

Complete Reversion of Cardiac Functional Adaptation Induced by Exercise Training

ATTILA OLÁH, DALMA KELLERMAYER, CSABA MÁTYÁS, BALÁZS TAMÁS NÉMETH, ÁRPÁD LUX, LILLA SZABÓ, MARIANNA TÖRÖK, MIHÁLY RUPPERT, ANNA MELTZER, ALEX ALI SAYOUR, KÁLMÁN BENKE, ISTVÁN HARTYÁNSZKY, BÉLA MERKELY, and TAMÁS RADOVITS

Heart and Vascular Center, Semmelweis University, Budapest, HUNGARY

ABSTRACT

OLÁH, A., D. KELLERMAYER, C. MÁTYÁS, B. T. NÉMETH, Á. LUX, L. SZABÓ, M. TÖRÖK, M. RUPPERT, A. MELTZER, A. A. SAYOUR, K. BENKE, I. HARTYÁNSZKY, B. MERKELY, AND T. RADOVITS. Complete Reversion of Cardiac Functional Adaptation Induced by Exercise Training. *Med. Sci. Sports Exerc.*, Vol. 49, No. 3, pp. 420–429, 2017. **Purpose:** Long-term exercise training is associated with characteristic cardiac adaptation, termed athlete's heart. Our research group previously characterized *in vivo* left ventricular (LV) function of exercise-induced cardiac hypertrophy in detail in a rat model; however, the effect of detraining on LV function is still unclear. We aimed at evaluating the reversibility of functional alterations of athlete's heart after detraining. **Methods:** Rats ($n = 16$) were divided into detrained exercised (DEX) and detrained control (DCo) groups. Trained rats swam $200 \text{ min} \cdot \text{d}^{-1}$ for 12 wk, and control rats were taken into water for $5 \text{ min} \cdot \text{d}^{-1}$. After the training period, both groups remained sedentary for 8 wk. We performed echocardiography at weeks 12 and 20 to investigate the development and regression of exercise-induced structural changes. LV pressure–volume analysis was performed to calculate cardiac functional parameters. LV samples were harvested for histological examination. **Results:** Echocardiography showed robust LV hypertrophy after completing the training protocol (LV mass index = 2.61 ± 0.08 DEX vs $2.04 \pm 0.04 \text{ g} \cdot \text{kg}^{-1}$ DCo, $P < 0.05$). This adaptation regressed after detraining (LV mass index = 2.01 ± 0.03 vs $1.97 \pm 0.05 \text{ g} \cdot \text{kg}^{-1}$, n.s.), which was confirmed by postmortem measured heart weight and histological morphometry. After the 8-wk-long detraining period, a regression of the previously described exercise-induced cardiac functional alterations was observed (DEX vs DCo): stroke volume (SV; 144.8 ± 9.0 vs $143.9 \pm 9.6 \mu\text{L}$, $P = 0.949$), active relaxation ($\tau = 11.5 \pm 0.3$ vs $11.3 \pm 0.4 \text{ ms}$, $P = 0.760$), contractility (preload recruitable stroke work = 69.5 ± 2.7 vs $70.9 \pm 2.4 \text{ mm Hg}$, $P = 0.709$), and mechanoenergetic (mechanical efficiency = 68.7 ± 1.2 vs 69.4 ± 1.8 , $P = 0.742$) enhancement reverted completely to control values. Myocardial stiffness remained unchanged; moreover, no fibrosis was observed after the detraining period. **Conclusion:** Functional consequences of exercise-induced physiological LV hypertrophy completely regressed after 8 wk of deconditioning. **Key Words:** PHYSIOLOGICAL HYPERTROPHY, DETRAINING, PRESSURE–VOLUME ANALYSIS, FUNCTIONAL REVERSIBILITY

The beneficial effects of regular exercise training and the harmful effect of physical inactivity are already extensively recognized (10,20). As a result of long-term physical training, the heart undergoes physiological ventricular remodeling, which is an adaptation to the biomechanical

stress (increased workload) imposed on the cardiovascular system during exercise sessions (8,41). This benign adaptation process results in increased cardiac mass produced by the hypertrophy of cardiomyocytes and new capillary formation by endothelial cells (8,40). Physiological hypertrophy is associated with increased stroke volume (SV) and ameliorated cardiac function. Our research group previously reported the first *in vivo* hemodynamic characterization of athlete's heart, demonstrating enhancement of left ventricular (LV) contractility, early diastolic function, and mechanoenergetics after a 12-wk-long swimming training program in rodents (34).

