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ORIGINAL ARTICLE

One year of exercise training promotes distinct adaptations in right and left ventricle of female Sprague-Dawley rats

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Abstract

Aerobic exercise training induces a unique cardioprotective phenotype, but it is becoming clear that it does not promote the same structural, functional, and molecular adaptations in both ventricles. In the present study, we aimed to better characterize and compare the molecular pathways involved in the exercise-induced remodeling of both ventricles. Female Sprague-Dawley rats were randomly assigned to control and exercise groups. Animals in the exercise group were submitted to low-intensity treadmill exercise for 54 weeks. After the experimental period, biventricular hemodynamic analysis was performed and right and left ventricles were harvested for morphological and biochemical analyses. Data showed that long-term low-intensity exercise training improves cardiac function, especially left ventricular diastolic function; however, the expression of connexin-43, CCAAT-enhancer binding protein β, and c-kit did not change in none of the ventricles. In the right ventricle, long-term exercise training induced an increase of manganese superoxide dismutase and sirtuin 3 protein expression, suggestive of improved antioxidant capacity. Our results also support that long-term aerobic exercise training imposes greater metabolic remodeling to the right ventricle, mainly by increasing mitochondrial ability to produce ATP, with no association to estrogen-related receptor α regulation.

Keywords Cardiac adaptation · Hemodynamics · Metabolism · Mitochondria · Oxidative stress · Treadmill exercise

Introduction

Endurance exercise training is widely recognized to induce a unique cardioprotective phenotype, making it a useful intervention for the prevention and treatment of cardiovascular diseases [14]. Cardiac hypertrophy is one of the major adaptations promoted by aerobic exercise, which confers mechanical advantages as it normalizes wall stress, decreases oxygen consumption, and increases work capacity [14]. Cross-sectional cardiac magnetic resonance imaging studies have consistently reported an increase in cardiac mass and volumes of both ventricles in endurance-trained athletes compared with that in age- and sex-matched sedentary subjects [4, 41]. Increase in cardiomyocyte length and width are considered the greater contributors to this physiologic cardiac growth but recent findings also support a role for the generation of new cardiomyocytes [27].

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Exercise-induced intrinsic changes in the left ventricle (LV) have been well characterized, but data depicting the changes that occur in the right ventricle (RV) following aerobic exercise training is scarcer. This is very important as there is growing body of evidence suggesting that adaptations of the RV do not always match those observed in the LV [23]. Indeed, for the same cardiac output, the RV has to deal with higher load and stroke work during endurance exercise as the pulmonary vascular bed can only reduce its resistance by 30–50% during exercise as compared with greater reductions in systemic vascular resistance (> 75%). Moreover, RV wall stress at peak exercise intensities rises by 170% compared with the rest, with only a 23% increase in the LV wall stress (reviewed by [23]). The greater hemodynamic overload imposed to the RV may justify distinct ventricular adaptations to exercise in the long term. In this sense, 1 year of endurance exercise training in previously untrained individuals was shown to result in a more pronounced increase in RV mass and volume than in the LV, paralleled with improved function [4]. Strenuous exercise was also associated with RV structural, electrical, and functional changes that were related with proarrhythmogenic remodeling in some athletes [18, 23]. Evidence from animal studies further strengthens the notion that exercise differently impacts the RV and LV by showing that long-term endurance exercise resulted in the development of myocardial fibrosis, myocardial stiffness, and diastolic dysfunction in the RV, but not in the LV [7, 40]. Moreover, despite the awareness that cardiac adaptations to exercise training are influenced by sex, single sex studies of males still predominate [6]. Among the few studies performed in female subjects, there are evidences of smaller increases in LV wall thickness, eccentric hypertrophy, and cavity size in female athletes compared with those in male ones [16]. The over-reliance on male animals in preclinical research poses a bias on the comprehension on the mechanisms underlying the beneficial effects of exercise that may be sex-dependent and thus could guide clinical studies [11].

Thus, while it is becoming clear that the structural and functional adaptations that occur in the RV following endurance exercise training may not be parallel with those observed in the LV, it is important to clarify what happens at the molecular level. So, the purpose of the present work was to compare the functional, structural, and biochemical changes induced by 54 weeks of low-intensity aerobic exercise training on the RV and LV, using female Sprague-Dawley rats.

Material and methods

Animals and experimental design

Housing and experimental treatment were in accordance with the *Guide for the Care and Use of Laboratory Animals* from the Institute for Laboratory Animal Research (ILAR 2011). Twenty female Sprague-Dawley rats (age, 5 weeks; weight, 130 ± 2.4 g at the beginning of the experiment), provided by Harlan Laboratories Models (Barcelona, Spain), were randomly assigned into the following two groups: sedentary (SED; $n = 10$; with restricted movement to the cage space) and exercised (EX; $n = 10$; submitted to treadmill exercise training). All animals were maintained in a controlled environment at a room temperature of 22 °C, on a 12:12-h light-dark reverse cycle, and with food (standard diet 4RF21®, Mucedola, Italy) and water ad libitum.

Exercise training program

Animals in the exercise-training group were habituated to treadmill running for 2 weeks. During this period, the running time and intensity were gradually increased until the animals were running 60 min/day at 20 m/min. After the habituation period, the EX animals ran 5 days/week for 60 min/day at 20 m/min (low-intensity exercise) [26], and this speed was maintained for 54 weeks. All the EX animals completed the training program.

Experimental preparations for hemodynamic evaluation

Twenty-four hours after the end of the exercise training protocol, all animals were anesthetized by inhalation with a mixture of 4% sevoflurane with oxygen and placed over a heating pad, to maintain the body temperature at 37 °C. Animals were tracheostomized for mechanical ventilation with oxygen-enriched air (60 cpm, tidal volume set at 1 mL/100 g, model 683: TOPO, Kent Scientific, Torrington, USA). The right jugular vein was cannulated under binocular surgical microscopy (Wild M651.MS-D; Leica, Herbugg, Switzerland) for administration of prewarmed 0.9% NaCl solution in order to balance the perioperative fluid losses. The heart was exposed by a median sternotomy, and the pericardium was extensively opened. Lastly, two conductance catheters (SPR-324; Millar Instruments, Houston, USA) were positioned on the LV and RV to assess cardiac performance in baseline conditions. The animals were allowed to stabilize for 15 min before the collection of the hemodynamic data.

