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Rat model of exercise-induced cardiac hypertrophy: hemodynamic characterization using left ventricular pressure-volume analysis

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Radovits T, Oláh A, Lux Á, Németh BT, Hidi L, Birtalan E, Kellermayer D, Mátyás C, Szabó G, Merkely B. Rat model of exercise-induced cardiac hypertrophy: hemodynamic characterization using left ventricular pressure-volume analysis. *Am J Physiol Heart Circ Physiol* 305: H124–H134, 2013. First published May 3, 2013; doi:10.1152/ajpheart.00108.2013.—Long-term exercise training is associated with characteristic structural and functional changes of the myocardium, termed athlete's heart. Several research groups investigated exercise training-induced left ventricular (LV) hypertrophy in animal models; however, only sporadic data exist about detailed hemodynamics. We aimed to provide functional characterization of exercise-induced cardiac hypertrophy in a rat model using the in vivo method of LV pressure-volume (P-V) analysis. After inducing LV hypertrophy by swim training, we assessed LV morphometry by echocardiography and performed LV P-V analysis using a pressure-conductance microcatheter to investigate in vivo cardiac function. Echocardiography showed LV hypertrophy (LV mass index: 2.41 ± 0.09 vs. 2.03 ± 0.08 g/kg, $P < 0.01$), which was confirmed by heart weight data and histomorphometry. Invasive hemodynamic measurements showed unaltered heart rate, arterial pressure, and LV end-diastolic volume along with decreased LV end-systolic volume, thus increased stroke volume and ejection fraction (73.7 ± 0.8 vs. $64.1 \pm 1.5\%$, $P < 0.01$) in trained versus untrained control rats. The P-V loop-derived sensitive, load-independent contractility indexes, such as slope of end-systolic P-V relationship or preload recruitable stroke work (77.0 ± 6.8 vs. 54.3 ± 4.8 mmHg, $P = 0.01$) were found to be significantly increased. The observed improvement of ventriculoarterial coupling (0.37 ± 0.02 vs. 0.65 ± 0.08 , $P < 0.01$), along with increased LV stroke work and mechanical efficiency, reflects improved mechanoenergetics of exercise-induced cardiac hypertrophy. Despite the significant hypertrophy, we observed unaltered LV stiffness (slope of end-diastolic P-V relationship: 0.043 ± 0.007 vs. 0.040 ± 0.006 mmHg/ μ l) and improved LV active relaxation (τ : 10.1 ± 0.6 vs. 11.9 ± 0.2 ms, $P < 0.01$). According to our knowledge, this is the first study that provides characterization of functional changes and hemodynamic relations in exercise-induced cardiac hypertrophy.

exercise-induced cardiac hypertrophy; pressure-volume analysis; systolic function; diastolic function; cardiac mechanoenergetics

ATHLETE'S HEART HAS BEEN DESCRIBED as the complex structural, functional, and electrical cardiac remodeling induced by long-term exercise training (40). Exercise training-induced cardiac hypertrophy is an important physiological adaption, which includes balanced increase of left ventricular (LV) and left atrial diameters, cardiac mass, and LV wall thicknesses ef-

fects by myocyte hypertrophy and neoangiogenesis (10, 12, 25, 36, 37).

Cardiac enlargement in athletes has been reported since the late 1890s (6), and several aspects of athlete's heart have been intensively investigated in humans (28, 40) as well as in experimental animals (48). A number of animal models of exercise-induced cardiac hypertrophy have been developed, and several types of endurance exercise training have been recognized to effectively induce physiological cardiac hypertrophy in experimental animals, such as treadmill running, voluntary wheel running, and swim training (11, 15, 30, 48). Swim training appears equally effective as treadmill or voluntary wheel running programs for inducing physiological hypertrophy (48).

Although our understanding of athlete's heart has gradually expanded in parallel with the development of new research tools, a number of unanswered questions remain; therefore, athlete's heart has become the object of intensive investigations in recent years. Numerous studies concerning the cellular and molecular remodeling (10) as well as structural and morphological changes (22, 37, 40) have been published in recent years; however, we still have limited data about the functional aspects of exercise-induced cardiac hypertrophy.

Several in vitro experimental studies from isolated rodent hearts (13, 26), papillary muscles (31, 37), and isolated cardiomyocytes (23, 24, 50) have demonstrated positive effect of exercise training on myocardial function. The major limitation of the previously used invasive and noninvasive approaches to study cardiac function in small animal models is that the hemodynamic parameters measured were not objective enough (echocardiography) or were largely dependent on loading conditions. Therefore, appropriate, hemodynamically validated standards of in vivo experimental models of exercise training-induced cardiac hypertrophy are required for the reliable investigations.

Pressure-volume (P-V) analysis is a useful approach for examining intact ventricular function independently from loading conditions. This analysis has been widely used in large animal studies and in humans since the mid-1980s (20). Advances of the last decade in the development and validation of miniature P-V catheters have made it possible to use this approach for studies in small animals (14, 33–35). The technique of LV P-V analysis in rats has been introduced only recently, and very limited normative data are available.

In the present study, we aimed to provide a detailed characterization of the systolic and diastolic performance of the LV in a rat model of exercise-induced cardiac hypertrophy using classical echocardiography and in vivo LV P-V analysis with a P-V conductance catheter system.

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MATERIALS AND METHODS

Ethical Approval and Animals

All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, Revised 1996). All procedures and handling of the animals during the study were reviewed and approved by the Ethical Committee of Hungary for Animal Experimentation.

Young adult (12 wk old, body weight = 275–300 g) male Wistar rats ($n = 32$) (Charles River, Sulzfeld, Germany) were housed in a room with constant temperature of $22 \pm 2^\circ\text{C}$ with a 12-h:12-h light-dark cycle and fed a standard laboratory rat diet ad libitum and free access to water.

Experimental Groups

After acclimation, rats of the exercise group ($n = 9$) underwent a 12-wk swim training program, and untrained animals served as controls (untrained control group, $n = 11$). Body weight was measured three times a week during the whole study period. Echocardiographic assessments and invasive hemodynamic experiments were performed after completion of the training program. Hearts were removed, and heart weight was measured immediately after euthanization.

For testing the reversibility of exercise-induced changes, additional series of rats underwent the same 12-wk training program, followed by discontinuation of exercise and an 8-wk resting (detraining) (1) period (detrained exercise group, $n = 6$), and untrained animals served as controls (detrained control group, $n = 6$). Echocardiographic assessments were performed after the detraining period, and hearts were removed from the animals and heart weight was measured immediately after euthanization.

Swim Training Protocol: Rat Model of Exercise-Induced Cardiac Hypertrophy

We designed a swimming apparatus (Fig. 1A) specially planned for exercise training of rats. A 150-liter water tank was divided into six lanes with a surface area of 20×25 cm and a depth of 45 cm per lane and filled with tap water maintained at 30 – 32°C to allow individual swim training. The dimensions of the lanes were selected to avoid floating of the rats by reclining to the walls. Based on data in the literature (11, 48) and on the results of our own preliminary pilot studies, we provided a training plan to establish a rat model for inducing robust cardiac hypertrophy. Exercised rats swam for a total period of 12 wk, 200 min session/day, and 5 days/wk (Fig. 1B). For

adequate adaptation, the duration of swim training was limited to 15 min on the first day and increased by 15 min every second training session until the maximal swim duration (200 min) was reached. Untrained control rats were placed into the water for 5 min each day during the 12-wk training program.