In contrast to the long-term exercise-induced physiological cardiac adaptation, the effects of training reduction or cessation are less understood. Detraining has been defined as the partial or complete loss of long-term exercise-induced adaptation as a consequence of training termination (24,25,31).

Address for correspondence: Attila Oláh, M.D., Ph.D., Experimental Research Laboratory Heart and Vascular Center, Semmelweis University, Városmajor u. 68, Budapest 1122, Hungary; E-mail: o.attilio@gmail.com. Submitted for publication June 2016. Accepted for publication October 2016.

0195-9131/17/4903-0420/0

MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Copyright © 2016 by the American College of Sports Medicine

DOI: 10.1249/MSS.0000000000001127

Various investigations in athletes (7,21,31) and in experimental animals (2,3,15) showed an obvious regression of training-induced morphological alterations. In a subset of elite athletes, the so-called gray zone appears where morphological characteristics of athlete's heart and mild hypertrophic cardiomyopathy overlap. In these cases, reversibility can be crucial to distinguish pronounced physiological hypertrophy from the pathological state (32). On the other hand, reversion of exercise-induced myocardial functional enhancement has been demonstrated by only few experimental studies using isolated cardiomyocytes (15) and papillary muscle (3). The influence of detraining on the performance of an intact heart still has not been reported; solely a few human echocardiographic investigations using traditional load-dependent parameters suggest the possible functional reversibility of athlete's heart (12,31).

LV pressure–volume (P–V) analysis provides the possibility to characterize *in vivo* cardiac mechanics in human and experimental animals: different aspects of LV function, such as contractility, active relaxation, stiffness, and mechanoenergetics can be reliably characterized (28). The use of this sensitive technique might answer the essential question of sports cardiology, whether after cessation of high-intensity training; after reverse remodeling, the improved cardiac performance would still persist or an impaired function would be developed in the heart of athletes compared with healthy subjects. However, considering the invasive nature of this method, because of ethical concerns, animal models are required to investigate such benign physiological cardiovascular conditions of athlete's heart. We aimed at investigating the effects of detraining on different aspects of LV performance *in vivo* using LV P–V analysis in a rat model of athlete's heart.

MATERIALS AND METHODS

Animals. All experimental procedures were approved by the Ethical Committee of Hungary for Animal Experimentation in accordance with the “Principles of Laboratory Animal Care” defined by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, provided by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (publication no. 86-23, revised 1996). All animals received human care.

Young adult Wistar male rats ($n = 16$, $m = 275$ – 325 g) were housed in standard rat cages at a constant room temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with a 12-h light–12-h dark cycle. Rats were fed with standard rodent chow and water *ad libitum*.

Model of exercise-induced cardiac hypertrophy and detraining protocol. After acclimation, rats were randomly divided into detrained control (DCo, $n = 8$) and detrained exercised (DEx, $n = 8$) groups. Swim training was performed in a divided container filled with tap water (45 cm deep) maintained at 30°C – 32°C . Rats of DEx group were exposed to $200 \text{ min} \cdot \text{d}^{-1}$ swimming $5 \text{ d} \cdot \text{wk}^{-1}$ for 12 wk to induce physiological LV hypertrophy as described previously

(34). For the appropriate adaptation, the duration of swimming was increased 15 min every second training day from a basic 15 min on the first day, until achieving the maximal $200 \text{ min} \cdot \text{d}^{-1}$. In this 12-wk-long period, DCo animals were habituated to water $5 \text{ min} \cdot \text{d}^{-1}$ $5 \text{ d} \cdot \text{wk}^{-1}$ to minimize the possible differences induced by the stress of water contact.

Thereafter, during the detraining period, animals of both groups remained sedentary for 8 wk. The length of the detraining period has been chosen according to previous echocardiographic results and corresponding literature data (13,39), aiming to obtain complete morphological regression of exercise-induced cardiac changes.

Body weight was measured regularly (three times a week) during the experimental period. At week 20 (after training and detraining period), we performed *in vivo* experiments (echocardiographic examination and P–V analysis to characterize LV morphology and function, respectively). The rats were euthanized after completion of *in vivo* experiments and heart weight was measured immediately.