Hemodynamic measurements

Hemodynamic data collection was performed as previously described in detail [32]. Parameters were recorded and online converted to a digital data with a sample frequency of 1000 Hz. Hemodynamic parameters included peak systolic pressure (Pmax), end-diastolic (EDP) and end-systolic

(ESP) pressure, and peak rate of pressure rise (dP/dtmax) and peak rate of pressure decay (dP/dtmin). The tau (time constant of relaxation rate) was estimated by fitting the isovolumetric pressure fall to a monoexponential function. Due to technical limitations, no volume-dependent parameters were obtained. All animals completed the experimental protocol. Data were stored and analyzed with Millar conductance data acquisition and analysis software (PVAN3.5).

Tissue collection

After completing the acquisition of the hemodynamic data, all animals were sacrificed through exsanguination. The heart and lungs were excised and weighed. Under binocular magnification (× 3.5), the LV free wall and RV free wall were dissected and weighed independently. Heart weight, LV, and RV were normalized to tibia length. The left *gastrocnemius* was also removed and weighed. Samples from lungs, LV, and RV from all animals were collected and fixed in a solution of 4% (v/v) buffered paraformaldehyde for latter histological analysis. Also, samples of LV and RV from all animals were collected for biochemical analysis.

Histologic and immunohistochemical analysis

Cubic pieces from cardiac muscle (RV and LV) and right lung were fixed (4% (v/v) buffered paraformaldehyde) by diffusion for 24 h and subsequently dehydrated with graded ethanol and included in paraffin blocks. Serial sections (5 μm of thickness) of paraffin blocks were cut by using a microtome and mounted on silane-coated slides. The slides were dewaxed in xylene and hydrated through graded alcohols finishing in phosphate-buffered saline solution. Deparaffinized sections of the heart were stained for hematoxylin-eosin or Sirius Red for the analysis of cross-sectional area (CSA) and fibrosis, and sections of the lungs were stained with hematoxylin-eosin for the analysis of medial hypertrophy of pulmonary artery, as previously described by us [33]. Sections were observed at light microscopy (Leitz, Wetzlar, Dialux 20, Germany) and photographed with a digital camera (XC30, Olympus, Germany). Cardiomyocyte CSA and medial hypertrophy of the pulmonary arteries were measured using the Cell B Olympus Software, and fibrosis was measured using Image J. Six images of random microscopic fields (magnification of × 400) were obtained from each section. Additional deparaffinized sections of cardiac tissue were prepared for immunohistochemical staining of connexin-43 (ab11370; Abcam, Cambridge, UK) and c-kit (sc-168; Santa Cruz, Heidelberg, Germany), as previously described by us [31].

Analysis of myosin heavy chain (MHC) isoform content

Right and left ventricle sections were weighed and homogenized (in the proportion of 1:19) in 100 mM phosphate buffer, pH 7.4, containing 0.02% bovine serum albumin (BSA), with a tightly fitted Potter-Elvehjem homogenizer and Teflon pestle at 0–4 °C. Total protein concentration was spectrophotometrically assayed with the DC method (Bio-Rad, CA, USA), using BSA as a standard. MHC isoform content was evaluated as previously described [36].

Right and left ventricle muscle preparation for biochemical analysis

A portion (~ 10 mg) of the right and left ventricle muscle was homogenized in 100 mM phosphate buffer, pH 7.4, supplemented with protease inhibitor, using a Teflon pestle on a motor-driven Potter-Elvehjem glass homogenizer at 0–4 °C (3–5 times for 5 s at low speed, with a final burst at a higher speed). The protein content of the cardiac muscle homogenates was assayed with the Bio-Rad DC method, following the instructions of the manufacturer, using BSA as a standard.

Citrate synthase activity

Citrate synthase activity was measured in RV and LV homogenates using the method described by Coore et al. [12]. In brief, the CoASH released from the reaction of acetyl-CoA (A2056; Sigma, Saint Louis, MO, USA) with oxaloacetate (O7753; Sigma) was measured by its reaction with 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB, D8130; Sigma) at 412 nm (molar extinction coefficient of 13.6 mM^{-1} cm^{-1}).

Isolation of mitochondria from cardiac muscle

Mitochondria isolation was performed as previously described [13]. In brief, samples from both ventricles were minced in an ice-cold isolation medium containing 250 mM sucrose, 0.5 mM EGTA, 10 mM HEPES-KOH (pH 7.4), and 0.1% defatted BSA (Sigma). The minced blood-free tissue was resuspended in isolation medium containing subtilopeptidase A type VIII (1 mg/g tissue) and homogenized. The homogenate was centrifuged at 14,500*g* for 10 min, and the pellet was gently resuspended in isolation medium. The suspension was centrifuged (750*g*, 10 min), and the resulting supernatant was centrifuged again (12,000*g*, 10 min). The new pellet was resuspended and repelleted (12,000*g*, 10 min) in isolation medium minus the BSA. Finally, the pellet, containing the mitochondrial fraction, was resuspended in a medium containing 250 mM sucrose, 10 mM HEPES-KOH, at pH 7.4. Phosphatase and protease inhibitors (P2850, P5726, P8340; Sigma) were added, and all the procedures were performed at 4 °C. The protein content was assayed with the Bio-Rad DC assay using BSA as a standard. ATP synthase activity was evaluated in mitochondrial extracts as previously described [38].

