

Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics

Attila Oláh, Balázs Tamás Németh, Csaba Mátyás, László Hidi, Árpád Lux, Mihály Ruppert, Dalma Kellermayer, Alex Ali Sayour, Lilla Szabó, Marianna Török, Anna Meltzer, László Gellér, Béla Merkely,* and Tamás Radovits*

Heart and Vascular Center, Semmelweis University, Budapest, Hungary

Oláh A, Németh BT, Mátyás C, Hidi L, Lux Á, Ruppert M, Kellermayer D, Sayour AA, Szabó L, Török M, Meltzer A, Gellér L, Merkely B, Radovits T. Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics. *Am J Physiol Heart Circ Physiol* 310: H587–H597, 2016. First published December 28, 2015; doi:10.1152/ajpheart.00588.2015.—Left ventricular (LV) hypertrophy is a physiological or pathological response of LV myocardium to increased cardiac load. We aimed at investigating and comparing hemodynamic alterations in well-established rat models of physiological hypertrophy (PhyH) and pathological hypertrophy (PaH) by using LV pressure-volume (P-V) analysis. PhyH and PaH were induced in rats by swim training and by abdominal aortic banding, respectively. Morphology of the heart was investigated by echocardiography. Characterization of cardiac function was completed by LV P-V analysis. In addition, histological and molecular biological measurements were performed. Echocardiography revealed myocardial hypertrophy of similar degree in both models, which was confirmed by post-mortem heart weight data. In aortic-banded rats we detected subendocardial fibrosis. Reactivation of fetal gene program could be observed only in the PaH model. PhyH was associated with increased stroke volume, whereas unaltered stroke volume was detected in PaH along with markedly elevated end-systolic pressure values. Sensitive indexes of LV contractility were increased in both models, in parallel with the degree of hypertrophy. Active relaxation was ameliorated in athlete's heart, whereas it showed marked impairment in PaH. Mechanical efficiency and ventriculo-arterial coupling were improved in PhyH, whereas they remained unchanged in PaH. Myocardial gene expression of mitochondrial regulators showed marked differences between PaH and PhyH. We provided the first comparative hemodynamic characterization of PhyH and PaH in relevant rodent models. Increased LV contractility could be observed in both types of LV hypertrophy; characteristic distinction was detected in diastolic function (active relaxation) and mechanoenergetics (mechanical efficiency), which might be explained by mitochondrial differences.

physiological left ventricular hypertrophy; pathological left ventricular hypertrophy; contractility; active relaxation; mechanoenergetics

NEW & NOTEWORTHY

We provided the first detailed in vivo hemodynamic comparison of physiological and compensated pathological hypertrophy of similar degree in relevant rodent models using left ventricular pressure-volume analysis. Characteristic differences were detected in energy-dependent early diastolic function and mechanoenergetics, which could be explained by alterations in mitochondrial regulation.

* B. Merkely and T. Radovits contributed equally to this article.

Address for reprint requests and other correspondence: A. Oláh, Experimental Research Laboratory, Heart and Vascular Center, Semmelweis Univ., 1122 Budapest, Városmajor u. 68, Hungary (e-mail: o.attilio@gmail.com).

CARDIAC HYPERTROPHY HAS BEEN referred to as an increase of cardiac mass by cardiomyocyte enlargement, which can develop in response to increased load of the heart. Pathological and physiological cardiac hypertrophies are caused by different stimuli and are associated with distinct structural and molecular phenotypes (1).

When pressure or volume overload is induced by diseases (e.g., hypertension, valvular disorders), it results in pathological hypertrophy, which is initially a compensatory response to the increased biomechanical stress in an attempt to maintain normal cardiovascular function (24). Pathological hypertrophy is also associated with loss of cardiomyocytes (apoptosis and necrosis), increased interstitial fibrosis, and the reactivation of fetal cardiomyocyte gene program (1, 9). This condition can eventually decompensate leading to ventricular dilatation, which, over time, may progressively lead to systolic and diastolic myocardial dysfunction (heart failure). With the rising incidence among the aging population, heart failure has remained a leading cause of death, and this major health problem facilitated numerous investigations to identify new therapeutic targets to prevent or reverse pathological cardiac hypertrophy (25).

Long-term exercise training also induces a cardiac hypertrophy response, called athlete's heart. This balanced increase of myocardial mass is associated with preserved or even enhanced cardiac function without cardiac fibrosis, cardiomyocyte apoptosis, or changes in fetal gene expression (9, 24). Furthermore, the protective role of exercise in cardiovascular prevention and treatment has been widely recognized (35, 41). The use of physiological stimuli can be one potential therapeutic strategy to inhibit pathological processes leading to heart failure and sudden cardiac death (23).

Despite some morphological similarities, pathological and physiological cardiac hypertrophy are induced by substantially distinct signaling pathways and characterized by different gene and protein expression profile (1, 16, 43). Although numerous studies have investigated these distinctions, the functional differences between pathological and physiological hypertrophy are poorly described. So far noninvasive approaches have been used to distinguish pathological from physiological left ventricular (LV) hypertrophy in experimental animals (8, 24) and in human subjects (7, 15). These works describe several aspects of LV function in the two forms of hypertrophy; however, most of these noninvasively measured indexes are dependent on loading conditions, and these characterizations are not detailed enough to deeply understand LV mechanics. Assessment of LV pump properties can be fundamental to advance the understanding of cardiovascular pathophysiology and therapeutics. Pressure-volume analysis provides the most comprehensive method to evaluate in vivo cardiac systolic and

diastolic function as well as mechanoenergetics, less affected by arterial and venous loading conditions and heart rate (HR) (30). Advances in miniature sensor technology established this approach to be successfully translated to rodents; thus LV pressure-volume analysis has become standard in basic cardiovascular research for detailed characterization of cardiovascular function (14).

The aim of this study was to give a direct, reliable hemodynamic comparison of exercise- (physiological) and pressure overload-induced (pathological) LV hypertrophy using the sophisticated method of LV pressure-volume analysis in two widely used, well-established rat models.

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 (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 male Wistar rats ($n = 41$, $m = 200$ – 250 g; Toxi-Coop, Dunakeszi, Hungary) 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 standard laboratory rat diet ad libitum and free access to water.

Swimming training: rat model of physiological cardiac hypertrophy. For long-term exercise training, after an adequate adaptation, exercised (Ex; $n = 12$) rats swam for a total period of 12 wk, for 200 min/day, 5 days a week as previously described (28). Untrained control (Co; $n = 11$) rats were placed into the water for 5 min each day during the 12-wk-long training program.

Abdominal aortic banding: rat model of pathological cardiac hypertrophy. Pressure overload-induced cardiac hypertrophy was produced by abdominal aortic banding (AB; $n = 10$). All surgical procedures were performed under anesthesia with pentobarbital sodium (60 mg/kg) administered by intraperitoneal injection. Abdominal aortic constriction was performed as described before (17), with some modification. Briefly, after proper anesthesia, a midline laparotomy was performed. The abdominal aorta was constricted at suprarenal level by tightening a 2-0 surgical suture against a blunted 20-gauge needle, which was pulled out thereafter. The abdominal musculature and the skin incisions were closed by standard surgery techniques. Sham-operated rats (Sham; $n = 8$) served as controls and underwent an identical procedure, but without ligation of abdominal aorta. Thereafter, animals were observed for a 6-wk period after surgical processes.

Echocardiography. At the completion of swimming training program or 6 wk after abdominal aortic banding procedure, LV morphological alterations were observed by echocardiography as described before (21). Rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Animals were placed on controlled heating pads, and the core temperature 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 using a 13-MHz linear transducer (12L-RS; GE Healthcare, Horten, Norway), connected to a commercially available system (Vivid I; GE Healthcare). Standard two-dimensional and M-mode long- and short-axis (at midpapillary level) images were acquired. Archived recordings were analyzed by a blinded investigator using a dedicated software (EchoPac v113; GE Healthcare). On two-dimensional recordings of the short axis at the midpapillary level, LV anterior wall thickness (LVAWT) and posterior wall thickness (LVPWT) in diastole (index: d) and systole (index: s) as well as LV end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were mea-

sured. 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.

Fractional shortening (FS) was determined from the measurements of LV chamber diameters: $\text{FS} = [(\text{LVEDD} - \text{LVESD})/\text{LVEDD}] \times 100$. LV mass was calculated according to the following formula: $\text{LVmass} = [(\text{LVEDD} + \text{LVAWTd} + \text{LVPWTd})^3 - \text{LVEDD}^3] \times 1.04 \times 0.8 + 0.14$. LV volume values were estimated according to a biplane ellipsoid model as previously described (30), and LV ejection fraction (EF) was calculated as the ratio of stroke volume (SV) and end-diastolic volume. To calculate LV mass index, we normalized the LV mass values to the tibial length (TL) of the animal.