Echocardiography

Rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Animals were placed on controlled heating pads, and the core temperature, measured via rectal probe, was maintained at 37°C . After the anterior chest was shaved, transthoracic echocardiography was performed in the supine position by one investigator blinded to the experimental groups. Two-dimensional and M-mode echocardiographic images of long- and short (mid-papillary level)-axis were recorded, using a 13-MHz linear transducer (GE 12L-RS, GE Healthcare), connected to an echocardiographic imaging unit (Vivid i, GE Healthcare). Digital images were analyzed by an investigator in blinded fashion using an image analysis software (EchoPac, GE Healthcare). On two-dimensional recordings of the short-axis at the mid-papillary muscle level, LV anterior wall thickness (AWT), posterior wall thickness (PWT), interventricular septal wall thickness in diastole (index: d) and systole (index: s), and LV end-diastolic (LVEDD) and end-systolic (LVESD) diameters were measured. In addition, end-diastolic and end-systolic LV areas were planimetered from short- and long-axis two-dimensional recordings. End systole was defined as the time point of minimal LV dimensions, and end diastole as the time point of maximal dimensions. All values were averaged over three consecutive cycles.

The following parameters were derived from these measurements (41). Fractional shortening (FS) was calculated as $[(\text{LVEDD} - \text{LVESD})/\text{LVEDD}] \times 100$. End-diastolic (LVEDV) and end-systolic (LVESV) LV volumes were estimated according to two different validated geometrical models as previously described: the single plain ellipsoid model and the biplane ellipsoid model (45). Stroke volume (SV) was calculated as $\text{LVEDV} - \text{LVESV}$. Ejection fraction (EF) was determined as $(\text{SV}/\text{LVEDV}) \times 100$. LV mass (LV mass) was calculated according to a cubic formula, suggested by Devereux et al. (8): $\text{LV mass} = \{[(\text{LVEDD} + \text{AWTd} + \text{PWTD})^3 - \text{LVEDD}^3] \times 1.04\} \times 0.8 + 0.14$. To calculate LV mass index and SV index, we normalized the LV mass and SV values to the body weight of the animal, respectively.

Hemodynamic Measurements: LV P-V Analysis

Rats were anesthetized with a mixture of ketamine (100 mg/kg ip) and xylazine (3 mg/kg ip), tracheotomized, and intubated to facilitate breathing. Animals were placed on controlled heating pads, and the core temperature, measured via rectal probe, was maintained at 37°C .

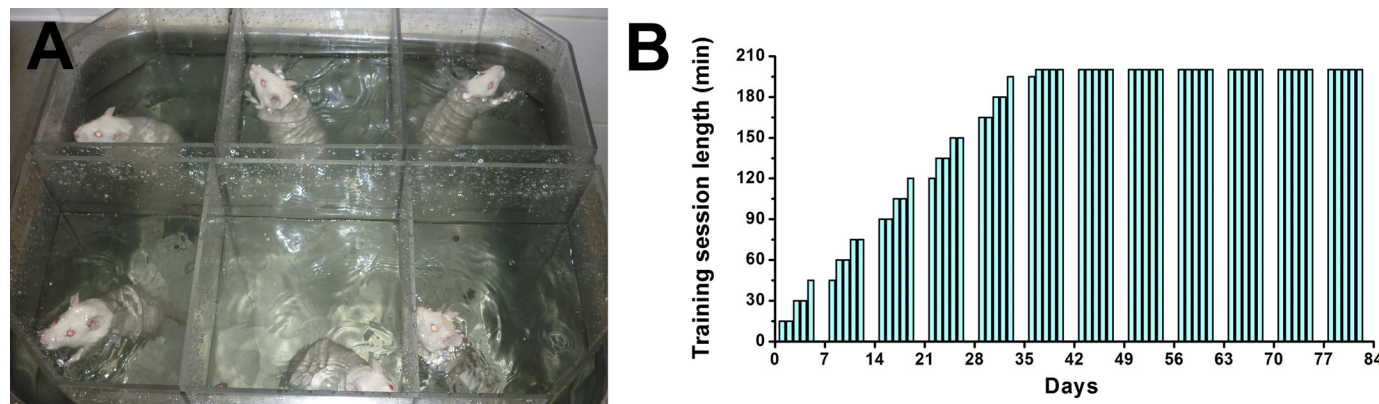


Fig. 1. Swimming apparatus and training plan. Our swimming apparatus (A) ensured 6 lanes for parallel swimming training. The training plan of exercised animals (B) included 5 wk of adaptation phase and 7 wk of intense exercise training, 5 days/wk, 200 min per daily swimming session.

A polyethylene catheter was inserted into the left external jugular vein for fluid administration. A 2-Fr microtip pressure-conductance catheter (SPR-838, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the ascending aorta. After stabilization for 5 min, mean arterial blood pressure (MAP) and heart rate (HR) were recorded. After that, the catheter was advanced into the LV under pressure control. After stabilization for 5 min, signals were continuously recorded at a sampling rate of 1,000 samples/s using a P-V conductance system (MPVS-Ultra, Millar Instruments) connected to the PowerLab 16/30 data acquisition system (AD Instruments, Colorado Springs, CO), stored, and displayed on a personal computer by the LabChart5 Software System (AD Instruments).

With the use of a special P-V analysis program (PVAN, Millar Instruments), LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), the maximal slope of LV systolic pressure increment (dp/dt_{max}) and diastolic pressure decrement (dp/dt_{min}), time constant of LV pressure decay τ ; according to the Weiss method and Glantz method (34)], EF, stroke work (SW), and P-V area (PVA) were computed and calculated. SV and cardiac output (CO) were calculated and corrected according to in vitro and in vivo volume calibrations using PVAN software. The total peripheral resistance (TPR) was calculated by the following equation: $TPR = MAP/CO$. To exclude the influence of body weight differences, CO, SW, and TPR were

normalized to body weight [cardiac index (CI), SW index (SWI), and TPR index].

In addition to the above parameters, P-V loops recorded at different preloads can be used to derive other useful systolic function indexes that are less influenced by loading conditions and cardiac mass (16, 34). Therefore, LV P-V relations were measured by transiently compressing the inferior vena cava (reducing preload) under the diaphragm with a cotton-tipped applicator. The slope [end-systolic elastance (E_{es})] of the LV end-systolic P-V relationship [ESPVR; according to the parabolic curvilinear model (17)], preload recruitable SW (PRSW), and the slope of the dp/dt_{max} -end-diastolic volume relationship (dp/dt_{max} -EDV) were calculated as load-independent indexes of LV contractility. The slope of the LV end-diastolic P-V relationship (EDPVR) was calculated as a reliable index of LV stiffness (16).

Arterial elastance (E_a) was calculated as $LVESP/SV$. Ventriculo-arterial coupling was described by the quotient of E_a and E_{es} . According to Sunagawa and associates (44) mechanical efficiency was calculated as the ratio of SW and PVA.

At the end of each experiment, 100 μ l of hypertonic saline were injected intravenously, and from the shift of P-V relations, parallel conductance volume was calculated by the software and used for the correction of the cardiac mass volume. The volume calibration of the conductance system was performed as previously described (16).