Immunoblotting analysis

Equivalent amounts of right and left ventricle protein or mitochondrial protein were electrophoresed on a 12.5% SDS-PAGE as described by Laemmli [24]. Gels were blotted onto nitrocellulose membranes (Whatman®, Protan®, Merck, Darmstadt, Germany) in transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3, and 20% methanol) for 2 h (200 mA). Then, nonspecific binding was blocked with 5% (w/v) nonfat dry milk in TBS-T (100 mM Tris, 1.5 mM NaCl, pH 8.0, and 0.5% Tween 20). Membranes were incubated with primary antibody solution diluted 1:1000 in 5% (w/v) nonfat dry milk in TBS-T (mouse anti-ATP synthase subunit beta, ab14730, Abcam; rabbit anti-GAPDH, ab9485, Abcam; rabbit anti-ETFDH, ab91508, Abcam; rabbit anti-Estrogen-related Receptor α (ErRα), 07-662, Merck; mouse anti-MnSOD, ALX-804-265-C100, Alexis, Farmingdale, NY; rabbit anti-PGC1 alpha, ab54481, Abcam; rabbit anti-mtTFA, sc-28200, Santa Cruz; mouse anti-total OXPHOS, ab110413, Abcam; rabbit anti-c-kit, sc-168, Santa Cruz; rabbit anti-CEBP-beta, ab32358, Abcam; rabbit anti-connexin-43, ab63851, Abcam; rabbit anti-SIRT3, #2627, Cell Signaling, MA, USA; rabbit anti-RAF1, ab32025, Abcam). After a 2-h incubation at room temperature with agitation, membranes were washed with TBS-T and incubated, with agitation, with anti-mouse, or with anti-rabbit IgG peroxidase secondary antibody (Merck) diluted 1:1000 in 5% (w/v) nonfat dry milk in TBS-T.

For the protein carbonyl derivative assay, a given volume (V) of the sample containing 20 μg of protein was derivatized with 2,4-dinitrophenylhydrazine (DNPH). Briefly, the sample was mixed with 1 V of 12% SDS, 2 V of 2 mM DNPH/10% trifluoroacetic acid, followed by 30 min of incubation in the dark, after which 1.5 V of 2 M Tris-base/18.3% of β-mercaptoethanol was added for neutralization. After diluting the derivatized proteins in TBS to obtain a final concentration of 0.001 μg/μL, a 100 μL volume was slot-blotted into a nitrocellulose membrane. For 3-nitrotyrosine expression, 30 μg of protein from RV and LV samples were diluted in TBS to obtain a final protein concentration of 0.001 μg/μL and a volume of 100 μL was slot-blotted into a nitrocellulose membrane. The slot-blot membranes were processed as above using anti-3-nitrotyrosine (MAB 5404, EMD Millipore, Merck) or anti-DNP (MAB 2223, EMD Millipore, Merck) primary antibodies diluted 1:1000 in 5% w/v nonfat dry milk in TBS-T.

Immunoreactive bands were detected with enhanced chemiluminescence reagents (ECL, Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's procedure, and images were recorded using X-ray films (Amersham Hyperfilm ECL, GE Healthcare). The films were scanned in Molecular Imager Gel Doc XR + System (Bio-Rad) and analyzed with Image Lab software (v4.1, Bio-Rad). Protein loading was controlled by Ponceau S staining once the expression of structural proteins as actin or tubulin was previously noticed to change with exercise training.

Statistical analysis

Values are given as mean ± standard deviation for all variables. Kolmogorov-Smirnov test was performed to check the normality of the data. When variables were normally distributed, significant differences between the groups were evaluated using an unpaired *t* test (data sets with 2 variables) or a two-way analysis of variance (data sets with 4 variables) followed by the Bonferroni post hoc test. Kruskal-Wallis test followed by Dunn's Multiple Comparison Test was used for nonparametric variables (histological data). Results were considered significantly different when $P < 0.05$. Statistical analysis was performed with Graph Pad Prism software, version 5.0.

Results

Impact of exercise training on morphometric parameters and cardiac hemodynamics

Fifty-four weeks of endurance exercise training resulted in a significant increase of body weight ($P < 0.05$), *gastrocnemius* mass ($P < 0.001$), and a lower RV weight and RV/tibia ratio in comparison with its sedentary counterparts ($P < 0.01$; Table 1). No significant alterations were observed for lungs

Table 1 Morphometric characterization

	SED	EX
Body weight (kg)	0.291 ± 0.031	0.323 ± 0.036*
Gastrocnemius mass (g)	1.988 ± 0.120	2.230 ± 0.170***
Lungs mass (g)	1.946 ± 0.287	1.931 ± 0.356
Lungs/tibia (g/cm)	0.501 ± 0.076	0.472 ± 0.101
Heart mass (g)	1.051 ± 0.121	1.041 ± 0.120
Heart/tibia (g/cm)	0.271 ± 0.030	0.264 ± 0.035
Left ventricle (g)	0.572 ± 0.084	0.600 ± 0.109
Left ventricle/tibia (g/cm)	0.147 ± 0.020	0.145 ± 0.030
Right ventricle (g)	0.225 ± 0.068	0.170 ± 0.017**
Right ventricle/tibia (g/cm)	0.058 ± 0.016	0.043 ± 0.004**

SED sedentary, *EX* exercise. Values are presented as mean ± standard deviation ($n = 10$ *per* group). *$P < 0.05$ vs. SED; **$P < 0.01$ vs. SED; ***$P < 0.001$ vs. SED mass, lungs/tibia, heart weight, LV weight, heart/tibia, or LV/tibia length.

Hemodynamic RV and LV profile at baseline conditions is presented in Table 2. Long-term exercise training resulted in a significant decrease in resting heart rate ($P < 0.05$). In comparison with the SED group, the EX group had higher LV Pmax ($P < 0.01$) and ESP ($P < 0.01$) and lower EDP ($P < 0.05$). No significant alterations were detected in the remaining LV hemodynamic parameters between the SED and EX. Regarding the RV, no significant alterations were detected in the hemodynamic parameters between the SED and EX.

Impact of exercise training on histological parameters

The exercise-training program resulted in a significant increase in LV cardiomyocyte CSA when compared with the SED animals ($P < 0.001$; Fig. 1). In the EX group, higher cardiomyocyte CSA was observed in LV compared with that in RV ($P < 0.001$). Accumulation of total collagen was higher in the RV of the SED animals in comparison with that in LV ($P < 0.001$), while no significant changes were detected between ventricles in the EX group. Inter-group comparisons showed that the EX group presented significantly less accumulation of total collagen in the RV than in the SED group ($P < 0.001$), but no differences were observed in the LV. Medial hypertrophy of pulmonary arteries was also significantly reduced in the EX animals in comparison with that in the SED ($P < 0.01$; Fig. 1).