Hemodynamic measurements: LV pressure-volume analysis. After completion of the protocols to induce myocardial hypertrophy, in vivo hemodynamic measurements were performed (29). Rats were anesthetized with pentobarbital sodium (60 mg/kg), 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 . A polyethylene catheter was inserted into the left external jugular vein for fluid administration. A 2-Fr pressure-conductance microcatheter (SPR-838; Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the ascending aorta. After stabilization, aortic pressure curves were recorded to calculate mean arterial blood pressure (MAP).

The catheter was thereafter advanced into the LV under pressure control. After stabilization for 10 min, baseline P-V relations were recorded. Signals were continuously registered 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 LabChart7 Software System (AD Instruments). With the use of a special P-V analysis program (PVAN; Millar Instruments), HR, LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), the maximal slope of LV systolic pressure increment ($\text{dP}/\text{dt}_{\text{max}}$) and diastolic pressure decrement ($\text{dP}/\text{dt}_{\text{min}}$), time constant of LV pressure decay [τ ; according to the Glantz method (30)], LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), SV, EF, cardiac output (CO), and stroke work (SW) 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: $\text{TPR} = \text{MAP}/\text{CO}$. To exclude the influence of body weight differences, CO was normalized to body weight [cardiac index (CI)].

In addition to the above parameters, P-V loops recorded at different preloads can be used to derive useful indexes of LV function that are less influenced by loading conditions and cardiac mass. 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 of the LV end-systolic P-V relationship (ESPVR; according to the parabolic curvilinear model), preload recruitable SW (PRSW), and the slope of the $\text{dP}/\text{dt}_{\text{max}}$ -end-diastolic volume relationship ($\text{dP}/\text{dt}_{\text{max}}$ -EDV) were calculated as load-independent indexes of LV contractility. The slope of the LV end-diastolic PV relationship (EDPVR) was calculated as a reliable indicator of LV stiffness (18).

Cardiac mechanoenergetic parameters were calculated as suggested by Suga (37) and Sunagawa et al. (39). SW and P-V area (PVA) were calculated by the PVAN software, and LV mechanical efficiency was calculated as SW/PVA . Arterial elastance (E_a) was calculated as LVESP/SV . Ventriculoarterial coupling (VAC) was described by the quotient of E_a and ESPVR (44).

At the end of each experiment, 100 μl of hypertonic saline were injected intravenously, and from the shift of volume relations, parallel conductance was calculated by the software and used for the correction of the LV volume values. The volume calibration of the conductance system was performed as previously described (30). Shortly, 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.

To remove erythrocytes from myocardial tissue, an *in vivo* perfusion was performed. After the thoracic cavity was opened and the inferior caval vein in the thorax was dissected, a total volume of 40 ml oxygenated Ringer solution (37°C) was infused into the LV through the apex of the heart.

Thereafter, the heart was quickly removed and placed into cold (4°C) Ringer solution. Heart weight was measured, and LV myocardial tissue samples were collected immediately for histology and molecular biology. Subsequently, post-mortem TL measurements were done.

Histology. Hearts were harvested immediately after animals were euthanized. Heart tissue samples were fixed in buffered paraformaldehyde solution (4%) and embedded in paraffin. Transverse transmural slices of the ventricles were sectioned (5 μ m), deparaffinized, and processed conventionally for histological examination. The sections were stained with hematoxylin-eosin (HE) and picrosirius red staining. 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).

On the HE-stained LV myocardial sections, transverse transnuclear widths of randomly selected, longitudinally oriented cardiomyocytes were measured by a single investigator (with a magnification of 400 $\times$). The mean value of 100 LV cardiomyocytes represents each sample.

To investigate myocardial collagen content, picrosirius red positive area was calculated using ImageJ (National Institutes of Health, Bethesda, MD) image analysis software. Five subendocardial and five subepicardial images (magnification 200 $\times$) were taken randomly from the free LV wall on each sections. After background subtraction, eye controlled auto-threshold have been determined to detect positive areas. The fractional area (picrosirius red positive area-to-total area ratio) was determined on each image, and the mean value of the images represents each animal.

Cardiac mRNA analysis. LV myocardial tissue samples were harvested immediately after animals were euthanized, snap-frozen in liquid nitrogen, and stored at -80°C . LV tissue of eight animals from each group was homogenized in a lysis buffer (RLT buffer; Qiagen, Hilden, Germany); RNA was isolated from the ventricular samples using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions and quantified by measuring optical density (OD) at 260 nm. RNA purity was ensured by obtaining a 260/280 nm OD ratio ~ 2.0 . Reverse transcription reaction (1 μ g total RNA of each sample) was completed using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed with the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) in triplicates of each sample in a volume of 10 μ l in each well containing cDNA (1 μ l), TaqMan Universal PCR MasterMix (5 μ l), and a TaqMan Gene Expression Assay (0.5 μ l) (Applied Biosystems) for the following genes: myocardial hypertrophy markers, such as α -myosin heavy chain (α -MHC; assay ID: Rn00691721_g1), β -myosin heavy chain (β -MHC; assay ID: Rn00568328_m1), and atrial natriuretic factor (ANF; Rn00561661_m1); endogenous antioxidants, such as catalase (Cat; assay ID: Rn00560930_m1), SOD-2 (assay ID: Rn00690587_g1), and thioredoxin-1 (TRX-1; assay ID: Rn00587437_m1); inflammatory markers, such as IL-1 β (assay ID: Rn00580432_m1), TNF- α (assay ID: Rn99999017_m1), and transforming growth factor β (assay ID: Rn00572010_m1); and markers related to mitochondrial function, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α ; assay ID: Rn00580241_m1), nuclear respiratory factor 1 (NRF1; assay ID: Rn01455958_m1), estrogen-related receptor α (ERR- α ; assay ID: Rn00433142_m1), peroxisome proliferator-activated receptor α

(PPAR- α ; assay ID: Rn00566193_m1), and cytochrome *c* (CytC; assay ID: Rn00470541_g1). Gene expression data were normalized to GAPDH (reference gene; assay ID: Rn01775763_g1), and expression levels were calculated using the CT comparative method (2- Δ CT). All results are expressed as values normalized to a positive calibrator (a pool of cDNA from all samples of the control group).

Statistical analysis. Statistical analysis was performed on a personal computer by using GraphPad Prism software (GraphPad Software, San Diego, CA). Results are expressed as means $\pm$ SE. We confirmed normal distribution of our data by the Shapiro-Wilk test. Thereafter, an unpaired two-sided Student's *t*-test was used to compare parameters of control and corresponding hypertrophic rats (Co vs. Ex, Sham vs. AB).

To compare hypertrophy-induced alterations of cardiac function and myocardial gene expression between the two hypertrophy models, individual values of animals with cardiac hypertrophy were normalized to the mean value of the corresponding control group. These normalized data (given in percentage) were compared by using two-sided Student's *t*-test after confirming normal distribution. Differences were considered statistically significant when $P < 0.05$.

The relations between LV mass values and sensitive indexes of LV contractility were examined by Pearson's correlation.

RESULTS

Body weight and heart weight data. Body weight was decreased in exercised rats compared with control ones (402 ± 10 g Ex vs. 469 ± 10 g Co; $P < 0.001$), whereas it did not differ in the model of pathological hypertrophy (421 ± 14 g Sham vs. 435 ± 16 g AB; $P = 0.517$). Post-mortem measured heart weight values normalized to TL were increased in both physiological and pathological hypertrophy (Fig. 1A).

Echocardiography. Echocardiography data are shown in Table 1. There was no difference in HR between the hypertrophied and corresponding control groups. Anterior and posterior wall thickness values, either in diastole or systole, were increased in both models. LVEDD remained unaltered, whereas LVESD was decreased in physiological hypertrophy. Pathological hypertrophy was associated with unaltered ventricular dimensions, despite a tendency toward higher values that could be observed. Consequently, FS and EF, two conventional parameters of systolic function, were improved in exercised animals, whereas they remained unchanged in AB rats. The calculated LV mass and LV mass index (normalized to TL) values were markedly elevated in the hypertrophic groups, clearly underpinning LV hypertrophy (Table 1 and Fig. 1A).

According to LV mass index values, the degree of LV hypertrophy was similar in these two models (Table 1 and Fig. 1A).

Hemodynamic measurements. Figure 2 shows typical steady-state P-V loops obtained from Co, Ex, Sham, and AB animals. The widening of the baseline loops can be observed in Ex rats compared with controls. A slight shift of the P-V loops to the right and markedly increased systolic pressure values in the AB rats indicate the pressure overload-induced baseline alterations.