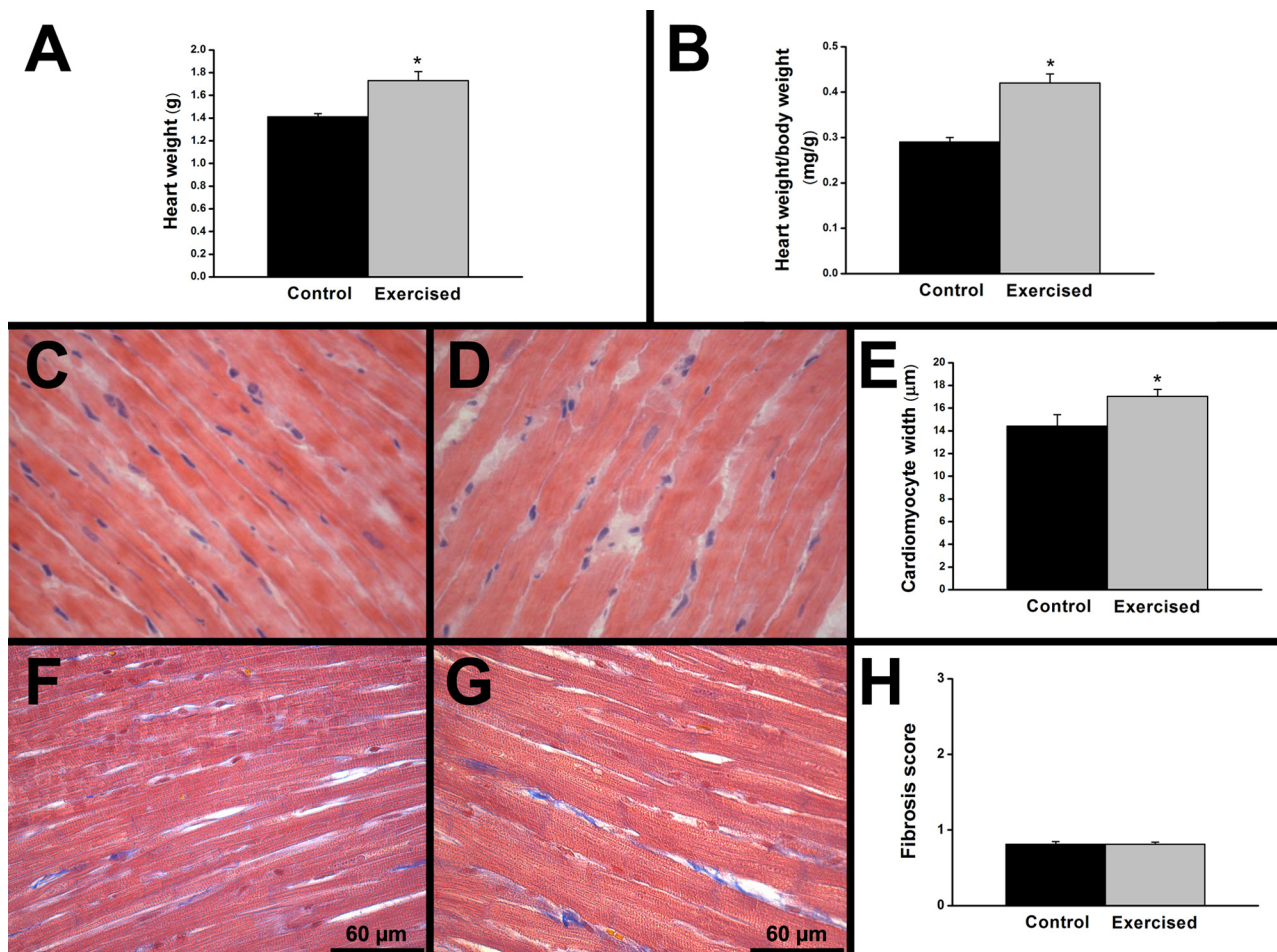


Fig. 2. Evaluation of physiological cardiac hypertrophy. The increased heart weight values (A) and heart weight-to-body weight ratio (B) in trained rats showed robust cardiac hypertrophy. Representative photomicrographs of left ventricular (LV) myocardium after hematoxylin and eosin (C and D), as well as Masson's trichrome staining (F and G). Longitudinally oriented cardiomyocytes are displayed for control (C) and exercised (D) animals. An increase of LV cardiomyocyte width was observed in exercised rats compared with untrained controls (E). Masson's trichrome-stained myocardium showed low, physiological amounts of blue collagen staining both in the control (F) and exercised (G) groups. Additionally, semiquantitative scoring values indicated no difference between the groups (H). Magnification, $\times 400$; scale bar, 60 μ m. * $P < 0.05$.

Briefly, nine cylindrical holes in a block 1 cm deep and with known diameters ranging from 2 to 11 mm were filled with fresh heparinized whole rat blood. In this calibration, the linear volume-conductance regression of the absolute volume in each cylinder versus the raw signal acquired by the conductance catheter was used as the volume calibration formula.

After completion of the hemodynamic measurements all animals were euthanized by exsanguination.

Histology

Hearts were harvested immediately after euthanization, snap frozen in liquid nitrogen, and stored at -80°C . Transverse transmural slices of the ventricles were sectioned ($5\ \mu\text{m}$) and conventionally processed for histological examination. The sections were stained with hematoxylin and eosin and Masson's trichrome. Light microscopic examination was performed with a Zeiss microscope (Axio Observer.Z1, Carl Zeiss, Jena, Germany), and digital images were captured using an imaging software (QCapture Pro 6.0, QImaging, Canada). The transverse transnuclear widths of randomly selected, longitudinally oriented cardiomyocytes were measured by a single investigator after calibrating the system. The mean value of 100 LV cardiomyocytes represents each sample. The amount of myocardial collagen was determined by semiquantitative morphometry scoring of Masson's trichrome-stained sections by one blinded observer as follows: 0, absent; 1, slight; 2, moderate; and 3, intense. The mean value of twenty randomly selected visual fields (magnification, $\times 400$) of free LV wall represents each sample.

Biochemical Measurements

After hemodynamic measurements were completed, blood samples from the inferior caval vein were collected in tubes prerinsed with EDTA. The blood samples were centrifuged at $3,000\ \text{g}$ for 15 min at 4°C . Plasma adrenocorticotrophic hormone (ACTH) and cortisol levels were determined by electrochemiluminescence immunoassay using commercial kits (Elecys ACTH Kit and Elecys Cortisol Test, Cobas, Roche Diagnostics, Mannheim, Germany).

Cardiac mRNA Analysis

LV myocardial tissue samples were harvested immediately after euthanasia, snap frozen in liquid nitrogen, and stored at -80°C . LV tissue was homogenized in RLT buffer, and RNA was isolated from the ventricular samples using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and quantified by measuring optical density at 260 nm. RNA purity was ensured by obtaining a 260/280 nm optical density ratio of ~ 2.0 . Reverse transcription reaction ($1\ \mu\text{g}$ total RNA of each sample) was completed using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed with the StepOne-Plus Real-Time PCR System (Applied Biosystems) in triplicates of each sample in a volume of $10\ \mu\text{l}$ in each well containing cDNA ($1\ \mu\text{l}$), TaqMan Universal PCR MasterMix ($5\ \mu\text{l}$), and a TaqMan Gene Expression Assay for the following targets ($0.5\ \mu\text{l}$): β -isoform of myosin heavy chain (β -MHC, assay ID: Rn00568328_m1) and transforming growth factor- β (TGF- β , assay ID: Rn00572010_m1), all purchased from Applied Biosystems. Gene expression data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; reference gene; assay ID: Rn01775763_g1), and expression levels were calculated using the C_T comparative method ($2^{-\Delta C_T}$). All results are expressed as values normalized to a positive calibrator (a pool of cDNAs from all samples of the control group).

Statistical Analysis

Statistical analysis was performed on a personal computer with a commercially available software (Origin 7G; OriginLab, Northampton, MA). All data are expressed as means $\pm$ SE. An unpaired

two-sided Student's *t*-test was used to compare parameters of exercised and control rats, as well as detrained exercised and detrained control rats, respectively. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Body Weight and Heart Weight

When compared with untrained controls, exercise trained animals were associated with decreased body weight gain during the study period (body weight at the end of the training program: 409 ± 7 exercised vs. 490 ± 14 g untrained control, $P = 0.0005$). Heart weight measured immediately after the hemodynamic measurements was markedly increased in exercised animals (Fig. 2A). The heart weight-to-body weight ratio showed an even more significant increase in the exercised group reflecting cardiac hypertrophy (Fig. 2B).