Echocardiography. Echocardiographic measurements were performed after the training (12 wk) and detraining period (20 wk) under pentobarbital sodium ($60 \text{ mg} \cdot \text{kg}^{-1}$ ip.) anesthesia as described previously (16). Shortly, animals were placed on controlled heating pads, and the core temperature, measured via rectal probe, was kept at 37°C . After shaving the anterior chest, transthoracic echocardiographic examination 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 (midpapillary-level) axis were recorded by using a 13-MHz linear transducer (GE 12L-RS; GE Healthcare, Horten, Norway). The transducer was attached to an echocardiographic imaging unit (Vivid i, GE Healthcare). Digital images were analyzed by a blinded investigator using an image analyzing software (EchoPac, GE Healthcare). Using two-dimensional recordings of the short axis at the midpapillary muscle level, LV anterior wall thickness (AWT), posterior wall thickness (PWT) in diastole (index d) and systole (index s), and LV end-diastolic (LVEDD) and end-systolic diameters (LVESD) were measured. All values were averaged over three consecutive cycles. LV mass was calculated according to a cubic formula: $\text{LV mass} = \{[(\text{LVEDD} + \text{AWTd} + \text{PWTD})^3 - \text{LVEDD}^3] \times 1.04\} \times 0.8 + 0.14$. LV mass values were standardized to the animal's body weight to calculate LV mass index. To characterize LV systolic function, LV fractional shortening [FS = $(\text{LVEDD} - \text{LVESD})/\text{LVEDD} \times 100$] and LV ejection fraction (EF; according to the Teichholz method) were calculated.

Hemodynamic measurements - LV P–V analysis. LV P–V analysis was performed with the help of a 2-F pressure–conductance microcatheter (SPR-838; Millar Instruments, Houston, TX) to investigate *in vivo* cardiac function as described in detail previously (28,34). Animals were anesthetized with pentobarbital sodium ($60 \text{ mg} \cdot \text{kg}^{-1}$ ip.), tracheotomized and intubated to ease breathing. The rats were placed on automatically controlled heating pads, with a

maintained core temperature at 37°C, measured via rectal probe. A polyethylene catheter was inserted into the left external jugular vein for fluid administration. The pressure–conductance catheter was inserted into the right carotid artery and advanced into the ascending aorta. After 5 min stabilization, mean arterial pressure (MAP) and heart rate (HR) were recorded. The catheter was further inserted to the LV, under pressure control. Signals were continuously recorded after a 5-min stabilization by 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).

After steady-state P–V loops were recorded, baseline parameters of LV performance were calculated with 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 Glantz method), LV end-systolic volume (LVESV), LV end-diastolic volume (LVEDV), SV, cardiac output (CO), EF, stroke work (SW), and arterial elastance (E_a). CO was normalized to body weight (cardiac index [CI]). Total peripheral resistance (TPR) was calculated as follows: $TPR = MAP/CO$.

P–V loops recorded during decreasing preload (by transiently occluding the inferior caval vein) can be used to determine useful load-independent contractility indices: the slope of the LV end-systolic P–V relationship (ESPVR, parabolic curvilinear model), the preload recruitable SW (PRSW, slope of the linear relation between SW and EDV), and the slope of $dP/dt_{\max}$ –end-diastolic volume relationship ($dP/dt_{\max}$ –EDV). Furthermore, the slope of end-diastolic P–V relationship (EDPVR) was calculated, which is a reliable index of LV stiffness.

To characterize the mechanoenergetic status of the left ventricle, SW, P–V area (PVA), and mechanical efficiency ($Eff = SW/PVA$) were calculated. Ventriculoarterial coupling (VAC) was calculated as the ratio of E_a and ESPVR to describe the matching between the LV and the arterial system.

Histology. After euthanizing the rats, their hearts were removed from the chest, snap frozen in liquid nitrogen, and stored at -80°C . Transverse transmural ventricular slices (5 μm) were sectioned and conventionally processed for histological examination. The sections were stained with hematoxylin–eosin (HE) and picrosirius red. Light microscopic examination was performed with a Zeiss microscope (Axio Observer.Z1; Carl Zeiss, Jena, Germany) at a magnification of $\times 400$, and digital images were captured by an imaging software (Qcapture Pro 6.0, Qimaging, Canada).