We determined the protein expression levels of connexin-43 (Cx43; Fig. 2a) that was predominantly expressed at the desmosome level (Fig. 2b). No significant changes of Cx43 content and cellular location were observed in both ventricles of trained animals (Fig. 2a, b). However, the content of Cx43 was significantly higher in the LV than in the RV of EX animals ($P < 0.05$; Fig. 2a).

Moreover, after 54 weeks of endurance training, there were no signs of cardiac progenitor cell activation in both ventricles given by the expression levels of c-kit (Fig. 2c), a marker of a major type of cardiac progenitor cells [50], and of CCAAT-enhancer binding protein β (C/EBPβ) (Fig. 2d), a transcription factor involved in the repression of cardiomyocytes proliferation [9]. No differences in the pattern of c-kit immunostaining were observed among trained and sedentary animals (data not shown). No expression differences of c-kit and C/EBPβ were noticed among ventricles within each group.

Impact of exercise training on MHC isoform profiling

Figure 1d shows MHC isoforms' profile of the RV and LV muscles from sedentary and exercised animals. Two isoforms were observed in the gel, with predominance of alpha-MHC. No significant changes were observed regarding beta/alpha MHC ratio in both ventricles, despite the significant lower levels of this ratio in the LV of both the EX and SED in comparison with that in the RV ($P < 0.05$).

Impact of exercise training in cardiac metabolic remodeling

In order to evaluate the impact of 54 weeks of exercise training on heart metabolism, the levels of metabolic enzymes were evaluated by Western blotting in RV and LV total extracts. No changes were noted on the levels of ATP synthase while levels of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were significantly lower in the RV of exercised animals in comparison with those in sedentary rats ($P < 0.01$ vs. SED; Fig. 3a, b). The ratio of ATP synthase to

Table 2 Hemodynamic evaluation at basal conditions

HR (bpm)	SED		EX	
	313.10 ± 28.42		292.20 ± 17.83*	
	RV	LV	RV	LV
Pmax (mmHg)	32.48 ± 3.79	146.50 ± 16.92$^{\#\#\#}$	26.93 ± 1.43	160.40 ± 12.48^{**+++}
Pmin (mmHg)	0.22 ± 0.04	1.83 ± 1.72$^{\#}$	0.25 ± 0.14	1.76 ± 1.59^{+}
ESP (mmHg)	31.61 ± 4.56	143.00 ± 15.87$^{\#\#\#}$	25.75 ± 2.11	157.90 ± 12.06^{**+++}
EDP (mmHg)	5.18 ± 0.73	8.74 ± 4.36$^{\#}$	4.13 ± 1.56	5.80 ± 1.97*
dP/dtmax (mmHg/s)	1999.00 ± 118.70	9658.00 ± 1706.00$^{\#\#\#}$	1928.00 ± 224.00	9768.00 ± 764.80^{+++}
dP/dtmin (mmHg/s)	− 1654.00 ± 391.30	− 10.550.00 ± 1950.00$^{\#\#\#}$	− 1211.00 ± 265.80	− 11.070.00 ± 376.70^{+++}
Tau (ms)	11.62 ± 4.66	11.59 ± 1.39	11.53 ± 3.10	11.55 ± 0.95

SED sedentary, *EX* exercise, *LV* left ventricle, *RV* right ventricle, *HR* heart rate, *Pmax* maximum pressure, *Pmin* minimum pressure, *ESP* end systolic pressure, *EDP* end diastolic pressure, *dP/dtmax* maximum rate of pressure elevation, *dP/dtmin* maximum rate of pressure decay, *Tau* isovolumic relaxation constant. Values are presented as mean ± standard deviation ($n = 10$ *per* group). *$P < 0.05$ vs. respective SED group; **$P < 0.01$ vs. respective SED group; $^{\#}$ $P < 0.05$ vs. SEDRV; $^{\#\#\#}$ $P < 0.001$ vs. SEDRV; $^{+}$ $P < 0.05$ vs. EXRV; $^{+++}$ $P < 0.001$ vs. EXRV

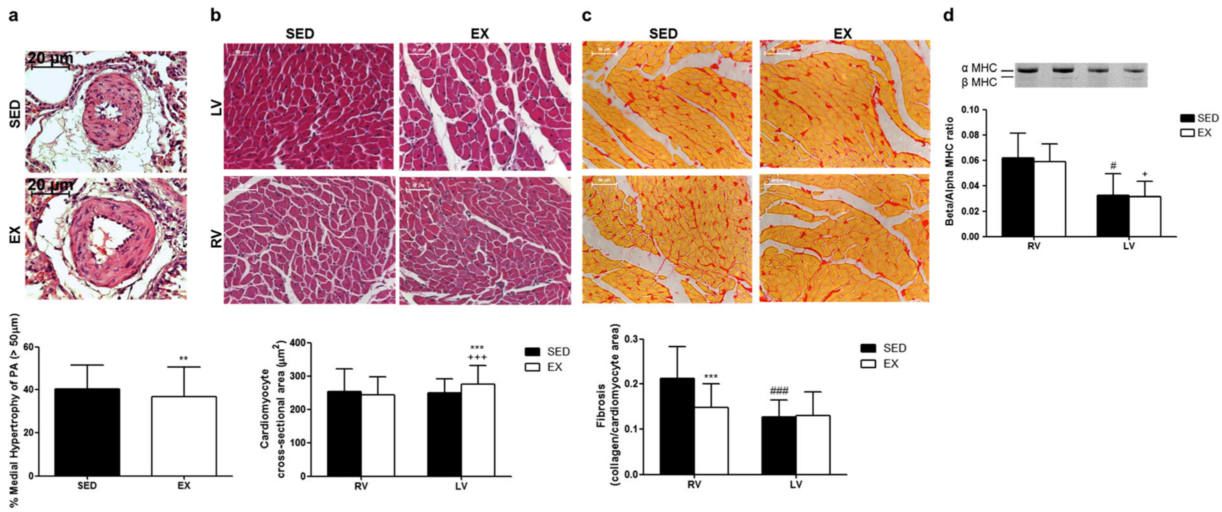

Fig. 1 Effect of exercise training on pulmonary artery hypertrophy (**a**), cardiomyocyte cross-sectional area (**b**), fibrosis (**c**), and MHC isoform ratio (**d**) in LV and RV. SED sedentary, EX exercise, LV left ventricle, RV right ventricle. Representative gel image of MHC isoform (alpha MHC, 223.5 kDa; beta MHC, 223.1 kDa) separation is shown above the correspondent graph (sample order has correspondence to the order of the groups presented in the graph). Values are presented as mean ± standard deviation ($n = 6$–10 *per* group). $^{**}P < 0.01$ vs. SED group, $^{***}P < 0.001$ vs. respective SED group, $^{\#}P < 0.05$ vs. SEDRV, $^{\#\#\#}P < 0.001$ vs. SEDRV, $^{+}P < 0.05$ vs. EXRV, $^{+++}P < 0.001$ vs. EXRV