Histology

Exercise training resulted in a significant increase in cardiomyocyte width compared with that in untrained control rats, indicating cardiac hypertrophy (Fig. 2, C–E). The semiquantitative evaluation of Masson's trichrome staining indicated unaltered cardiac collagen content in the exercised group (Fig.

Table 1. Echocardiography parameters in untrained control and exercised rats

	Control	Exercised	P Value Versus Control Group
<i>n</i>	6	6	
LVAWTd, mm	1.87 ± 0.03	$2.21 \pm 0.06^*$	0.0003
LVAWTs, mm	2.89 ± 0.16	$3.37 \pm 0.10^*$	0.0282
LVPWTd, mm	1.99 ± 0.07	$2.36 \pm 0.07^*$	0.0037
LVPWTs, mm	2.97 ± 0.05	$3.68 \pm 0.16^*$	0.0014
LVIVSTd, mm	2.00 ± 0.11	2.33 ± 0.13	0.0734
LVIVSTs, mm	2.68 ± 0.14	$3.41 \pm 0.16^*$	0.0054
LVEDD, mm	7.12 ± 0.09	6.85 ± 0.06	0.0666
LVESD, mm	4.40 ± 0.11	$3.75 \pm 0.15^*$	0.0129
FS, %	39.1 ± 2.2	$46.3 \pm 3.0^*$	0.0258
LV mass, g	0.94 ± 0.04	$1.06 \pm 0.04^*$	0.0443
LV mass index, g/kg body wt	2.03 ± 0.08	$2.41 \pm 0.09^*$	0.0058
LVEDVsp, μl	419.1 ± 10.5	419.4 ± 28.4	0.5493
LVESVsp, μl	162.4 ± 8.6	$123.7 \pm 10.5^*$	0.0179
SVsp, μl	256.7 ± 10.0	295.7 ± 25.2	0.1568
SVIsp, $\mu\text{l}/\text{mg}$	551.2 ± 20.9	662.2 ± 56.4	0.0785
EFsp, %	61.2 ± 1.8	$70.7 \pm 2.0^*$	0.0054
LVEDVbp, μl	467.4 ± 10.1	474.5 ± 35.3	0.8516
LVESVbp, μl	169.0 ± 11.9	$129.8 \pm 6.7^*$	0.0383
SVbp, μl	298.4 ± 8.1	$347.6 \pm 21.2^*$	0.0449
SVIbp, $\mu\text{l}/\text{mg}$	640.9 ± 18.3	$802.6 \pm 52.4^*$	0.0118
EFbp, %	62.5 ± 1.9	$72.6 \pm 2.3^*$	0.0109

Values are means $\pm$ SE. LVAWTd and LVAWTs, left ventricular (LV) anterior wall thickness at diastole and systole, respectively; LVPWTd and LVPWTs, LV posterior wall thickness at diastole and systole, respectively; LVIVSTd and LVIVSTs, LV interventricular septal wall thickness at diastole and systole, respectively; LVEDD, LV end diastolic dimension; LVESD, LV end-systolic dimension; FS, LV fractional shortening; LVEDVsp and LVEDVbp, LV end-diastolic volume (LVEDV) calculated using single-plane ellipsoid (sp) and biplane ellipsoid (bp) model, respectively; LVESVsp and LVESVbp, LV end-systolic volume (LVESV) calculated using sp and bp model, respectively; SVsp and SVbp, LV stroke volume (SV) calculated using sp and bp model, respectively; SVIsp and SVIbp, LV SV index calculated using sp and bp model, respectively; EFsp and EFbp, LV ejection fraction (EF) using sp and bp model, respectively. $*P < 0.05$ vs. untrained controls.

2, *F–H*). Figure 2 shows representative photomicrographs of LV myocardium in exercised and control rats.

Markers of Stress and Pathological Hypertrophy

Plasma ACTH (172.1 ± 32.3 exercised vs. 211.8 ± 58.4 pg/ml untrained control, $P = 0.5470$) and cortisol (87.4 ± 10.1 exercised vs. 95.6 ± 11.4 nmol/l untrained control, $P = 0.6000$) concentrations did not differ between the exercised and control rats.

Myocardial gene expressions of β -MHC (relative expression: 0.85 ± 0.11 exercised vs. 0.93 ± 0.04 untrained control, $P = 0.5516$) and TGF- β (relative expression: 0.84 ± 0.08 exercised vs. 0.84 ± 0.02 untrained control, $P = 0.9867$) were unaltered in exercised rats compared with untrained controls, indicating an absence of pathological hypertrophy and remodeling, respectively.

Echocardiographic Parameters

The morphological and functional echocardiographic results are shown in Table 1. At the end of the exercise training protocol, the LV wall thickness values were significantly higher in the exercised rats, except the septal thickness that showed only a strong tendency toward higher values, com-

pared with the control animals. Figure 3 shows representative long- and short-axis two-dimensional end-systolic snapshots in exercised and control rats. Irrespective of the geometrical model used for volume calculation, swim training was associated with significantly decreased LVESV along with unchanged LVEDV; thus EF was significantly increased in trained animals. The SV and SV index values showed a marked and significant increase using the single plane and the biplane model, respectively, in exercised rats compared with untrained control rats. The LV mass and the LV mass index were significantly higher in exercised animals versus controls, indicating robust cardiac hypertrophy. The FS was increased in exercised animals, suggesting increased systolic performance.

Hemodynamic Parameters

Baseline hemodynamic data. HR, MAP, LVESP, LVEDP, and $dP/dt_{\min}$ were not different in exercised animals compared with the control group (Table 2). The $dP/dt_{\max}$, as a classic contractility parameter, showed only an increasing tendency in trained rats without reaching the level of statistical significance. Decreased τ was also detected in exercised rats, suggesting improved active relaxation in trained animals. Figure 4A shows representative original steady-state P-V loops obtained from

Fig. 3. Echocardiographic assessment. Representative two-dimensional echocardiographic images at end systole (long axis, *A* and *B*; and short axis, *C* and *D*) and M-mode recordings (*E* and *F*) are shown. The images demonstrate increased wall thickness values and decreased LV end-systolic dimensions (LVESD) along with unaltered LV end-diastolic dimensions (LVEDD) in exercised rats (*B*, *D*, and *F*) compared with untrained controls (*A*, *C*, and *E*).