After calibration, transverse transnuclear widths of 100 randomly selected, longitudinally oriented cardiomyocytes were measured by a blinded investigator to detect myocardial hypertrophy.

The amount of myocardial collagen was determined by using picrosirius red–stained sections with ImageJ (National

Institutes of Health, Bethesda, MD) image analysis software. Four subendocardial and four subepicardial images (magnification $\times 200$) were taken randomly from the free LV wall on each section from six animals of each group. After background subtraction and threshold adjustment, 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.

Statistical analysis. Data were analyzed with a personal computer software (GraphPad Prism 6; GraphPad Software, La Jolla, CA). All data are presented as mean $\pm$ SEM.

After testing normal distribution of echocardiographic data by the Shapiro–Wilk normality test, mixed ANOVA was conducted (with time/detraining as the within-subject factor and training as the between-subject factor). Where statistically significant interaction was found ($P_{\text{interaction}} < 0.05$), *post hoc* pairwise comparisons with Bonferroni correction were performed to determine differences between groups (DCo vs DEx) at one time point and to compare data within a group at different time points (week 12 vs week 20).

To compare postmortem, histological, and hemodynamic parameters of DCo and DEx animals, unpaired Student's *t*-test was used. $P < 0.05$ was considered to be statistically significant.

RESULTS

Markers of Myocardial Hypertrophy

Figure 1A shows representative M-mode echocardiographic images in animals from both groups after 12 and 20 wk. After the 12-wk-long training protocol, we observed LV hypertrophy in the DEx group. Both anterior and posterior wall thickness values were increased significantly in trained animals; however, a complete regression was revealed in the DEx group after the detraining period (Fig. 1A). Consistently, calculated LV mass and LV mass index values were significantly increased in exercised rats after the swim training period compared with control ones. However, after discontinuation of training, the marked differences regarding these values equalized between the groups (Fig. 1A). These parameters demonstrate the complete morphological reversibility of exercised-induced LV hypertrophy after 8 wk of detraining. Echocardiography also revealed systolic functional improvement (increased FS and EF) in exercised rats after completion of training program, along with a complete reversion after the resting period (Fig. 1A).

Comparing the heart weight in DCo and DEx groups, we found equal values. Also, the heart weight-to-body weight ratio was similar in the two groups after the 8-wk-long detraining period (Fig. 1B).

The cardiomyocyte diameters also did not differ between DCo and DEx groups (Fig. 1C). The evaluation of picrosirius red staining reflects unaltered LV collagen in both groups after the detraining period (Fig. 1D). Figure 1C and D shows representative HE- and picrosirius red–stained

