ca$^{2+}$] cycling observed in our study explain the changes in the shortening and relaxation rates of the LV myocytes observed in response to LIET.

**Ca$^{2+}$ handling proteins.** The cardiomyocyte [Ca$^{2+}$] transient and the contraction-relaxation process are controlled by proteins that regulate calcium handling (5–7). We demonstrated that LIET increased the LV expression of SERCA2a, phosphorylation of PLBser16, and reduced the PLBt-to-SERCA2a ratio without altering the NCX and PLBt levels. None of these changes were observed in the RV of T rats. These effects of LIET on the LV protein expression caused a decrease in the PLBt-to-SERCA2a ratio and therefore permitted an increase in SERCA2a activity. This implicates in increased SR Ca$^{2+}$ load, which leads to greater SR Ca$^{2+}$ release during systole and diastole. Our results point out a greater amount of Ca$^{2+}$ stored and released during systole in LV myocytes from LIET rats. Additionally, LIET increased the phosphorylation status of PLB (i.e., PLBser16), allowing further activation of SERCA2a contributing to diminish diastolic [Ca$^{2+}$]. This adaptation would be effective to avoid myocardial spontaneous activity. PLB phosphorylation relieves SERCA2a from inhibition by PLB. Serine 16 and threonine 17 are independently phosphorylated by cAMP-dependent protein kinase A and Ca$^{2+}$/calmodulin-dependent protein kinase II, respectively (21). We did not measure the PLB phosphorylation at threonine 17, and the increase in PLBser16 phosphorylation observed only in the LV in response to LIET was unexpected; thus further studies are needed to better clarify these points. It is known that activation of β$_1$-adrenergic receptors improves ventricular inotropic and lusitropic properties via phosphorylation of L-type voltage-dependent Ca$^{2+}$ channels, PLBser16, and troponin I (6). Although we have no data on
adrenergic signaling, it has been shown that there is difference neither in density nor in gene expression of \( \beta \)-adrenergic receptors between RV and LV in pigs (22) and rats (40). Voluntary exercise was shown not to alter gene expression of \( \beta_1 \)-adrenergic receptors but to reduce \( \beta_2 \)-adrenergic receptors in the LV of rats (41).

Our findings suggest enhanced SR-cytosol \( \mathrm{Ca}^{2+} \) cycling and provide a likely explanation for the faster \([\mathrm{Ca}^{2+}]_i\) decay and relengthening, as was observed in this study in T animals.

RV myocytes. The above-mentioned parameters did not change significantly in the RV myocytes in response to LIET. Under normal circumstances, the RV is connected serially to the LV and, therefore, is obligated to pump the same effective stroke volume. However, the RV wall is thinner and more compliant than the LV wall, and the RV has a unique physiology related to the low hydraulic impedance characteristics of the pulmonary vascular bed. The output of the LV and RV are similar; however, the RV output requires \( \sim 25\% \) of stroke work (implicating less energy cost) because of the low-pressure pulmonary system, in addition to the unique characteristics of the RV pressure-volume relationship (42). All of these specific characteristics of the RV chamber could potentially explain the absence of adaptations in response to LIET at the cellular and molecular levels.

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Fig. 3. Cardiomyocyte calcium transients. A: \( \mathrm{Ca}^{2+} \) transient amplitude. B: time to peak. C: time to one-half decay. Values are means ± SE of 90 cells in each group. \*P < 0.05 vs. C group in the same ventricular chamber. \#P < 0.05 vs. respective RV group.

Fig. 4. Cardiomyocyte contractile function. A: shortening. B: maximal velocity of shortening. C: maximal velocity of relengthening. Values are means ± SE of 90 cells in each group. \*P < 0.05 vs. C group in the same ventricular chamber. \#P < 0.05 vs. respective RV group.
Clinical implication. The regional analysis of cell function, including structural, mechanical, and molecular properties, assists to characterize the functional and morphological changes that accompany the endurance training-induced cardiac remodeling. Furthermore, advances in the comprehension of such cardiac remodeling will cooperate with clinical assessments of exercise performance and may help to distinguish the athlete’s heart from pathological forms of RV and LV hypertrophy.

Limitations. The absence of structural and mechanical adaptations in the RV may depend on the exercise type and intensity. We cannot rule out different results with other exercise types, such as swimming. Another possibility is that adaptive responses of the RV myocytes depend on more prolonged or intense exercise protocols. Moreover, we did not include a power calculation (β-error) when experiments were planned. Therefore, some statistical differences should not be detected because of the small number of animals in each group. However, all data presented normal distribution, and the sizes of our samples are consistent with the majority of the published studies in this issue. Our data should be viewed as a preliminary approach on this subject, since additional studies are necessary to indicate the specific effects of aerobic exercise on the structure and function of the RV.

Conclusions. Eight weeks of LIET improves the structural and mechanical properties of the LV myocytes in adult rats, which is associated with increased cellular length, volume, and length/width; improved Ca$^{2+}$ handling and cellular contractility via the upregulation of SERCA2a expression and PLB\textsubscript{ser16} phosphorylation; and reduction in PLBt-to-SERCA2a ratio. These adaptations, however, are restricted to the LV, suggesting that LIET for 8 wk exhibits limited capacity to produce these adaptations in the RV.

Fig. 5. Calcium regulatory proteins expression. Values are means ± SE of 6–7 animals in each group. A: total phospholamban (PLBt)/GAPDH. B: phospholamban phosphorylated at serine 16 (PLB\textsubscript{ser16})/GAPDH. C: sarcoplasmic reticulum Ca$^{2+}$-ATPase (SERCA2a)/GAPDH. D: Na$^+$/Ca$^{2+}$ exchanger (NCX)/GAPDH. E: PLBt/SERCA2a. *P < 0.05 vs. C group in the same ventricular chamber. #P < 0.05 vs. respective RV group.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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