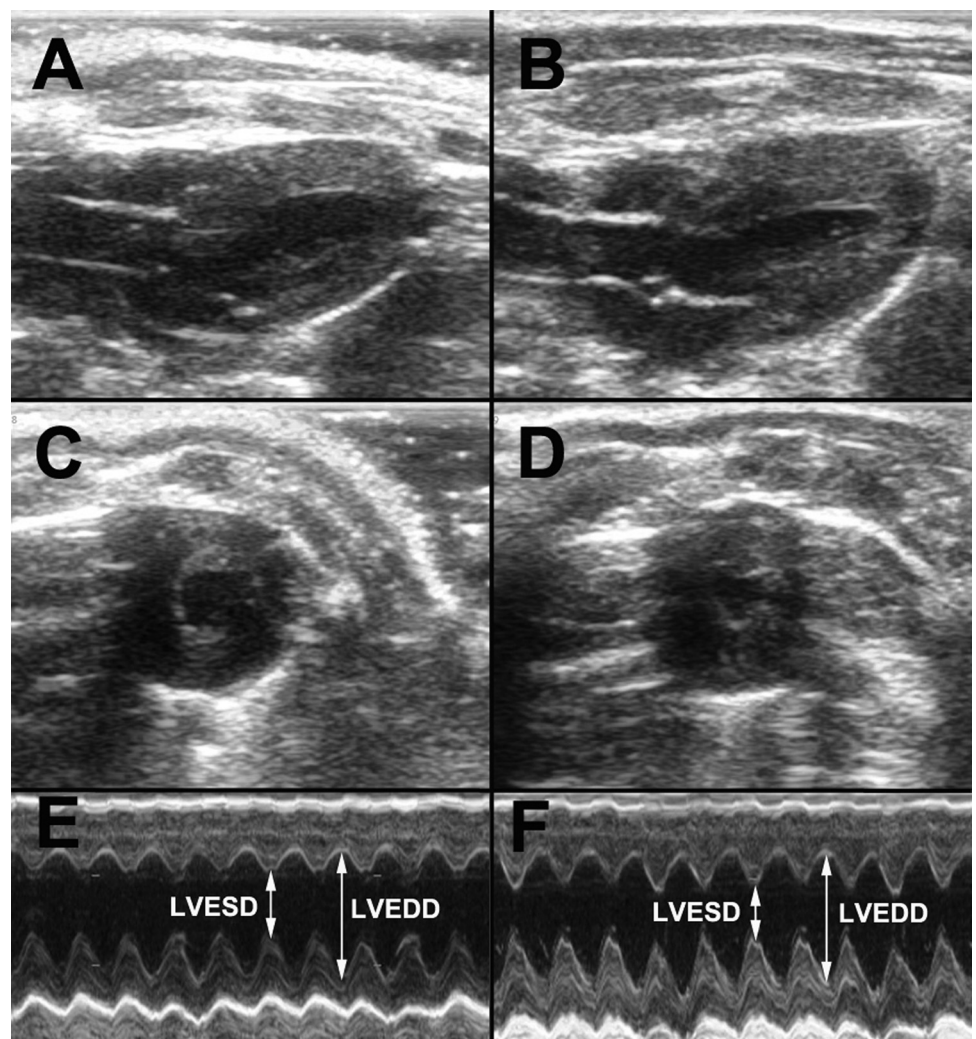


Table 2. Hemodynamic parameters in untrained control and exercised rats

	Control	Exercised	P Value Versus Control Group
<i>n</i>	9	11	
HR, beats/min	234.6 ± 6.5	236.1 ± 6.7	0.8736
MAP, mmHg	81.8 ± 2.5	83.4 ± 2.4	0.6475
LVESP, mmHg	95.1 ± 3.2	91.5 ± 2.4	0.4131
LVEDP, mmHg	8.2 ± 0.8	8.5 ± 0.5	0.7614
LVEDV, μ l	206.0 ± 8.0	209.4 ± 11.9	0.8099
LVESV, μ l	99.5 ± 6.7	75.4 ± 5.0*	0.0126
SV, μ l	145.4 ± 7.7	175.4 ± 8.5*	0.0172
CO, ml/min	32.25 ± 1.86	44.68 ± 3.71*	0.0053
CI (ml/min)/100 g body wt	6.65 ± 0.40	9.98 ± 0.78*	0.0008
EF, %	64.1 ± 1.5	73.7 ± 0.8*	0.0001
SW, mmHg·ml	11.08 ± 0.79	15.08 ± 0.86*	0.0030
SWI, (mmHg·ml)/100 g body wt	2.28 ± 0.17	3.37 ± 0.18*	0.0003
dP/dt _{max} , mmHg/s	7295 ± 189	7668 ± 196	0.1936
dP/dt _{min} , mmHg/s	6771 ± 396	6878 ± 470	0.8367
τ (Weiss), ms	10.7 ± 0.2	9.6 ± 0.3*	0.0109
τ (Glanz), ms	11.9 ± 0.2	10.1 ± 0.6*	0.0099
TPR, mmHg/(ml/min)	2.63 ± 0.20	1.98 ± 0.19*	0.0283
TPRI, mmHg/(ml/min)/100 g body wt	0.55 ± 0.05	0.45 ± 0.05	0.1694
E_{es} , mmHg/ μ l	1.16 ± 0.11	1.52 ± 0.08*	0.0270
Slope of EDPVR, mmHg/ μ l	0.040 ± 0.006	0.043 ± 0.007	0.7113
PRSW, mmHg	54.3 ± 4.8	77.0 ± 6.8*	0.0116
Slope of dP/dt _{max} -EDV, (mmHg/s)/ μ l	25.84 ± 2.83	35.94 ± 2.57*	0.0214
E_a , mmHg/ μ l	0.69 ± 0.05	0.53 ± 0.03*	0.0294
Maximal power, mW	59.8 ± 5.6	91.7 ± 9.3*	0.0067
PVA, mmHg·ml	25.9 ± 0.9	28.3 ± 2.6	0.3448
VAC	0.65 ± 0.08	0.37 ± 0.02*	0.0088
Eff, %	44.6 ± 2.1	53.0 ± 2.4*	0.0191

Values are means ± SE. Hemodynamic parameters were measured by the Millar pressure-volume conductance catheter system. HR, heart rate; MAP, mean arterial pressure; LVESP, LV end-systolic pressure; LVEDP, LV end-diastolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; CO, cardiac output; CI, cardiac index; EF, ejection fraction; SW, stroke work; SWI, SW index; dP/dt_{max} and dP/dt_{min}, maximal slope of the systolic pressure increment and the diastolic pressure decrement, respectively; τ , time constant of LV pressure decay; TPR, total peripheral resistance; TPRI, TPR index; E_{es} , slope of the end-systolic pressure-volume relationship; EDPVR, end-diastolic pressure-volume relationship; PRSW, preload recruitable SW; EDV, end-diastolic volume; E_a , arterial elastance; PVA, pressure-volume area; VAC, ventriculoarterial coupling; Eff, mechanical efficiency. * $P < 0.05$ vs. untrained controls.

exercised and untrained control rats. The wider baseline P-V loops in exercise-induced cardiac hypertrophy reflect increased SV along with unaltered LVEDV and decreased LVESV. The EF increased significantly, suggesting increased systolic performance in trained rats. CO, CI, SW, SWI, and also the LV maximal power were increased in swimming animals.

Functional indexes derived from P-V analysis at different preloads. Figure 4 shows representative original P-V loops recorded during transient occlusion of the inferior vena cava in exercised and untrained control animals. Overall results of EDPVR and ESPVR (E_{es}) are depicted in Table 2. As shown in Fig. 4, ESPVR was steeper in exercised than in control animals, suggesting increased systolic performance in trained heart. The EDPVR did not differ between the groups, indicating unchanged LV stiffness in exercised rats.

Figure 5 shows PRSW (the slope of the linear relation between SW and EDV, a sensitive contractility parameter) in a representative exercised and control animal. The slope was

steeper in trained rats than in control rats, indicating increased systolic performance. When compared with the corresponding control animals, the overall PRSW values were significantly higher in exercised rats (Fig. 5 and Table 2).

We also determined the relation between dP/dt_{max} and EDV. dP/dt_{max} is a classic contractility parameter, but it is dependent on changes in preload. Analysis of dP/dt_{max}-EDV allowed us to compare dP/dt_{max} values of exercised and control rats at a given EDV. As Fig. 5 shows, the slope of this relation was steeper in trained animals than in untrained animals, indicating increased contractility in exercise-induced hypertrophy. The overall dP/dt_{max}-EDV slope values were significantly higher in exercised rats (Fig. 5 and Table 2).