GAPDH, a rough marker of oxidative metabolism, was also increased in the RV of exercised animals though without statistical significance (Fig. 3c). As shown in Fig. 3d, exercise training did not promote significant alterations in the cardiac levels of the subunits NDUFB8 from complex I, SDHB from complex II, UQCRC2 from complex III, MTCO1 from complex IV, and ATP5A from complex V of oxidative phosphorylation (OXPHOS), respectively, in none of the ventricles. However, when the content of ATP5B was analyzed in mitochondria isolated from cardiac muscle (pool of both

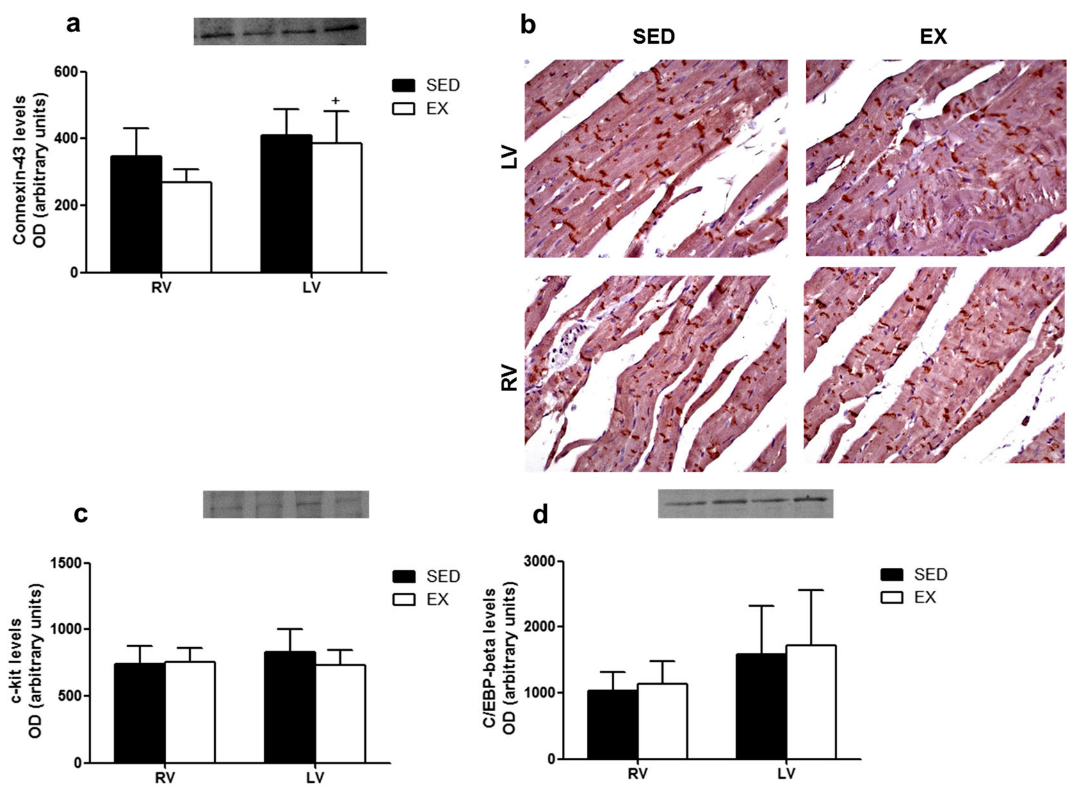

Fig. 2 Effect of exercise training on connexin-43 (43 kDa) content (**a**), cell location in RV and LV (**b**), on c-kit (145 kDa) (**c**), and C/EBP-beta (36 kDa) content (**d**). SED sedentary, EX exercise, LV left ventricle, RV right ventricle, C/EBP-beta CCAAT-enhancer binding protein β. Representative immunoblots are shown above the correspondent graph (sample order has correspondence to the order of the groups presented in the graph). Values are presented as mean ± standard deviation ($n = 4$–6 *per* group). $^{+}P < 0.05$ vs. EXRV