As shown in Table 2, exercise training was associated with decreased LVESV along with unaltered HR, MAP, LV pressure values, $\text{dP/dt}_{\text{max}}$, and $\text{dP/dt}_{\text{min}}$. Consequently, SV, EF, CO, and CI were increased in Ex rats compared with Co rats, suggesting increased systolic performance. In the model of pressure overload-induced hypertrophy, increased MAP, LVESP, and $\text{dP/dt}_{\text{max}}$ values were observed along with un-

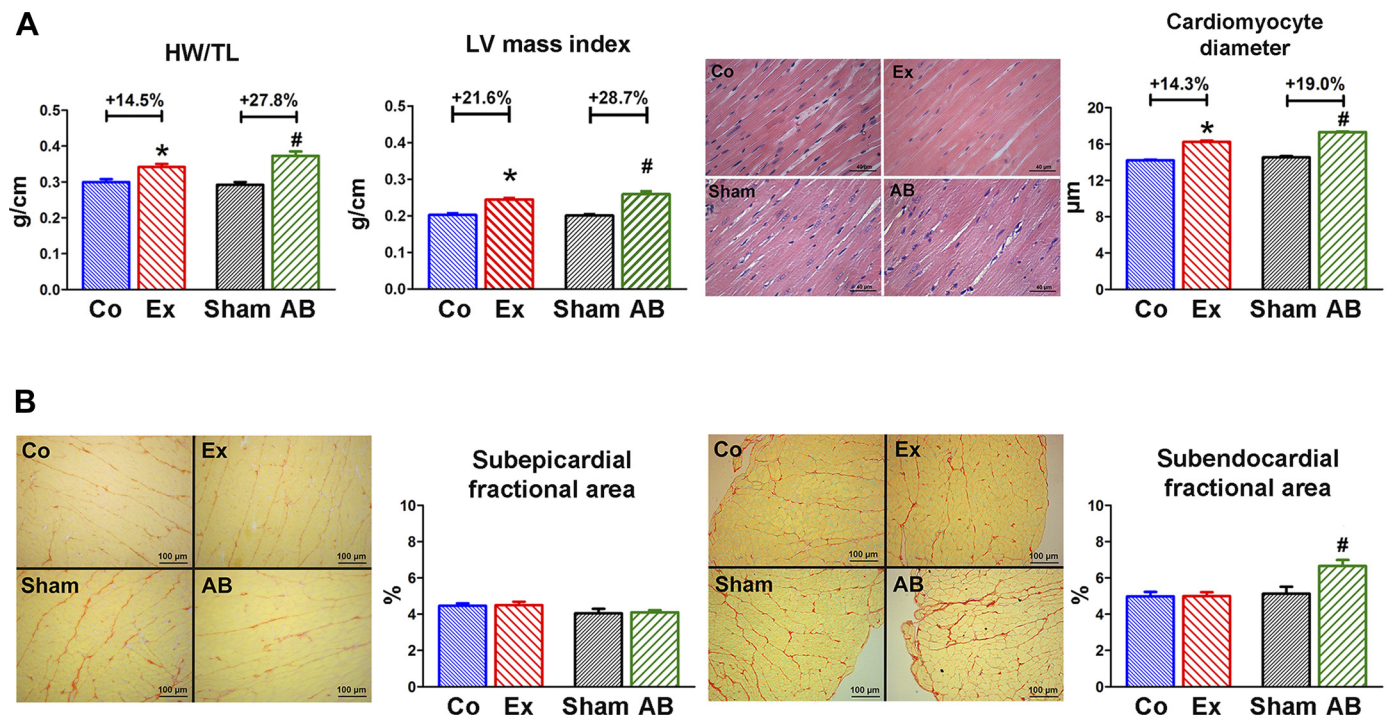


Fig. 1. Characterization of left ventricular (LV) hypertrophy. A: post-mortem measured heart weight (HW) values and echocardiographic LV mass index [LV mass normalized to tibial length (TL)] and mean LV cardiomyocyte width of histological analysis showed increased values in exercised (Ex) and aortic-banded (AB) animals compared with control (Co) and sham-operated (Sham) rats, respectively. These data clearly indicate cardiac hypertrophy in both models. B: picrosirius staining showed unaltered collagen density (red color indicates collagen fibers) in the model of physiological hypertrophy. In contrast, subendocardial fibrosis was observed in pathological hypertrophy. Values are means $\pm$ SE. * $P < 0.05$ vs. Co; # $P < 0.05$ vs. Sham.

changed HR, LVEDP, and dP/dt_{min} . LV end-diastolic volume, LVESV, and SV tended toward higher values, but without reaching statistical significance; thus EF, CO, and CI did not differ between the Sham and AB groups. TPR and E_a were decreased in Ex rats compared with Co rats, whereas higher values were shown in AB rats compared with Sham ones (Table 2).

A load-independent index of LV relaxation, τ , was significantly improved in physiological hypertrophy, whereas it showed a marked deterioration in pathological hypertrophy, resulting in an important difference between the two kinds of hypertrophy (Table 2 and Fig. 4).

Figure 3 displays representative P-V loops obtained during inferior vena cava occlusions in Co, Ex, Sham, and AB rats. Overall results of ESPVR, PRSW, and dP/dt_{max} -EDV are depicted in Fig. 3. All of these sensitive contractility parameters were increased in both hypertrophy models, and no significant difference was calculated between the two kinds of hypertrophy (Figs. 3. and 4). Moreover, load-independent contractility parameters showed positive linear correlation with calculated LV mass values involving the data of all animals (LV mass vs. ESPVR: $r = 0.535$, $P < 0.001$, Fig. 3; LV mass vs. PRSW: $r = 0.490$, $P = 0.003$; LV mass vs. dP/dt_{max} -EDV: $r = 0.434$, $P = 0.012$).

Table 1. Echocardiographic data

	Physiological Hypertrophy			Pathological Hypertrophy		
	Co	Ex	P Value	Sham	AB	P Value
HR, beats/min	357 $\pm$ 15	351 $\pm$ 10	0.719	378 $\pm$ 24	382 $\pm$ 11	0.874
LVAWTd, mm	1.96 $\pm$ 0.02	2.17 $\pm$ 0.02	<0.001	1.95 $\pm$ 0.02	2.19 $\pm$ 0.03	<0.001
LVAWTs, mm	3.02 $\pm$ 0.05	3.37 $\pm$ 0.06	<0.001	2.94 $\pm$ 0.10	3.32 $\pm$ 0.07	0.005
LVPWTd, mm	1.82 $\pm$ 0.03	1.93 $\pm$ 0.02	0.001	1.78 $\pm$ 0.01	2.06 $\pm$ 0.04	<0.001
LVPWTs, mm	2.91 $\pm$ 0.04	3.09 $\pm$ 0.04	0.007	2.84 $\pm$ 0.06	3.09 $\pm$ 0.04	0.002
LVEDD, mm	6.85 $\pm$ 0.05	6.89 $\pm$ 0.03	0.527	6.79 $\pm$ 0.08	6.95 $\pm$ 0.08	0.199
LVESD, mm	4.06 $\pm$ 0.08	3.45 $\pm$ 0.09	<0.001	3.99 $\pm$ 0.17	4.22 $\pm$ 0.11	0.245
FS, %	41.4 $\pm$ 0.7	50.0 $\pm$ 1.1	<0.001	41.3 $\pm$ 2.3	40.5 $\pm$ 1.3	0.739
EF, %	65.5 $\pm$ 1.3	73.0 $\pm$ 1.3	<0.001	65.1 $\pm$ 1.5	62.4 $\pm$ 1.3	0.191
LV mass, g	0.87 $\pm$ 0.01	0.98 $\pm$ 0.01	<0.001	0.85 $\pm$ 0.02	1.03 $\pm$ 0.02	<0.001
LV mass index, g/cm	0.202 $\pm$ 0.004	0.246 $\pm$ 0.003	<0.001	0.202 $\pm$ 0.003	0.260 $\pm$ 0.007	<0.001

Values are means $\pm$ SE. Co, control; Ex, exercised; AB, aortic banding; HR, heart rate; 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; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, LV fractional shortening; EF, LV ejection fraction. LV mass index is LV mass normalized to tibial length.

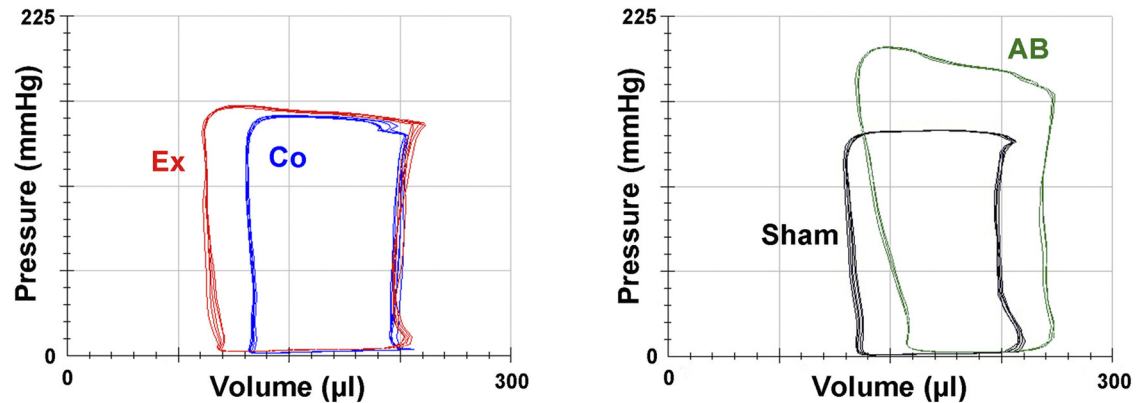


Fig. 2. Baseline pressure-volume (P-V) loops. Original recordings of steady-state P-V loops obtained from 1 representative rat from the Ex, Co, AB, and Sham groups. As shown by the left recording, the wider P-V loop indicates increased stroke volume along with unaltered end-diastolic volume and pressure relations and decreased end-systolic volume in exercised rats compared with untrained controls. In pressure overload-induced pathological hypertrophy, markedly increased LV systolic pressure and a tendency toward increased end-systolic and end-diastolic volume were observed. These alterations resulted in a slight shift rightward on the P-V diagram.