Cardiac mechanoenergetics. P-V analysis revealed a significant increase in E_{es} and a decrease of E_a . Subsequently, the ventriculoarterial coupling ratio (E_a/E_{es}) was significantly decreased in trained rats, suggesting improved mechanoenergetics (Fig. 6 and Table 2). The SW was increased in exercised rats, and the PVA did not differ between the groups. The mechanical efficiency and the maximum power of LV work was increased in trained rats compared with control rats (Fig. 6 and Table 2).

Reversibility of Exercise-Induced Cardiac Hypertrophy

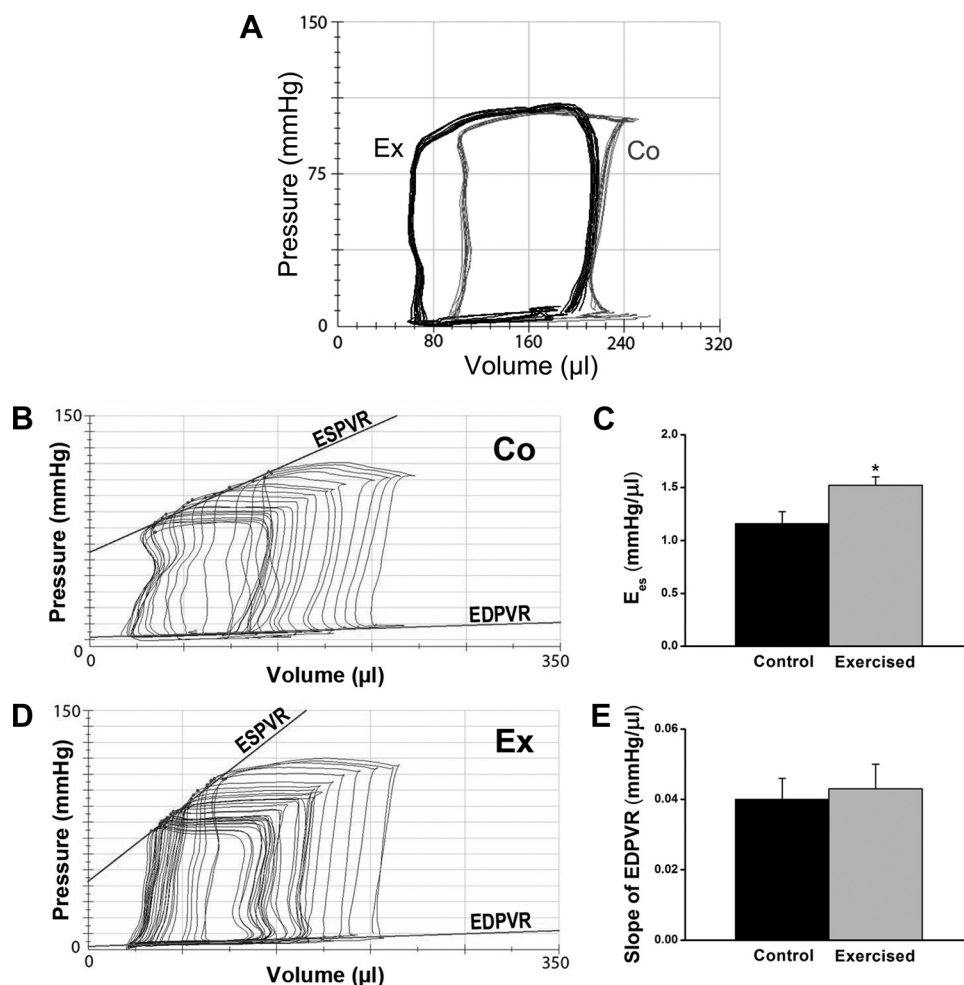
As shown in Table 3, body weight and heart weight did not differ between the detrained exercised and detrained control groups. The mean cardiomyocyte width regressed to control levels after discontinuation of the training. No differences could be observed between the detrained exercised and detrained control groups regarding morphological and functional echocardiographic parameters, which suggests the complete reversibility of exercise-induced changes (Table 3).

DISCUSSION

In the present study we provide a reliable characterization of systolic and diastolic cardiac function in a rodent model of endurance exercise training using the method of LV P-V analysis and measuring load-independent functional indexes. Here, we show that swim training-induced cardiac hypertrophy is characterized by increased systolic performance accompanied by improved active relaxation, unaltered diastolic stiffness, and ameliorated mechanoenergetics of the heart.

The adaptation of myocardial tissue that accompanies exercise training in experimental animal models depends on factors such as modality, intensity, duration, and frequency of the physical activity (47). According to data in the literature and results from our own previous pilot studies, we established a rat model of robust cardiac hypertrophy induced by swim training. Although the duration of swim exercise considerably varies in previously published works, it generally induces hypertrophy of the myocardium by as much as 15% (15, 30, 49, 50). Our present data on rats that underwent a 12-wk intensive (200 min/day) swim exercise training program showed a more robust cardiac hypertrophy as indicated by heart weight and calculated LV mass index values (Fig. 2). According to the results of echocardiography measurements, we observed significantly increased LV wall thickness and LV mass index values in exercised animals compared with controls, which is in line with previous studies (2, 47). The histomorphometry of LV myocardium showed increased cardiomyocyte diameter (Fig. 2E) in exercise-induced cardiac hypertrophy. This

Fig. 4. LV pressure-volume (P-V) loops. Original recordings of steady-state P-V loops (A) obtained with the Millar P-V conductance catheter system from 1 representative rat from the exercised (Ex) and control (Co) groups. The wider P-V loop indicates increased stroke volume along with unaltered end-diastolic volume (EDV) and decreased end-systolic volume, thus increased ejection fraction in exercised rats compared with untrained controls. End-systolic P-V relationship (ESPVR) and end-diastolic P-V relationship (EDPVR) in 1 representative animal from the exercised (B) and control (D) groups are shown. Original recordings of representative P-V loops were obtained with a P-V conductance catheter system at different preloads during vena cava occlusion and showed increased slope [E_{es} (end-systolic elastance)] of ESPVR (C) in exercised rats compared with untrained controls. The steeper ESPVR indicated increased contractile function. The slope of EDPVR did not differ between the groups, indicating unaltered diastolic stiffness (E). * $P < 0.05$.



finding is consistent with the features described in isolated cardiomyocytes (47, 50) as well as in LV myocardium (30) derived from animals that underwent endurance training.

To confirm the physiological nature of the observed myocardial hypertrophy and exclude the possible role of the swimming-associated stress response, we examined conventional markers of pathological hypertrophy [TGF- β (9), β -MHC, and Masson's trichrome staining (29)], as well as biomarkers of stress [ACTH (3) and cortisol (5)]. The observed unaltered amount of myocardial collagen, unchanged TGF- β and β -MHC expression, and stress biomarker values serve as evidence to rule out stress-induced remodeling and prove the physiological background of massive LV hypertrophy observed in our swimming rats.

Previous studies showed increased LV end-diastolic dimensions in human athletes (12) as well as in trained experimental animals (1), suggesting an exercise-induced dilation of the LV. In contrast, other researchers reported unaltered LV end-diastolic dimensions after long-term endurance exercise training (22, 47). Also, controversial data in the literature exist about LV end-systolic dimensions (2, 21). In the present study, both in vivo echocardiography and invasive hemodynamic measurements showed unaltered LVEDV and decreased LVESV in our exercised rats (Tables 1 and 2). Recent data suggesting the importance of the training program characteristics (type, duration, intensity) might provide a reasonable explanation to these differences (32, 38).