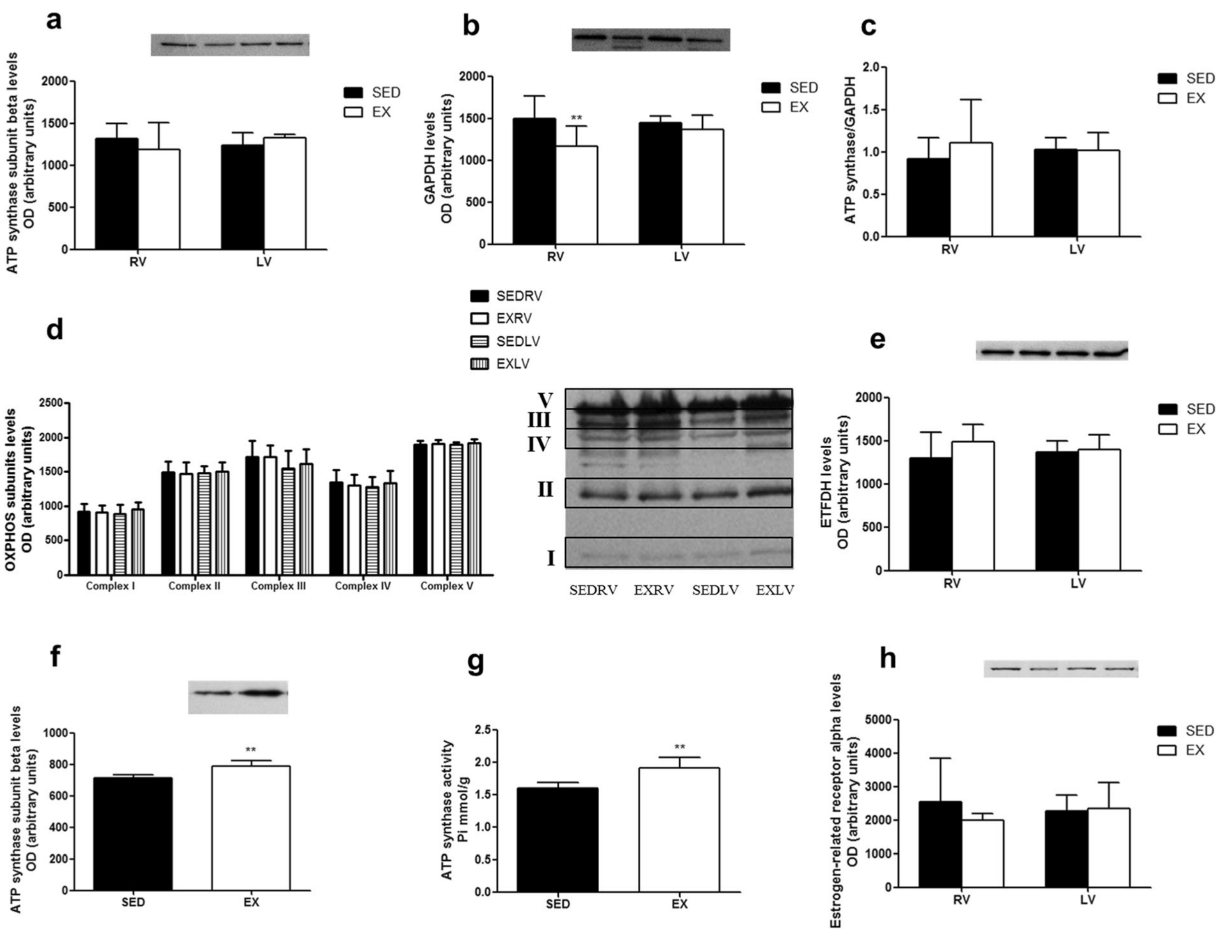

Fig. 3 Effect of exercise training on the levels of ATP synthase subunit β (52 kDa) (**a**); GAPDH (37 kDa) (**b**); ratio ATP synthase to GAPDH (**c**); OXPHOS subunits (complex V-ATP5A (55 kDa), complex III-UQCRC2 (48 kDa), complex IV-MTCO1 (40 kDa), complex II-SDHB (30 kDa), complex I-NDUFB8 (20 kDa)) (**d**), ETFDH (69 kDa) (**e**), and on the levels of mitochondrial ATP synthase subunit β (**f**), on the ATP synthase activity (**g**), and on the levels of estrogen-related receptor alpha (53 kDa) (**h**) in RV and LV. SED sedentary, EX exercise, LV left ventricle, RV right ventricle, OXPHOS oxidative phosphorylation, GAPDH glyceraldehyde 3-phosphate dehydrogenase, ETFDH electron transfer flavoprotein:ubiquinone oxidoreductase. Representative immunoblots are shown above the correspondent graph (sample order has correspondence to the order of the groups presented in the graph). Values are presented as mean ± standard deviation ($n = 4$–6 *per* group). $^{**}P < 0.01$ vs. SED group

ventricles), a significant increase of its content and of complex V activity in trained animals was noticed ($P < 0.01$ vs. SED; Fig. 3f, g). These data suggest that despite no alterations in OXPHOS subunits levels in the RV and LV, exercise increased mitochondrial ability to produce ATP. Moreover, the content of the electron transfer flavoprotein:ubiquinone oxidoreductase (ETFDH), which conducts electrons from nine different mitochondrial FAD-containing acyl-CoA dehydrogenases of fatty acid β-oxidation to the ubiquinone pool of the main respiratory chain [3], was not modulated by exercise training in none of the ventricles (Fig. 3e), suggesting no alterations in fatty acid oxidation. Moreover, 54 weeks of exercise did not impact the content of estrogen-related receptor α (ErRα) in both ventricles. No differences of ErRα levels were observed between ventricles (Fig. 3h).

Regarding citrate synthase (CS) activity, we observed a significant increase in the LV of both the SED and EX groups in comparison with that in their RV ($P < 0.001$ vs. SEDRV and $P < 0.01$ vs. EXRV), but no differences were noted with training (Fig. 4a). CS activity has been suggested as a marker of mitochondrial content [8], so data points to no alterations in the pool of mitochondria from each ventricle due to prolonged exercise training. The levels of peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α), a key player in the regulation of mitochondria biogenesis [15], were significantly lower in the LV of exercised animals in comparison with those in their RV ($P < 0.05$) and in comparison with those in the LV of sedentary animals ($P < 0.01$; Fig. 4b). Differences between RV and LV of exercised animals were more expressive when the levels of PGC-1α were normalized to the number of mitochondria, indirectly assessed by CS activity ($P < 0.001$; Fig. 4c). So, data suggest that mitochondrial biogenesis is decreased in exercised LV. A similar trend of PGC-1α levels was noticed for the content of the