We also determined EDPVR, a sensitive marker of LV stiffness, which tended toward higher values in both hypertrophy (Table 2). This slight increase did not differ between the two models (Fig. 4).

Regarding mechanoenergetics, our results showed increased SW in both models. Values of PVA were elevated in only pathological hypertrophy and remained unchanged in athlete's heart (Fig. 5). Consequently, LV mechanical efficiency was improved in physiological hypertrophy, whereas pathological hypertrophy was associated with unchanged mechanical efficiency of LV performance (Fig. 5). Additionally, VAC showed a more optimized ventriculo-arterial interaction in Ex rats than in controls and was unchanged in pressure overload-induced hypertrophy (Table 2).

Histology. Increased cardiomyocyte width values were observed in exercised and aortic banding groups compared with control and sham-operated groups, respectively (Fig. 1A).

Picrosirius staining revealed no collagen deposition in the myocardium of exercise-trained rats. In contrast, increased subendocardial fractional area was observed in pathological hypertrophy, whereas the subepicardial collagen content did not differ between the Sham and AB rats (Fig. 1B).

Cardiac mRNA analysis. Myocardial gene expressions of ANF and β -MHC were markedly increased, whereas expression of α -MHC was markedly decreased (resulting in decreased α/β -MHC ratio) in the model of pathological hypertrophy; the expression values of these pathological hypertrophy markers did not differ between the control and exercised groups (Fig. 6A). There was no difference neither within nor between the models regarding the myocardial expression of major antioxidants (catalase, SOD-2, thioredoxin-1) and inflammation-related markers (TGF- β , TNF- α , and IL-1 β) (Fig. 6, B and C). Expression of markers related to mitochondrial function (PGC-1 α , NRF1, ERR- α , PPAR- α , and CytC) were

Table 2. Hemodynamic data

	Physiological Hypertrophy			Pathological Hypertrophy		
	Co	Ex	P Value	Sham	AB	P Value
HR, beats/min	412 $\pm$ 9	400 $\pm$ 8	0.329	429 $\pm$ 15	434 $\pm$ 11	0.794
MAP, mmHg	145.3 $\pm$ 2.8	142.1 $\pm$ 5.2	0.586	142.0 $\pm$ 2.0	182.3 $\pm$ 7.9	<0.001
LVESP, mmHg	154.8 $\pm$ 3.4	152.2 $\pm$ 8.2	0.761	150.8 $\pm$ 2.0	213.4 $\pm$ 6.8	<0.001
LVEDP, mmHg	3.0 $\pm$ 0.2	3.7 $\pm$ 0.4	0.151	3.9 $\pm$ 0.7	4.4 $\pm$ 0.4	0.374
dP/dt _{max} , mmHg/s	9,237 $\pm$ 397	9,847 $\pm$ 659	0.439	9,103 $\pm$ 424	12,185 $\pm$ 399	<0.001
dP/dt _{min} , mmHg/s	-12,191 $\pm$ 443	-12,213 $\pm$ 670	0.979	-12,390 $\pm$ 261	-12,506 $\pm$ 676	0.887
τ , ms	12.1 $\pm$ 0.3	11.2 $\pm$ 0.3	0.036	11.7 $\pm$ 0.6	16.6 $\pm$ 1.3	0.006
LVEDV, μ l	234.4 $\pm$ 5.2	239.2 $\pm$ 6.1	0.552	239.9 $\pm$ 7.2	261.7 $\pm$ 9.1	0.100
LVESV, μ l	111.5 $\pm$ 2.0	98.6 $\pm$ 2.3	<0.001	105.7 $\pm$ 4.8	114.4 $\pm$ 5.1	0.179
SV, μ l	123.1 $\pm$ 5.5	140.6 $\pm$ 4.8	0.026	134.2 $\pm$ 7.0	147.2 $\pm$ 6.3	0.193
EF, %	52.2 $\pm$ 1.4	58.3 $\pm$ 0.8	0.001	55.9 $\pm$ 2.1	56.3 $\pm$ 1.3	0.861
CO, ml/min	50.6 $\pm$ 2.0	56.2 $\pm$ 1.5	0.037	58.5 $\pm$ 3.9	63.7 $\pm$ 2.8	0.281
CI, (ml/min)/100 g BW	10.9 $\pm$ 0.5	14.2 $\pm$ 0.4	<0.001	13.8 $\pm$ 1.1	14.7 $\pm$ 0.7	0.485
SW, mmHg·ml	14.7 $\pm$ 0.8	18.0 $\pm$ 0.8	0.009	15.6 $\pm$ 1.2	22.7 $\pm$ 1.2	0.001
Slope of EDPVR, mmHg/ μ l	0.036 $\pm$ 0.003	0.042 $\pm$ 0.003	0.131	0.034 $\pm$ 0.004	0.042 $\pm$ 0.006	0.256
TPR, mmHg/(ml/min)	2.91 $\pm$ 0.09	2.54 $\pm$ 0.12	0.034	2.52 $\pm$ 0.18	2.95 $\pm$ 0.22	0.167
E _a , mmHg/ μ l	1.33 $\pm$ 0.05	1.09 $\pm$ 0.08	0.021	1.07 $\pm$ 0.05	1.48 $\pm$ 0.07	0.001
VAC	0.53 $\pm$ 0.03	0.32 $\pm$ 0.03	<0.001	0.42 $\pm$ 0.02	0.37 $\pm$ 0.03	0.188

Values are means $\pm$ SE. Hemodynamic parameters were measured by the Millar pressure-volume conductance catheter system. MAP, mean arterial pressure; LVESP, LV end-systolic pressure; LVEDP, LV end-diastolic pressure; 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; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; SV, stroke volume; CO, cardiac output; CI, cardiac index; BW, body weight; SW, stroke work; EDPVR, end-diastolic pressure-volume relationship; TPR, total peripheral resistance; E_a, arterial elastance; VAC, ventriculoarterial coupling.