Our cardiac morphometric and echocardiographic results after discontinuation of intense exercise training and 8 wk of detraining (Table 3) confirm the complete reversibility of the observed cardiac phenotype (and reflect the age-related continuous increase in rat body dimensions). These findings further support the physiological nature of exercise-induced myocardial hypertrophy in our rat model and are in accordance with previous observations (1, 2).

Systolic Function and Cardiac Contractility

Previous studies showed improved contractility on isolated cardiomyocytes (24, 50) as well as on retrogradely perfused Langendorff hearts (26) of endurance trained animals; however, there is limited available data on sensitive contractility parameters assessed in vivo.

We found in our rat model that exercise training was associated with markedly increased dP/dt_{max} and significantly increased EF (Table 2). Although dP/dt_{max} has been widely used as a cardiac contractility parameter, it is well recognized that it is dependent on loading conditions, especially on changes in preload (16, 19). Classical echocardiographic parameters of cardiac contractility, FS, and EF are also known to be influenced by both preload and afterload and therefore cannot reliably be used to assess contractile function in models where loading is altered. The method of simultaneous LV pressure and volume analysis by the miniatur-

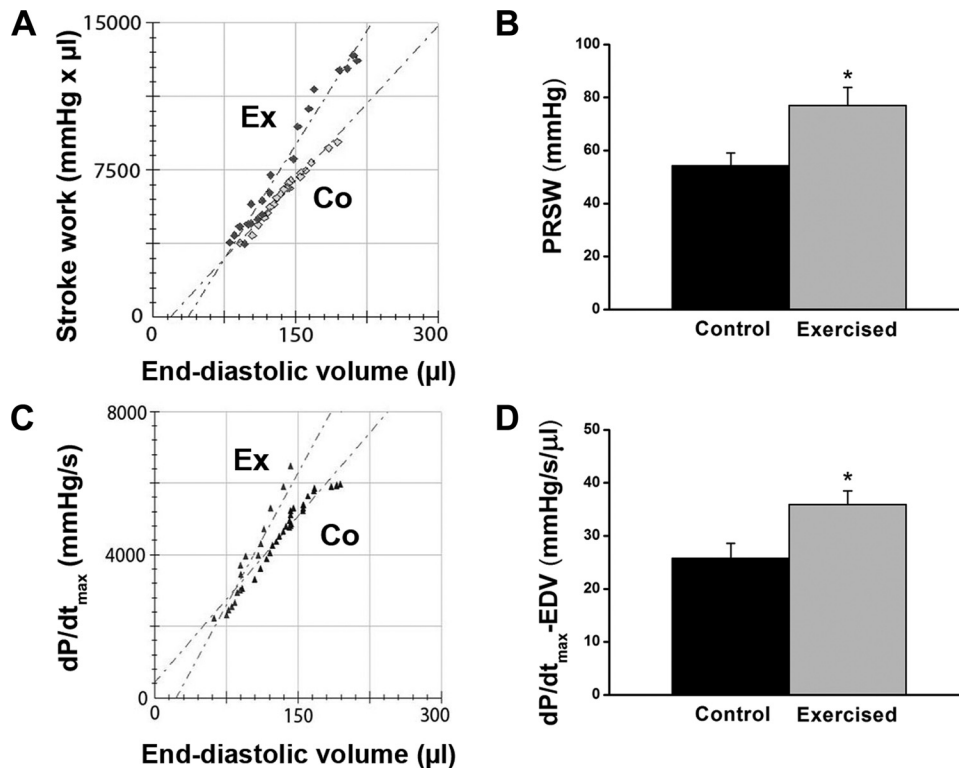


Fig. 5. P-V loop-derived LV contractility parameters. Preload recruitable stroke work (PRSW), the slope of the relationship between stroke work (SW), and EDV (A) and maximal slope of the systolic pressure increment (dP/dt_{max})-EDV relationship (C) in 1 representative rat from the exercised (Ex) and control (Co) groups are shown. Note that for both relationships, slope values are increased in exercised rats compared with untrained controls (B and D), suggesting increased systolic performance in exercised animals. * $P < 0.05$.

ized pressure-conductance catheters allowed us to calculate highly sensitive and reliable load-independent indexes of LV contractility.

Historically, ESPVR (E_{es}) has been proposed as a fairly load-insensitive index of ventricular contractility. According to

present results, E_{es} was significantly increased in exercised animals, indicating an improved contractile state of the LV myocardium (Fig. 4 and Table 2). However, because this relation can be altered not only by changes in the inotropic

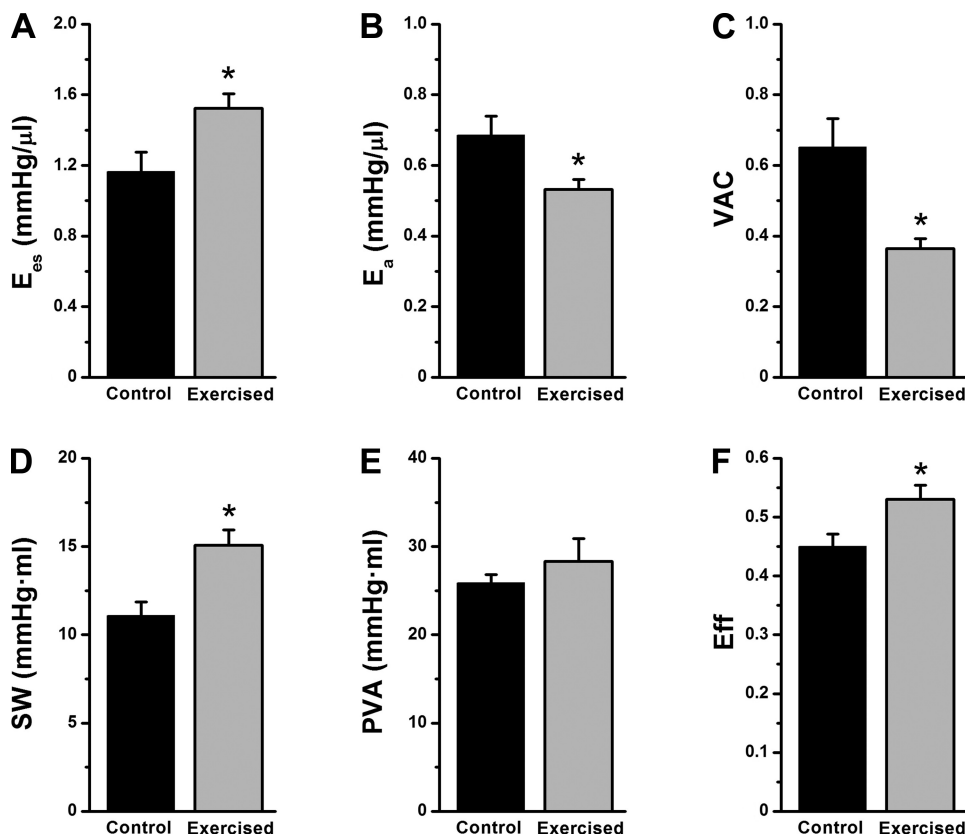


Fig. 6. Cardiac mechanoenergetics. The increased E_{es} (A) and the decreased arterial elastance (E_a ; B) resulted in improved ventriculoarterial coupling ($VAC = E_a/E_{es}$; C) in exercised rats compared with untrained controls. The increased SW (D) along with unaltered P-V area (PVA; E) led to an amelioration of mechanical efficiency ($Eff = SW/PVA$; F) in trained rats. Note that all of these parameters suggest improved LV mechanoenergetics in exercise-induced cardiac hypertrophy. * $P < 0.05$.