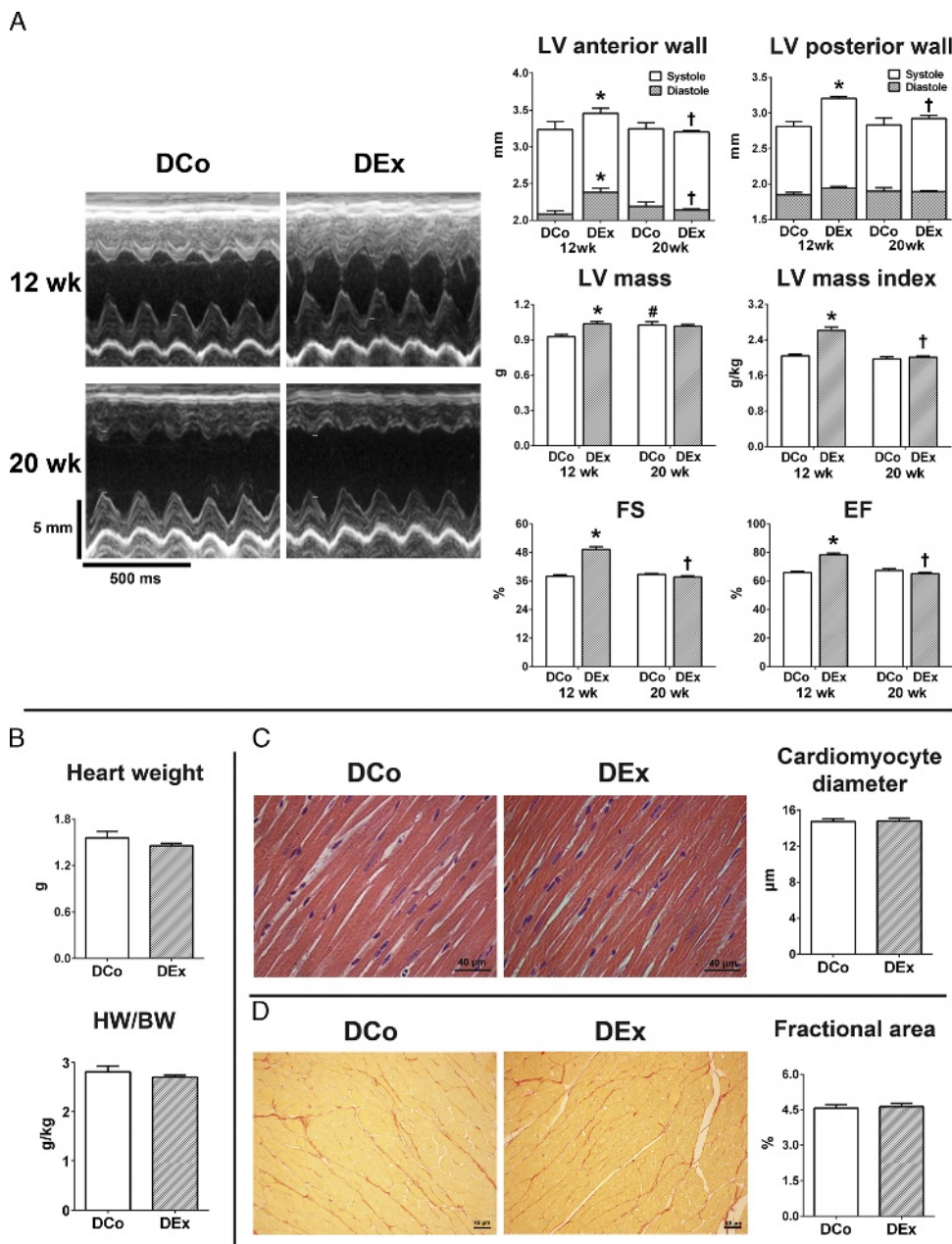


FIGURE 1—Markers of myocardial hypertrophy. A, LV M-mode echocardiography recordings are shown at weeks 12 and 20 from one-one representative DCo and DEx rat. LV anterior and posterior wall thickness markedly increased after training (12 wk), as well as calculated LV mass and LV mass index values. Traditional LV systolic parameters (FS and EF) showed an improvement after completion of the training protocol. There is a complete regression regarding these parameters after the detraining period. *P* value of pretraining–time (detraining) interaction by mixed ANOVA: LV mass $P_{\text{interaction}} = 0.004$, LV mass index $P_{\text{interaction}} < 0.001$, LV anterior wall diastole $P_{\text{interaction}} < 0.001$, LV anterior wall systole $P_{\text{interaction}} = 0.004$, LV posterior wall diastole $P_{\text{interaction}} = 0.108$, LV posterior wall systole $P_{\text{interaction}} = 0.008$, FS $P_{\text{interaction}} < 0.001$, and EF $P_{\text{interaction}} < 0.001$. B, Postmortem measured heart weight and heart weight-to-body weight (HW/BW) ratio were similar in detrained groups. C, Representative photomicrographs of LV myocardium after HE staining. Cardiomyocyte diameters showed no difference between DEx and DCo groups. Magnification $\times 400$; scale bar, 40 μm . D, Representative photomicrographs of picrosirius red–stained LV myocardium demonstrate physiological amounts of collagen (red staining) in both groups. Collagen fractional area did not differ in DEx and DCo rats. Magnification $\times 200$; scale bar, 40 μm . * $P < 0.05$ DEx vs DCo at week 12; # $P < 0.05$ DCo at week 20 vs DCo at week 12; † $P < 0.05$ DEx at week 20 vs DEx at week 12.

photomicrographs of the LV myocardium in case of one DCo and DEx rats.