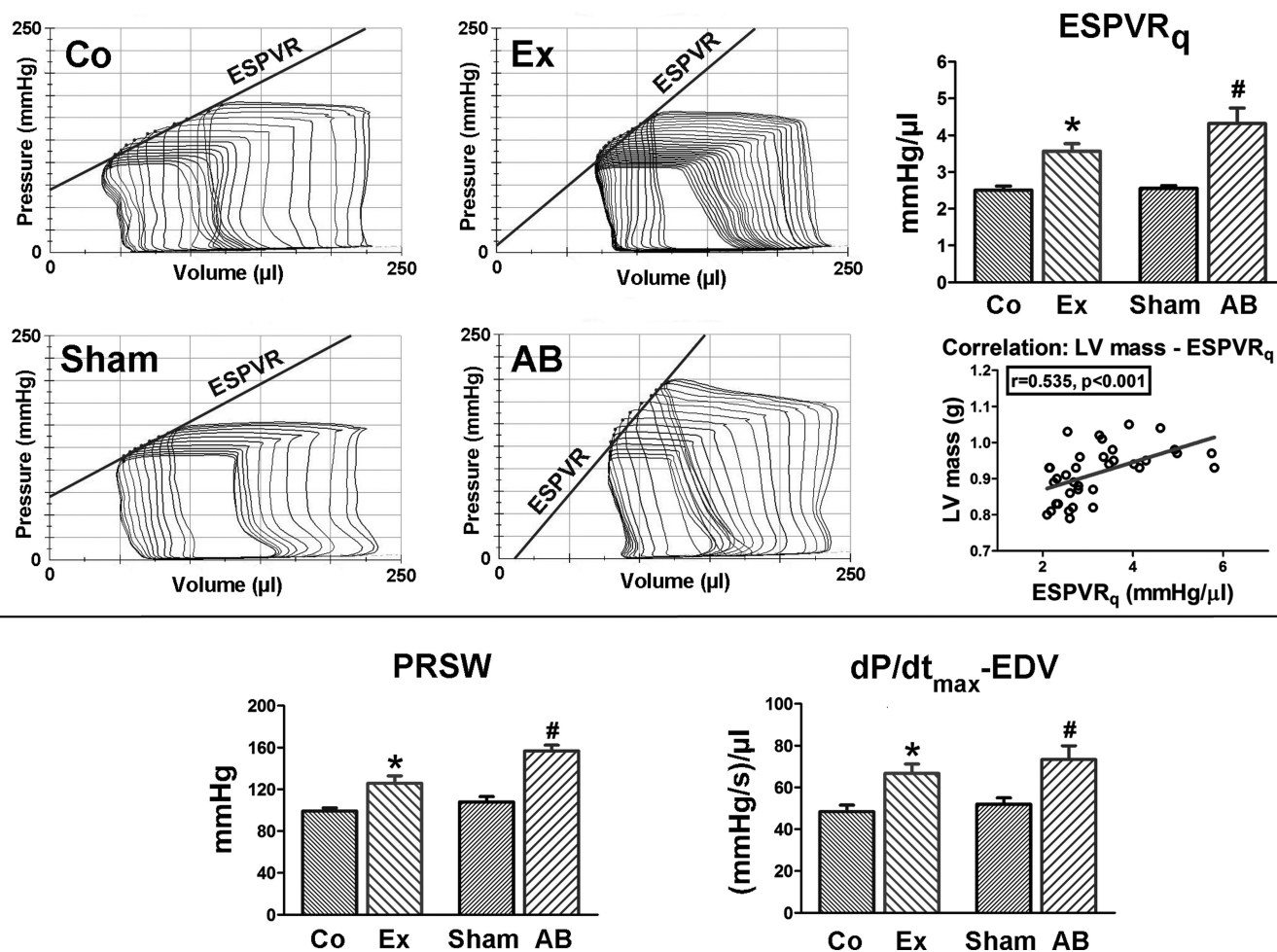


Fig. 3. LV contractility in physiological and pathological hypertrophy. Sensitive, load-independent indexes of LV contractility could be obtained by P-V analysis during reducing cardiac preload by the transient occlusion of vena cava inferior. Representative original recordings of P-V relations during this maneuver are depicted at *top*. The widely used slope of end-systolic P-V relationship (ESPVR) was increased in Ex and AB rats compared with Co and Sham animals, respectively. Preload recruitable stroke work (PRSW) and the slope of maximal dP/dt-end-diastolic volume relationship (dP/dt_{max}-EDV) were increased in both models of hypertrophy. Values are means $\pm$ SE. * $P < 0.05$ vs. Co; # $P < 0.05$ vs. Sham.

not altered in physiological hypertrophy. In contrast, pathological hypertrophy was associated with myocardial down-regulation of these genes, which led to characteristic differences between the two hypertrophy models (Fig. 6D).

DISCUSSION

According to our knowledge, this is the first study to provide a direct hemodynamic comparison of physiological and pathological LV hypertrophy using pressure-conductance catheter system in relevant small animal models. Despite the similar degree of LV hypertrophy, distinct characteristics of energy-dependent LV performance were observed. According to our data, different myocardial expression of mitochondrial markers might indicate the alteration of mitochondrial regulation as a possible underlying mechanism.

Characterization of LV hypertrophy. Cardiac hypertrophy is described as the response of the heart to a variety of stimuli that impose increased biomechanical stress. An increase in cardiomyocyte size, enhanced protein synthesis, and a higher organization of the sarcomere lead to morphological alterations resulting in hypertrophic phenotype (12). According to our

results, in response to chronic pressure overload or exercise training, the heart weight values of rats increased significantly. Both aortic banding and intense swimming training resulted in a significant increase of LV wall thickness values (Table 1). LV mass index data revealed the degree of LV hypertrophy ≈ 20 –25% after exercise training and ≈ 25 –30% after chronic pressure overload stimulus (Fig. 1A), which are comparable with the results of our previous study (29) and other experimental investigations (24, 42, 45). Histological evaluation of LV cardiomyocyte width on HE-stained sections underpinned the LV hypertrophy in both models (Fig. 1). All of these data suggest marked LV hypertrophy in our animal models with similar degree of myocardial enlargement. Relatively unchanged LV cavity dimensions as well as FS and EF revealed no pathological hypertrophy-associated dilatation in AB rats, which is in line with previous investigations using abdominal aortic banding (Table 1) (8, 17).

The increase in collagen was localized only in subendocardial area after 6-wk banding period (Fig. 1B). This is in line with a previous investigation using abdominal aortic banding model, where subendocardial collagen deposition was ob-

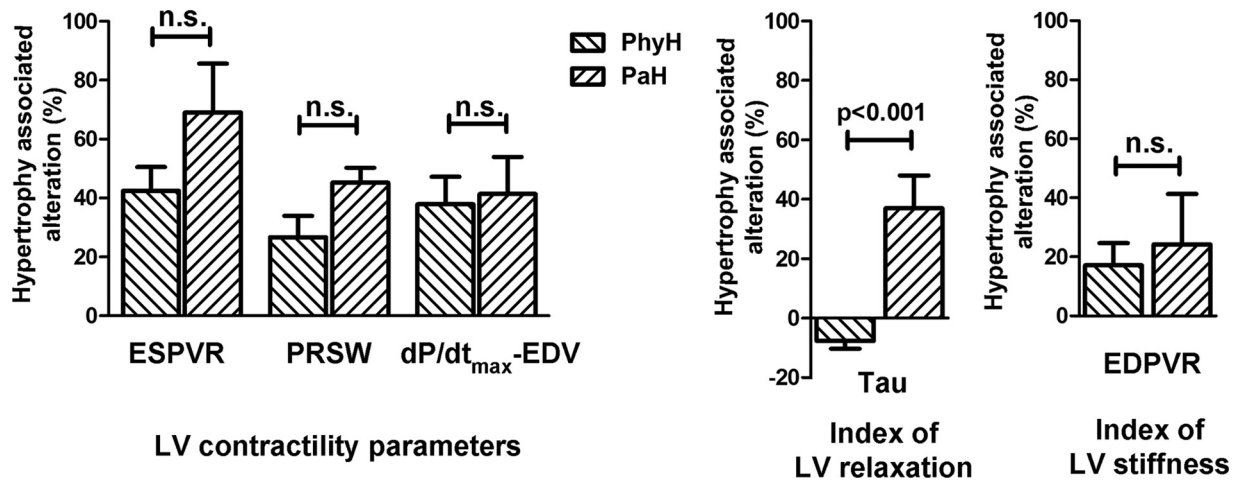


Fig. 4. Load-independent indexes of LV systolic and diastolic function. Sensitive contractility indexes, such as the slope of ESPVR, PRSW, and $dP/dt_{\max}$ -EDV, revealed comparable LV systolic improvement between the model of physiological hypertrophy (PhyH) and pathological hypertrophy (PaH). There was a marked difference regarding Tau (τ), a load-independent parameter of LV active relaxation. In PhyH a diastolic improvement was shown as indicated by decreased τ values, whereas hearts of aortic-banded animals showed a marked deterioration in active relaxation (increased τ). A tendency toward increased LV stiffness values was observed in both hypertrophy, without any difference between the models. Values are means $\pm$ SE. n.s., not significant.

served after 2 mo of chronic pressure overload (8). The absence of fibrotic remodeling supports the physiologic nature of LV hypertrophy after long-term exercise training (45).

In contrast with exercise training-induced cardiac hypertrophy, in the case of pathological hypertrophy the observed LV enlargement is accompanied by the reinduction of myocardial fetal gene program (12). Myocardial gene expression values of widely used pathological hypertrophy markers α -MHC, β -MHC, and ANF clearly demonstrated the physiological and pathological nature of LV hypertrophy in Ex and AB rats, respectively (Fig. 6A) (16, 24). Pathological hypertrophy advanced to heart failure is associated with myocardial oxidative stress and activated inflammatory mechanisms (1). Unaltered myocardial gene expressions of endogenous antioxidants and inflammatory cytokines after 6 wk of aortic banding might indicate that intensive oxidative stress and consecutive inflammatory response are not present in this compensated phase (Fig. 6, B and C). This is in line with results of an experiment comparing compensated myocardial hypertrophy with failing heart in a pressure overload model (2). No alterations in expression of oxidative stress- and inflammatory-related markers were expected in exercise-induced hypertrophy according to literature data (16).

Baseline hemodynamics. As it is clearly depicted on Fig. 2, unaltered end-diastolic dimensions along with decreased end-systolic dimensions—accompanied by similar LV pressure relations and heart rate—were observed in exercised animals under anesthesia (Fig. 2 and Table 2), which is contradictory to observations in humans, where athlete's heart induced by aerobic training was associated with decreased heart rate and increased end-diastolic volume (22). As a substantial point, in line with human studies using noninvasive methods, baseline pressure-volume relations revealed increased stroke volume in athlete's heart (34, 36).