Table 3. Cardiac morphometric and echocardiography parameters in detrained control and detrained exercised rats

	Detrained Control	Detrained Exercised	P Value Versus Detrained Control Group
<i>n</i>	5	6	
HW, g	1.58 ± 0.13	1.50 ± 0.03	0.5228
BW, g	524 ± 37	561 ± 10	0.3170
Cardiomyocyte width, μm	15.13 ± 0.12	15.31 ± 0.35	0.6368
LVAWTd, mm	2.26 ± 0.12	2.28 ± 0.03	0.8080
LVAWTs, mm	3.24 ± 0.19	3.37 ± 0.03	0.4857
LVPWTd, mm	2.03 ± 0.13	2.10 ± 0.03	0.6073
LVPWTs, mm	2.92 ± 0.11	2.89 ± 0.03	0.7919
LIVISTd, mm	2.00 ± 0.12	2.09 ± 0.05	0.4857
LIVISTs, mm	2.85 ± 0.13	2.93 ± 0.08	0.6219
LVEDD, mm	7.52 ± 0.13	7.40 ± 0.18	0.6138
LVESD, mm	5.08 ± 0.24	4.96 ± 0.14	0.6632
FS, %	32.6 ± 2.4	33.0 ± 1.0	0.8626
LV mass, g	1.17 ± 0.10	1.17 ± 0.04	0.9875
LV mass index, g/kg body wt	2.40 ± 0.10	2.26 ± 0.07	0.2558
LVEDVsp, μl	492.4 ± 34.6	472.3 ± 33.4	0.6876
LVESVsp, μl	220.2 ± 8.3	206.2 ± 10.0	0.3223
SVsp, μl	272.2 ± 26.8	266.1 ± 26.2	0.8752
SVIsp, $\mu\text{l}/\text{mg}$	508.8 ± 18.1	470.4 ± 48.1	0.5076
EFsp, %	54.9 ± 1.3	55.8 ± 1.9	0.7188
LVEDVbp, μl	527.4 ± 43.7	519.3 ± 30.1	0.8785
LVESVbp, μl	235.6 ± 17.9	211.5 ± 12.1	0.2801
SVbp, μl	291.8 ± 26.6	307.8 ± 22.7	0.6566
SVIbp, $\mu\text{l}/\text{mg}$	546.1 ± 16.5	543.2 ± 39.4	0.9522
EFbp, %	56.0 ± 0.5	59.1 ± 1.6	0.1730

Values are means ± SE. BW, body weight; HW, heart weight.

state but also by changes in chamber geometry and other diastolic factors, we also calculated other parameters (16). PRSW represents the slope of the linear relation between SW and EDV. It has been described as a parameter independent of chamber size and mass, and it is sensitive to the cardiac contractile function of the ventricle. Although it integrates data from the entire cardiac cycle, it is influenced most of all by the systole (16). PRSW was also significantly increased in exercised rats compared with control rats (Fig. 5 and Table 2). A previous investigation (27) has demonstrated that the slope of the relation between dP/dt_{max} and EDV, another P-V loop-derived index, represents a sensitive but less load-dependent parameter of LV contractility. This sensitive contractility index was also augmented in exercised animals compared with untrained controls (Fig. 5 and Table 2).

Diastolic Function

Diastolic LV stiffness is predominantly affected by alterations in myocardial structural changes (advanced glycation end-products deposition, interstitial fibrosis, and remodeling) (46). Indexes of LV stiffness and compliance (LVEDP and slope of EDPVR) were not significantly different between exercised and control animals despite the significant increase of ventricular wall thickness and myocardial hypertrophy. This is in accordance with Libonati (26), who described unaltered LV stiffness in isolated perfused hearts of trained rats (26).

Improved ventricular relaxation has been observed in exercised animals (as indicated by decreased τ ; Table 2). Ventricular relaxation is an ATP-dependent active process, depends mostly on Ca^{2+} uptake by the sarcoplasmic reticulum, during

the first third of diastole. The detected functional differences might reflect improved Ca^{2+} handlings as suggested by Kemi et al. (24) and improved energetic state of the myocardium.

Mechanoenergetics

We used P-V analysis to characterize mechanoenergetic changes in exercise-induced cardiac hypertrophy. We determined E_{es} and E_{a} , which are relative load-independent indexes of LV contractility and vascular loading, respectively (44). E_{a} is an integrative index that incorporates the principal elements of arterial load, including peripheral vascular resistance, total arterial compliance, characteristic impedance, and systolic and diastolic time intervals (44). The parallel increase of myocardial contractility (E_{es}) and the decrease of E_{a} led to a highly significant improvement of the ventriculoarterial coupling ratio in exercised animals (Fig. 6 and Table 2). $E_{\text{a}}/E_{\text{es}}$ is an important determinant of net cardiac performance and energetics (18, 42). Improved ventriculoarterial coupling in exercise-trained animals reflects a more appropriate matching between the LV and the arterial system, which results in an optimal transfer of blood from the LV to the periphery without excessive changes in pressure (4).

SW is determined as the area within the P-V loop and represents the external mechanical work performed by the LV during a single heart cycle. PVA has been described as an index of LV total mechanical energy and is linearly related to total myocardial oxygen consumption (43). Increased SW along with unaltered PVA leads to increased mechanical efficiency (SW/PVA) in exercised animals compared with controls (Fig. 6 and Table 2), suggesting an optimization of metabolic efficiency in exercise-induced cardiac hypertrophy: increased mechanical work along with similar myocardial oxygen consumption. According to our knowledge, the present study is the first to demonstrate increased mechanical efficiency in exercise-induced hypertrophy.

Limitations

The interpretation of results from the present study is limited to young male rats. The possible influence of sex, age, or species should be assessed in future studies. Swimming has been suggested to be effective to induce cardiac hypertrophy in animals even though it is associated with an important stress response (7). In order to minimize the influence of stress on the development of exercise-induced cardiac hypertrophy, rats of the exercised group were gradually familiarized with swimming and also untrained control rats were placed into the water for 5 min at every training session. According to the plasma concentrations of stress biomarkers [ACTH and cortisol (3, 5)], this regular swim training protocol was not associated with increased stress response in our rats.

P-V loop analysis, which is widely used in large animals and mice, has become a requirement for the assessment of LV function because it is the only technique that allows the measurement of LV performance independent from loading conditions. The combined pressure-conductance microcatheter for rats has been introduced only a few years ago and limited normative data are available (34). The proper measurements of absolute volumes is the most vulnerable part of the P-V technique. Nevertheless, as our results demonstrate, the baseline P-V data of control rats were very similar to those reported

in a recent methodological study (34). Moreover absolute volume data from the P-V analysis were in correspondence with those assessed by echocardiography.

Conclusions

In conclusion, we confirmed exercise-induced reversible physiological cardiac hypertrophy in our rat model and characterized cardiac function in detail using the P-V conductance catheter system. Based on reliable load-independent indexes of LV performance, in the present study we demonstrated systolic (improved contractility) and diastolic (improved active relaxation and unchanged LV stiffness) functional amelioration in exercised animals along with improved mechanoenergetics. The P-V methodology could be a very useful and reliable approach for the assessment of LV function in exercise-induced cardiac hypertrophy.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

T.R., G.S., and B.M. conception and design of research; T.R., A.O., Á.L. B.T.N., L.H., E.B., D.K., and C.M. performed experiments; T.R., A.O., Á.L. and C.M. analyzed data; T.R., A.O., G.S., and B.M. interpreted results of experiments; T.R. and A.O. edited and revised manuscript; T.R., A.O., Á.L. B.T.N., L.H., E.B., D.K., C.M., G.S., and B.M. approved final version of manuscript; A.O. prepared figures; A.O. drafted manuscript.

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