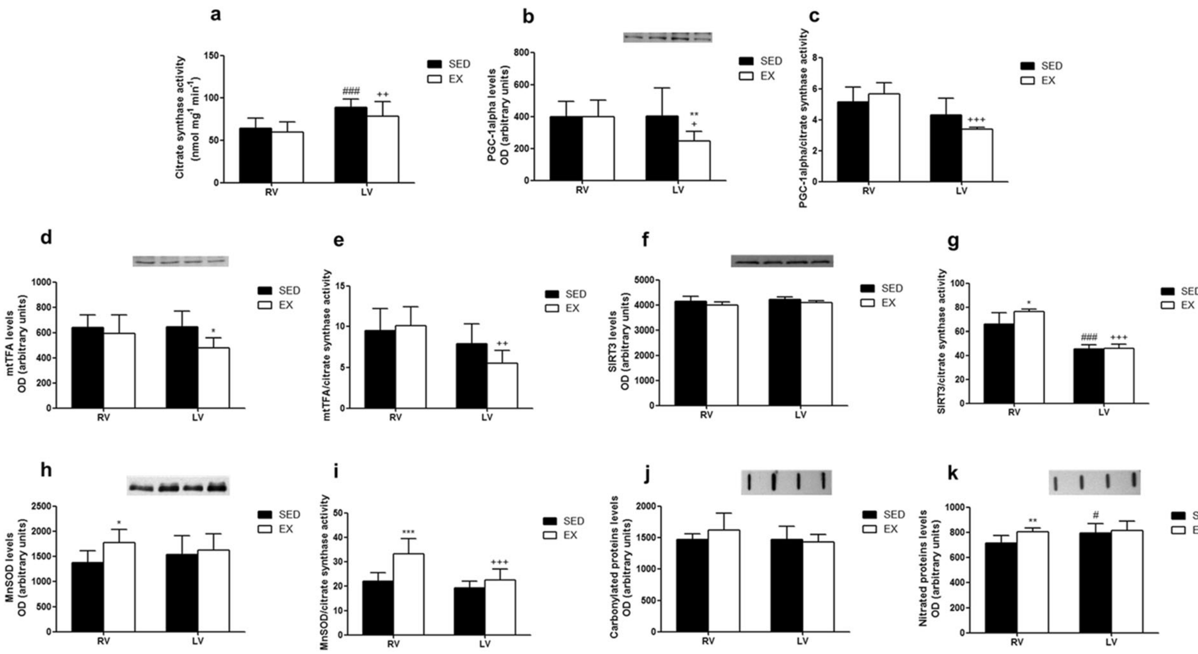

Fig. 4 Effect of exercise training on CS activity (**a**), PGC-1α (105 kDa) content (**b**), ratio PGC-1α to CS activity (**c**), Tfam (25 kDa) content (**d**), ratio Tfam to CS activity (**e**), SIRT3 (28 kDa) content (**f**), ratio SIRT3 to CS activity (**g**), MnSOD (24 kDa) content (**h**), and ratio MnSOD to CS activity (**i**) and on carbonylated (**j**) and nitrated (**k**) proteins in RV and LV. SED sedentary, EX exercise, LV left ventricle, RV right ventricle, CS citrate synthase, PGC-1α peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, mtTFA mitochondrial transcription factor A, MnSOD manganese-dependent superoxide dismutase, SIRT3 NAD-dependent deacetylase sirtuin-3. Representative immunoblots are shown above the correspondent graph (sample order has correspondence to the order of the groups presented in the graph). Values are presented as mean ± standard deviation ($n = 4$–6 *per* group). *$P < 0.05$ vs. respective SED group, **$P < 0.01$ vs. respective SED group, ***$P < 0.001$ vs. respective SED group, $^{+}P < 0.05$ vs. EXRV, $^{++}P < 0.01$ vs. EXRV, $^{+++}P < 0.001$ vs. EXRV, $^{\#}P < 0.05$ vs. SEDRV, $^{\#\#\#}P < 0.001$ vs. SEDRV

mitochondrial transcription factor mtTFA, which was lower in the exercised LV ($P < 0.05$; Fig. 4d).

The levels of RAF-1 were measured since this kinase has been implicated in the control of mitochondrial ROS and Ca^{2+} [14]. However, no expression differences were noticed among trained and sedentary animals in both ventricles (Supplementary Fig. 1), in opposition to the observed in isolated mitochondria (Supplementary Fig. 1).

The levels of the deacetylase SIRT3 were also determined in LV and RV extracts (Fig. 4f), considering its role in the regulation of mitochondrial metabolic pathways [2]. Exercise training did not change their expression levels in none of the ventricles (Fig. 4f). However, when data was normalized to CS activity, significant differences among ventricles were observed. Specifically, higher values were observed for RV compared with those for LV in both the EX and SED group ($P < 0.001$), and exercise training promoted a significant increase in SIRT3 *per* mitochondria in RV ($P < 0.05$; Fig. 4g). Data from the analysis of isolated mitochondria confirmed this exercise-related increase of SIRT3 levels (Supplementary Fig. 1). In the RV from exercised animals, there was a significant increase in MnSOD content in comparison with that in its sedentary counterparts ($P < 0.05$; Fig. 4h). More pronounced differences were detected when data was normalized to CS activity ($P < 0.001$; Fig. 4i). Moreover, the amount of this antioxidant enzyme *per* mitochondria was significantly higher in the RV than in the LV of exercised animals ($P < 0.001$; Fig. 4i).

In order to evaluate the impact of 54 weeks of endurance training on oxidative stress, we analyzed the content of oxidized proteins. In the RV of trained animals, a significant increase of nitrated proteins was observed ($P < 0.01$; Fig. 4k). The content of carbonylated proteins was also higher in the RV of exercised animals, though not statistically significant (Fig. 4j). In the LV, no alterations in the content of nitrated or carbonylated proteins were observed among experimental groups (Fig. 4j, k). However, the LV from the SED group showed greater levels of nitrated proteins in comparison with those in their RV ($P < 0.05$).

Discussion

Despite the well-documented benefits of endurance exercise training in promoting health, the molecular mechanisms underlying the long-term functional and structural changes that occur in the heart are not fully characterized, particularly in female subjects. Studies on the effects of exercise training have traditionally focused in the LV, even though overall cardiac performance is determined by two pumps in series. Both ventricles exhibit substantial differences in embryology, morphology, perfusion, workload, and downstream vascular beds, thus limiting any extrapolation of findings from the LV to the RV [1]. To add new insights on this topic, we analyzed RV and LV functional, structural, and molecular adaptations promoted by 1 year of endurance exercise training in female rats, which would correspond to approximately 35 years of training in humans [42], one of the longest exercise training programs performed in animal models.

In response to endurance exercise, the heart changes in size, shape, structure, and physiology. Indeed, we observed a 10% increase in the size of cardiomyocytes from the LV in exercised animals, despite no significant variation on LV mass (Table 1 and Fig. 1). This can be attributed in part to the low running intensity (20 m/min) as cardiac hypertrophy is known to be intensity-dependent. For instance, it has been shown that high-intensity aerobic exercise training (85–90% of VO_2max) resulted in 14% increase in cardiomyocytes' length, while moderate exercise intensity (65–70% of VO_2max) induced only a 5% increase [49]. Moreover, we maintained the same running speed throughout the entire duration of the study. The relative exercise load during an exercise training period decreases if the absolute load is kept constant as the exercise capacity improves, thus limiting more obvious changes in cardiac mass [47]. Trained animals also presented an increase in total body weight, which we attribute to maintenance of greater amounts of lean mass as suggested by their *gastrocnemius* mass.