As shown in Table 2, abdominal aortic banding was associated with a marked increase in MAP (≈ 40 mmHg) and in peak LV systolic pressure (≈ 60 mmHg), which degree is comparable with other studies using aortic constriction to induce pathological hypertrophy in rodents (19, 24), whereas LV end-diastolic pressure remained unchanged. The baseline pressure-volume loops showed a slight shift rightward since it is described in developing cardiac hypertrophy induced by pressure overload (26). However, no marked dilation was observed since LV volume values showed only a tendency toward higher values without reaching the level of significance,

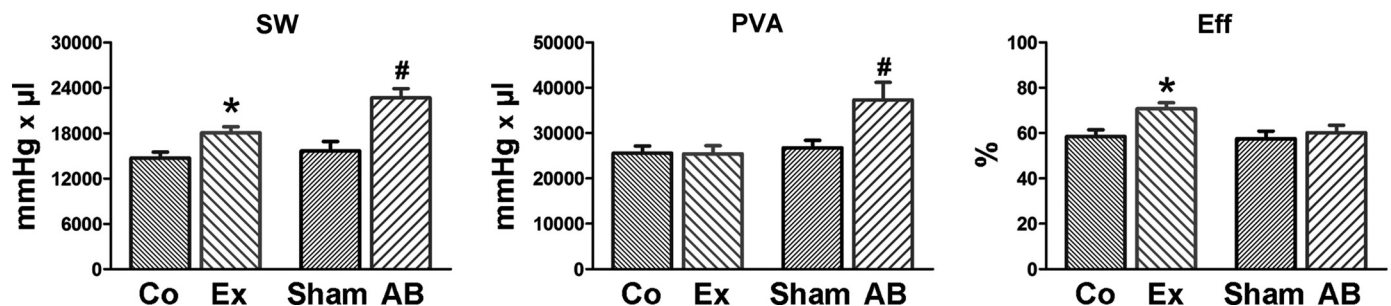


Fig. 5. Comparison of LV mechanoenergetics. Stroke work (SW), the mechanical work of LV, was increased in both models. PV area (PVA), the energy demand of LV performance, was increased in AB compared with Sham rats. However, this parameter did not differ between Ex and Co animals. Consequently, the LV mechanical efficiency (Eff) was improved in physiological hypertrophy, but remained unaltered in pathological hypertrophy. Values are means $\pm$ SE. * $P < 0.05$ vs. Co; # $P < 0.05$ vs. Sham.

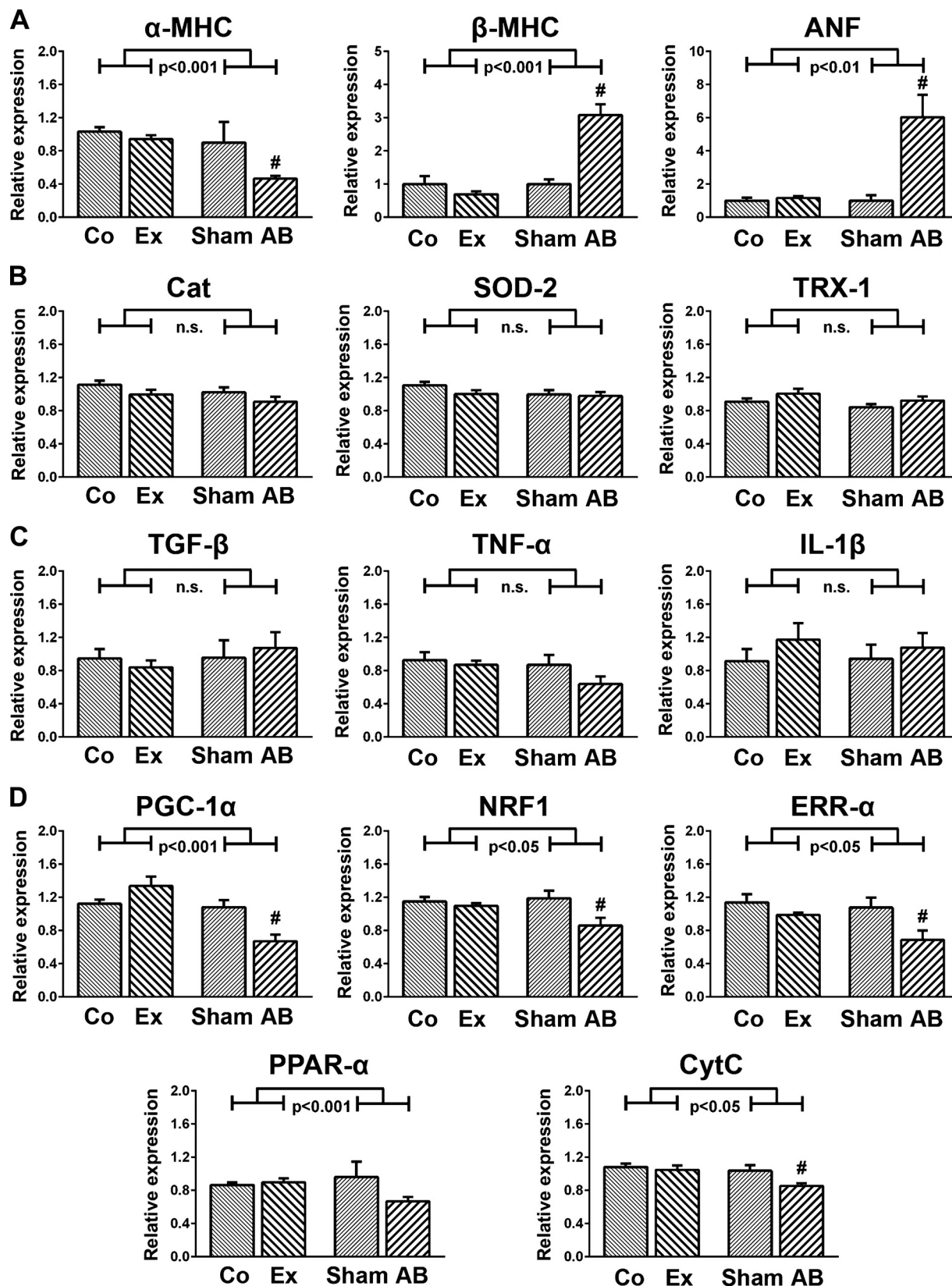


Fig. 6. Myocardial gene expression analysis. Relative myocardial expression of genes related to pathological hypertrophy [A: α -myosin heavy chain (α -MHC), β -myosin heavy chain (β -MHC), and atrial natriuretic factor (ANF)], oxidative stress [B: catalase (Cat), superoxide dismutase-2 (SOD-2), and thioredoxin-1 (TRX-1)], inflammation [C: transforming growth factor β (TGF- β), TNF- α , and IL-1 β], and mitochondrial function [D: peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), nuclear respiratory factor 1 (NRF1), estrogen-related receptor α (ERR- α), peroxisome proliferator-activated receptor α (PPAR- α), and cytochrome *c* (CytC)] in Co, Ex, Sham, and AB rats is shown. *P* values in the middle of the graphs indicate differences between the 2 models. Values are means $\pm$ SE. #*P* < 0.05 vs. Sham. n.s., not significant.

in which data suggest the compensated state of pathological hypertrophy.

In summary, when physiological and pathological stimuli-induced alterations of baseline pressure-volume loops are compared, the main characteristics are the widening of the P-V loop reflecting increased stroke volume in case of physiological hypertrophy, whereas increased systolic pressure values along with relatively unaltered stroke volume were shown in pathological hypertrophy by P-V analysis.

Systolic function and LV contractility. Conventional parameters of systolic function including LV FS and EF by echocardiography and EF by P-V analysis were elevated in the exercised rats, suggesting increased LV systolic performance in physiological hypertrophy (Table 2). These parameters remained unchanged, which demonstrated preserved systolic function after 6 wk of pressure overload, indicating clearly compensated pathological cardiac hypertrophy in accordance with a previous study using relatively short-term (2 mo) abdominal aortic banding (8). Another traditional index of LV systolic function is the peak rate of systolic pressure increment (dp/dt_{max}). However, it is influenced especially by preload but also by extreme grade of afterload and heart rate (5). Thus the potential alteration of cardiac preload in trained animals and the extremely increased LV afterload in AB rats turn this parameter unreliable.

LV contractility can be assessed precisely and reliably by pressure-volume analysis. By the help of pressure-volume relations being recorded during a transient preload reduction maneuver (vena cava inferior occlusion), the load-independent indicators of ventricular contractility can be calculated (18). Possibly the most widely used sensitive contractility index, ESPVR, was increased both in swim-trained and AB rats (Fig. 3). Stroke work and dp/dt_{max} , traditionally used systolic parameters, vary mostly with preload conditions of the heart. Preload dependence of these parameters can be effectively reduced by using regression to end-diastolic volume during preload-reduction maneuver (5). PRSW and dp/dt_{max} -EDV also were increased in both hypertrophy models, suggesting increased contractility in exercise training- and pressure overload-induced LV hypertrophy. These observations are in line with our previous results in physiological hypertrophy (31) and with formerly published data of pressure overload-induced pathological hypertrophy in rodents (4, 40).

There was no significant difference regarding contractility alterations compared with the corresponding control animals between the two models (Fig. 4). Moreover, contractility (indicated by ESPVR, PRSW, and dp/dt_{max} -EDV) was found to correlate with the degree of LV hypertrophy (pointed out by LV mass), which suggests that LV hypertrophy is an adaptive response required to sustain CO in the presence of increased biomechanical stress, whether the stimuli is physiological or pathological (12).

Diastolic function. The diastolic phase of the ventricular cycle contains two main components to characterize by pressure-volume analysis: active relaxation and myocardial stiffness.

LV relaxation is considered an active, energy-consuming process and depends mostly on calcium reuptake by the sarcoplasmic reticulum during the early diastole (46). Maximal velocity of LV pressure decline (dp/dt_{min}) was unchanged in both types of LV hypertrophy; however, the value of this

parameter is very limited because of dependence on many hemodynamic factors, such as cardiac loading conditions (13). In contrast, the time constant of LV pressure decay has been described as a relatively load-independent index of LV active relaxation (46). Physiological hypertrophy was associated with enhanced relaxation as shown by decreased τ , thus a shorter isovolumetric relaxation period (Table 2). This is in line with our previously published data in a rat model of athlete's heart (31). In contrast, we observed a markedly delayed relaxation in pathological hypertrophy (Table 2). In accordance, investigations for functional characterization of LV hypertrophy induced by pressure overload have yielded similar results regarding active relaxation. Animal models that included aortic constriction (19, 40) or spontaneously hypertensive rats (5) have described a marked impairment in active relaxation. This distinction might be the most characteristic difference between the two types of cardiac hypertrophy (Fig. 4). This is in accordance with recent human echocardiographic studies, where hypertrophic cardiomyopathy and athlete's heart could be distinguished by early diastolic components (3, 20).

Ventricular end-diastolic stiffness, although it can be influenced by different factors, is affected predominantly by alterations in myocardial intracellular and extracellular structural components (e.g., fibrosis, edema) (30). This passive viscoelastic property of the myocardium can be quantified by examination of the relationship between diastolic pressure and volume (47). EDPVR showed a slight tendency in both types of hypertrophy toward higher values—without reaching statistical significance—and no difference could be observed between the hypertrophy models (Table 2 and Fig. 4). These data are in good agreement with the lack of collagen deposition observed in the hearts of exercised animals. The observed slight increase of subendocardial (but not subepicardial) fibrosis in AB rats might be not so relevant as resulting in characteristic functional consequences (Fig. 1B). Unchanged LVEDP was also consistent with these observations (Table 2).

Mechanoenergetics. SW, the effective external mechanical work of LV in one cardiac cycle, can be calculated as the area enclosed by the PV loop. Increased mechanical work was observed both in exercise training- and pressure overload-induced LV hypertrophy due to increased stroke volume and elevated systolic pressure values, respectively (Fig. 5 and Table 2). PVA, the specific area in the P-V plane bounded by the end-systolic and end-diastolic P-V relationship lines and the systolic segment of the P-V loop, serves as a reliable index of total mechanical energy and is directly proportional to myocardial oxygen consumption (38). Although physiological hypertrophy was related to similar energy consumption as compared with control animals, pathological hypertrophy was associated with an increased energy consumption of LV myocardium (Fig. 5). As a consequence, mechanical efficiency of ventricular performance was increased in exercise training-induced hypertrophy, whereas it remained unaltered in pathological hypertrophy (Fig. 5). These results might reflect the previously described distinction of metabolic efficiency between physiological and pathological myocardial conditions: exercise training is associated with enhanced fatty acid and glucose oxidation, whereas pathological hypertrophy is related to a decrease in fatty acid oxidation and increase in glucose metabolism (1).

As an important factor of cardiac energetics, ventricular-arterial coupling expresses the interaction between left ventricle and arterial system by the ratio of arterial elastance and LV end-systolic elastance (39). Arterial system can be characterized by effective arterial elastance (E_a), an integrative index that includes, among others, peripheral vascular resistance, arterial compliance, and characteristic impedance. Decreased E_a in exercised rats reveals a better compliance of the arterial system in physiological hypertrophy, whereas increased E_a in pathological hypertrophy clearly reflects the increased afterload due to pressure overload (Table 2). These alterations were underpinned by changes in TPR (Table 2). ESPVR, the substantial index of contractility, corresponds to LV end-systolic elastance (32). Improved VAC 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. Unaltered VAC in pressure overload-induced pathological hypertrophy indicates that increased systolic LV performance compensates the increased arterial load (Table 2). These observations are in accordance with human investigations describing ventriculo-arterial relations in athletes and in patients with hypertension-induced LV hypertrophy (11, 27).

Mitochondrial function. The distinct cardiac functional parameters as active relaxation, SW, and mechanical efficiency have been each referred to as energy-dependent indexes. Thus we examined the myocardial expression of a mitochondrial transcriptional coactivator PGC-1 α , the master regulator of myocardial energy metabolism, by regulating mitochondrial biogenesis and function (10). In line with previous literature data (32), both PGC-1 α and its downstream coactivators and targets (ERR- β , NRF1, PPAR- α , and CytC) were downregulated in pathological hypertrophy, suggesting mitochondrial dysfunction and a shift from lipid to glucose utilization (Fig. 6D). Physiological cardiac hypertrophy is associated with normal or enhanced mitochondrial biogenesis (32). According to our data, there was no significant upregulation of these genes in our swim training model of athlete's heart (Fig. 6D). This difference between our cardiac hypertrophy models supports the concept that targeting regulator molecules of mitochondrial biogenesis and function might play an important role in the treatment of pathological hypertrophy.

Limitations and perspectives. This study was designed to compare functional and molecular alterations in the most widely used pressure overload-induced compensated pathological LV hypertrophy and dynamic exercise-induced LV hypertrophy models. To compare these conditions to decompensated hypertrophy or volume overload-induced pathological hypertrophy, further investigations are needed.

Changes in coronary angiogenesis and capillary density might be an important contributing factor to myocardial hypertrophic situations because the enlarged cardiac mass requires increased blood supply to maintain energy-dependent cellular processes; thus examining myocardial capillary density might be an important issue in future investigations comparing physiological and pathological hypertrophic phenotypes (28).

We should also mention that for a more accurate analysis of cardiomyocyte size, hearts should be arrested in diastole using intravenous injection of potassium chlorid solution, which was not possible in the present study, due to methodological reasons.

Conclusions. In conclusion, we provided the first detailed hemodynamic comparison of physiological and compensated pathological hypertrophy in relevant rodent models. We confirmed LV hypertrophy of comparable degree in swim-trained and AB animals with characteristic attributes of physiological and pathological stimuli-induced histological and gene expression alterations. Increased LV contractility could be observed in both types of myocardial conditions, and contractility correlated with the degree of hypertrophy. Although the degree of hypertrophy was similar in the two models, characteristic differences were detected in early diastolic function: active relaxation was improved in physiological hypertrophy, whereas a marked deterioration was observed in pathological hypertrophy. A tendency toward increased myocardial stiffness was observed in both types of myocardial hypertrophy. LV mechanoenergetics (mechanical efficiency and ventriculo-arterial coupling) were more optimal in exercise training-induced hypertrophy, whereas they remained unaltered in pressure overload-induced pathological hypertrophy. Altered myocardial expression of markers related to mitochondrial function and biogenesis might explain the described energy-dependent functional differences.

ACKNOWLEDGMENTS

We thank Henriett Biró, Tímea Fischinger, Viktória Gregor, Dóra Juhász, Gábor Fritz, and Gábor Alt for expert technical assistance and Dr. Gábor Kökény for scientific advice.

GRANTS

This work was supported by a grant from the National Development Agency of Hungary (TÁMOP-4.2.2/B-10/1-2010-0013), by the Hungarian Scientific Research Fund (OTKA 105555 to B. Merkely), and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (to T. Radovits).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

REFERENCES

- Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. *Pharmacol Ther* 128: 191–227, 2010.
- Brooks WW, Shen SS, Conrad CH, Goldstein RH, Bing OH. Transition from compensated hypertrophy to systolic heart failure in the spontaneously hypertensive rat: structure, function, and transcript analysis. *Genomics* 95: 84–92, 2010.
- Caselli S, Maron MS, Urbano-Moral JA, Pandian NG, Maron BJ, Pelliccia A. Differentiating left ventricular hypertrophy in athletes from that in patients with hypertrophic cardiomyopathy. *Am J Cardiol* 114: 1383–1389, 2014.
- Chen H, Hwang H, McKee LA, Perez JN, Regan JA, Constantopoulos E, Lafleur B, Konhilas JP. Temporal and morphological impact of pressure overload in transgenic FHC mice. *Front Physiol* 4: 205, 2013.
- Cingolani OH, Kass DA. Pressure-volume relation analysis of mouse ventricular function. *Am J Physiol Heart Circ Physiol* 301: H2198–H2206, 2011.