Exercise-induced LV cardiomyocyte hypertrophy was paralleled with greater LV maximum and end-systolic pressures, and a nonsignificant increase in dP/dtmax, which might indicate greater systolic function [5]. Regarding LV diastolic proprieties, the EX animals presented a lower EDP, suggesting a less stiff myocardium, which seems to be due to intrinsic changes to the cardiomyocytes [29], since no differences were found regarding total collagen deposition.

Regarding RV, we observed a significant increase of its mass in the SED group compared with that in the EX group, which we attribute to fibrosis. Indeed, while both groups presented similar RV cardiomyocyte size, total collagen levels were elevated only in the SED. Changes occurring in the RV of the sedentary heart could be a compensatory response to age-dependent pulmonary vascular remodeling [43]. Indeed, we found a 10% increase in pulmonary artery hypertrophy in the SED animals. Moreover, we observed that sedentary animals presented greater LV EDP, and it is known that downstream left heart filling pressures also contribute to RV afterload [25].

Besides hypertrophy of the pre-existing myocytes, it has been reported that exercise training induces the formation of new cardiomyocytes from progenitor/stem cells [48, 50]. Data from the present study does not support the effect of 1 year of treadmill exercise training in the activation of cardiac regeneration and repair. These apparently contradictory findings could be related to the low intensity of our exercise training protocol as it was shown, for instance, that c-kit positive cardiac stem cell activation is exercise-intensity-dependent [35, 48]. Further supporting this, we found no changes on the expression of C/EBPβ transcription factor. Its downregulation (and consequent activation of the exercise gene set involved in cardiomyocyte proliferation) was also shown to be intensity-dependent, with greater reductions of C/EBPβ mRNA and protein levels occurring following high-intensity (85–90% VO_2max) exercise compared with low- (45–50% of VO_2max) or moderate-intensity exercise (at 55–70% VO_2max) [35]. Long-term endurance training has been associated with arrhythmogenic cardiac remodeling of the RV [18, 22]. In the current study, we observed a significant reduction of Cx43 expression in the RV of exercised animals in comparison with that in their LV values, which might be an adaptive response to the increased vagal tone observed in the trained animals. Cx43 is the main gap junctional cardiac protein responsible for the rapid conduction of the action potential across the heart and its disturbance was implicated in the induction of arrhythmia [10, 20]. Downregulation of Cx43 expression was previously shown with the use of the nonspecific beta-blocker propranolol, indicating that sympathetic tone is involved in Cx43 regulation [44].

The main molecular adaptations of the heart to long-term exercise training were seen at the metabolic level, mainly in the RV. The oxidative metabolism was increased in the RV of trained animals, mainly due to a significant reduction of glycolysis without a significant compensation by fatty acid oxidation (Fig. 3). Previous data corroborate our findings by showing decreased RV myocardial glucose utilization while free fatty acid oxidation remained unaltered with exercise training in humans [19]. This seems to be a sex-specific adaptation as myocardial glucose uptake was reduced in female mice after exercise, whereas cardiac glucose uptake was unaltered in male counterparts [17]. Such sex-specific response to exercise training was previously reported to be modulated by estrogen receptors [21, 34]; however, no alterations in the cardiac content of ErRα were observed following 1 year of treadmill exercise.

Because of the metabolic adaptation of RV towards an oxidative phenotype, there was an increase of nitrated proteins, possibly due to an augmentation in NO levels and of MnSOD content. Increased generation of NO leads to the formation of peroxynitrite, which then reacts with proteins' tyrosine residues [45]. The upregulation of MnSOD and the increased production of NO associated with eNOS activity have been pointed out as a cardioprotective mechanism induced by exercise training [14, 37, 39].

Despite the described effect of exercise training in endorsing mitochondrial biogenesis [14], our data do not support this cardiac adaptation. PGC-1α, which plays a key role in regulating genes involved in myocardial fuel metabolism and mitochondrial biogenesis [15], and the content of mitochondria, roughly assessed by CS activity [8], were not significantly modulated by 54 weeks of exercise (Fig. 4). However, there is no consensus in the literature regarding the impact of exercise training on mitochondrial biogenesis in the heart, as it may vary with sex, intensity, duration, or mode of exercise. For instance, 6 weeks of swimming was shown to promote an upregulation of mitochondrial biogenesis in the heart [46], whereas 3 months of running did not impact mitochondrial density in the LV [28]. In the regulation of PGC-1α expression, the deacetylase activity of SIRT3 has a key role [28]. This deacetylase also regulates the activity of several metabolic pathways harbored in mitochondria, namely antioxidant enzymes [2], protecting the heart against oxidative stress [30]. Our exercise-training protocol induced an upregulation of SIRT3 *per* mitochondria in the RV (Fig. 4). Data suggest that RV metabolic adaptation to 54 weeks of exercise training occurs mainly at the mitochondria level, resulting in an increased ability of this organelle to produce ATP (Fig. 3) to support the cardiomyocytes' contractile apparatus. The lower mitochondrial density of the RV compared with that of the LV might explain, at least in part, the greater molecular alterations noticed in trained RV that could be considered a beneficial adaptation to meet the greater work requirements imposed by exercise [23].

We believe that a more marked effect of exercise training would be detected if we progressively adjusted the training intensity throughout the entire duration of the training period. However, our emphasis was on duration and we clearly show that 1 year of low-intensity aerobic exercise protocol was able to induce different biventricular changes at the functional, structural, and molecular level.

In conclusion, findings from the present study show that long-term exercise training in female Sprague-Dawley rats has beneficial effects on the heart, with different impacts in both ventricles: (i) it improves cardiac function, especially LV diastolic function; (ii) it promotes cardiac growth mainly due to cardiomyocyte hypertrophy, more marked in the LV, with no apparent involvement of cardiac stem cells; (iii) it prevents the accumulation of collagen in the RV; and (iv) it increases the antioxidant capacity of RV.

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Conflict of interest The authors declare that they have no conflict of interest.

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