6. Cingolani OH, Yang XP, Cavaasin MA, Carretero OA. Increased systolic performance with diastolic dysfunction in adult spontaneously hypertensive rats. *Hypertension* 41: 249–254, 2003.
7. D'Andrea A, D'Andrea L, Caso P, Scherillo M, Zeppilli P, Calabro R. The usefulness of Doppler myocardial imaging in the study of the athlete's heart and in the differential diagnosis between physiological and pathological ventricular hypertrophy. *Echocardiography* 23: 149–157, 2006.
8. Derumeaux G, Mulder P, Richard V, Chagraoui A, Nafeh C, Bauer F, Henry JP, Thuillez C. Tissue Doppler imaging differentiates physiological from pathological pressure-overload left ventricular hypertrophy in rats. *Circulation* 105: 1602–1608, 2002.
9. Ellison GM, Waring CD, Vicinanza C, Torella D. Physiological cardiac remodelling in response to endurance exercise training: cellular and molecular mechanisms. *Heart* 98: 5–10, 2012.
10. Finck BN, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) regulatory cascade in cardiac physiology and disease. *Circulation* 115: 2540–2548, 2007.
11. Florescu M, Stoicescu C, Magda S, Petcu I, Radu M, Palombo C, Cinteza M, Lichiardopol R, Vinereanu D. "Supranormal" cardiac function in athletes related to better arterial and endothelial function. *Echocardiography* 27: 659–667, 2010.
12. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 65: 45–79, 2003.
13. Garrido JM, Gerson MC, Hoit BD, Walsh RA. Load independence of early diastolic filling parameters in the anesthetized canine model. *J Nucl Med* 34: 1520–1528, 1993.
14. Georgakopoulos D, Kass DA. Estimation of parallel conductance by dual-frequency conductance catheter in mice. *Am J Physiol Heart Circ Physiol* 279: H443–H450, 2000.
15. Hildick-Smith DJ, Shapiro LM. Echocardiographic differentiation of pathological and physiological left ventricular hypertrophy. *Heart* 85: 615–619, 2001.
16. Iemitsu M, Miyauchi T, Maeda S, Sakai S, Kobayashi T, Fujii N, Miyazaki H, Matsuda M, Yamaguchi I. Physiological and pathological cardiac hypertrophy induce different molecular phenotypes in the rat. *Am J Physiol Regul Integr Comp Physiol* 281: R2029–R2036, 2001.
17. Juric D, Wojciechowski P, Das DK, Neticadan T. Prevention of concentric hypertrophy and diastolic impairment in aortic-banded rats treated with resveratrol. *Am J Physiol Heart Circ Physiol* 292: H2138–H2143, 2007.
18. Kass DA. Clinical ventricular pathophysiology: a pressure-volume view. In: *Ventricular Function*, edited by Wartler DC. Baltimore: Williams & Wilkins, 1995.
19. Kompa AR, Wang BH, Phrommintikul A, Ho PY, Kelly DJ, Behm DJ, Douglas SA, Krum H. Chronic urotensin II receptor antagonist treatment does not alter hypertrophy or fibrosis in a rat model of pressure-overload hypertrophy. *Peptides* 31: 1523–1530, 2010.
20. Kovacs A, Apor A, Nagy A, Vago H, Toth A, Nagy AI, Kovats T, Sax B, Szepplaki G, Becker D, Merkely B. Left ventricular untwisting in athlete's heart: key role in early diastolic filling? *Int J Sports Med* 35: 259–264, 2014.
21. Kovacs A, Olah A, Lux A, Matyas C, Nemeth BT, Kellermayer D, Ruppert M, Torok M, Szabo L, Meltzer A, Assabiny A, Birtalan E, Merkely B, Radovits T. Strain and strain rate by speckle-tracking echocardiography correlate with pressure-volume loop-derived contractility indices in a rat model of athlete's heart. *Am J Physiol Heart Circ Physiol* 308: H743–H748, 2015.
22. Maron BJ. Structural features of the athlete heart as defined by echocardiography. *J Am Coll Cardiol* 7: 190–203, 1986.
23. McMullen JR, Jennings GL. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. *Clin Exp Pharmacol Physiol* 34: 255–262, 2007.
24. McMullen JR, Shioi T, Zhang L, Tarnavski O, Sherwood MC, Kang PM, Izumo S. Phosphoinositide 3-kinase(p110alpha) plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy. *Proc Natl Acad Sci USA* 100: 12355–12360, 2003.
25. McMurray JJ, Pfeffer MA. Heart failure. *Lancet* 365: 1877–1889, 2005.
26. Moens AL, Takimoto E, Tocchetti CG, Chakir K, Bedja D, Cormaci G, Ketner EA, Majumdar M, Gabrielson K, Halushka MK, Mitchell JB, Biswal S, Channon KM, Wolin MS, Alp NJ, Paolucci N, Champion HC, Kass DA. Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy. *Circulation* 117: 2626–2636, 2008.
27. Nitenberg A, Loiseau A, Antony I. Left ventricular mechanical efficiency in hypertensive patients with and without increased myocardial mass and with normal pump function. *Am J Hypertens* 14: 1231–1238, 2001.
28. Oka T, Akazawa H, Naito AT, Komuro I. Angiogenesis and cardiac hypertrophy: maintenance of cardiac function and causative roles in heart failure. *Circ Res* 114: 565–571, 2014.
29. Olah A, Nemeth BT, Matyas C, Horvath EM, Hidi L, Birtalan E, Kellermayer D, Ruppert M, Merkely G, Szabo G, Merkely B, Radovits T. Cardiac effects of acute exhaustive exercise in a rat model. *Int J Cardiol* 182: 258–266, 2015.
30. Pacher P, Nagayama T, Mukhopadhyay P, Batkai S, Kass DA. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat Protoc* 3: 1422–1434, 2008.
31. Radovits T, Olah A, Lux A, Nemeth BT, Hidi L, Birtalan E, Kellermayer D, Matyas C, Szabo 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.
32. Rimbaud S, Garnier A, Ventura-Clapier R. Mitochondrial biogenesis in cardiac pathophysiology. *Pharmacol Rep* 61: 131–138, 2009.
33. Sagawa K. The ventricular pressure-volume diagram revisited. *Circ Res* 43: 677–687, 1978.
34. Scharhag J, Schneider G, Urhausen A, Rochette V, Kramann B, Kindermann W. Athlete's heart: right and left ventricular mass and function in male endurance athletes and untrained individuals determined by magnetic resonance imaging. *J Am Coll Cardiol* 40: 1856–1863, 2002.
35. Shephard RJ, Balady GJ. Exercise as cardiovascular therapy. *Circulation* 99: 963–972, 1999.
36. Spirito P, Pelliccia A, Proschan MA, Granata M, Spataro A, Bellone P, Caselli G, Biffi A, Vecchio C, Maron BJ. Morphology of the "athlete's heart" assessed by echocardiography in 947 elite athletes representing 27 sports. *Am J Cardiol* 74: 802–806, 1994.
37. Suga H. Cardiac energetics: from E_{max} to pressure-volume area. *Clin Exp Pharmacol Physiol* 30: 580–585, 2003.
38. Suga H, Hayashi T, Shirahata M. Ventricular systolic pressure-volume area as predictor of cardiac oxygen consumption. *Am J Physiol Heart Circ Physiol* 240: H39–H44, 1981.
39. Sunagawa K, Maughan WL, Burkhoff D, Sagawa K. Left ventricular interaction with arterial load studied in isolated canine ventricle. *Am J Physiol Heart Circ Physiol* 245: H773–H780, 1983.
40. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolucci N, Gabrielson KL, Wang Y, Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J Clin Invest* 115: 1221–1231, 2005.
41. Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B, Fletcher GF, Gordon NF, Pate RR, Rodriguez BL, Yancey AK, Wenger NK. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 107: 3109–3116, 2003.
42. Wang Y, Wisloff U, Kemi OJ. Animal models in the study of exercise-induced cardiac hypertrophy. *Physiol Res* 59: 633–644, 2010.
43. Weeks KL, McMullen JR. The athlete's heart vs. the failing heart: can signaling explain the two distinct outcomes? *Physiology (Bethesda)* 26: 97–105, 2011.
44. Weymann A, Radovits T, Schmack B, Korkmaz S, Li S, Chaimow N, Patzold I, Becher PM, Hartysanzky I, Soos P, Merkely G, Nemeth BT, Istok R, Veres G, Merkely B, Tertytze K, Karck M, Szabo G. Total aortic arch replacement: superior ventriculo-arterial coupling with deceleralized allografts compared with conventional prostheses. *PLoS One* 9: e103588, 2014.
45. Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, Jones F, Kimball TR, Molkenstein JD. Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. *Circ Res* 94: 110–118, 2004.
46. Zhao W, Choi JH, Hong GR, Vannan MA. Left ventricular relaxation. *Heart Fail Clin* 4: 37–46, 2008.
47. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 105: 1387–1393, 2002.