Functional Parameters

Baseline P–V relations. Figure 2 shows original steady-state P–V loops of detrained animals that demonstrate

no differences in baseline hemodynamics between the groups after cessation of swimming. One can observe similar pressure and dP/dt curves and that representative baseline P–V loops almost completely overlap each other (Fig. 2). Consequently, HR, MAP, LVESP, LVEDP, LVEDV, LVESV, and SV did not show any difference in the detrained groups after

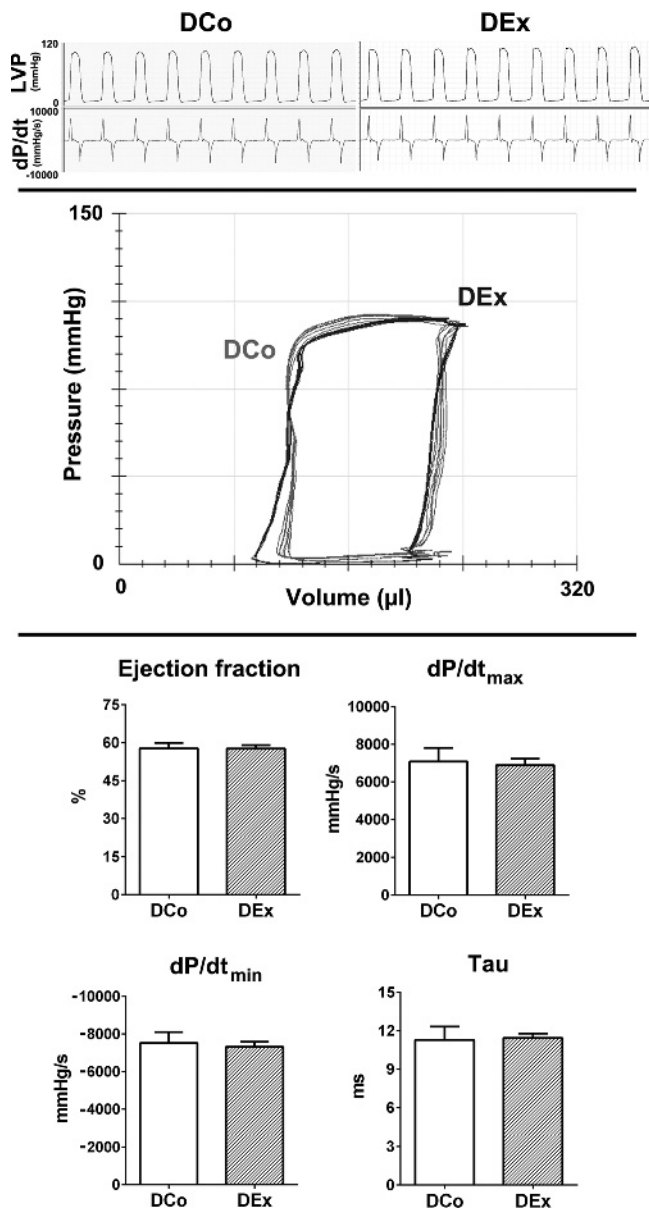


FIGURE 2—Steady-state P–V relations. *Upper panel:* original recordings of left ventricular pressure (LVP) and dP/dt signals in one representative rat from the DCo and DEx groups. *Mid panel:* original recordings of steady-state P–V loops obtained from one representative rat from the DCo and DEx groups. The similar P–V loops almost overlap each other, indicating similar pressure and volume values after detraining. *Lower panel:* classic parameters of systolic (EF; dP/dt_{max} , maximal slope of the systolic pressure increment) and diastolic (dP/dt_{min} , maximal slope of the diastolic pressure decrement; Tau: time constant of LV pressure decay) function did not differ between the detrained groups.

discontinuation of swim training (Table 1). Moreover, we found similar TPR values in the two groups.

Systolic function and contractility. After the 8-wk-long resting period, EF, CO, and CI values of DEx rats were similar to parameters of DCo animals. Moreover, dP/dt_{max} , which is a widely used classic contractility parameter, was unaltered in DCo and DEx rats (Table 1 and Fig. 2).

Figure 3 shows representative original P–V loops recorded during the transient occlusion of the vena cava inferior (upper panel) and obtained sensitive contractility indices in one-one representative DCo and DEx animals. The load-independent specific contractility parameters ESPVR, PRSW, and dP/dt_{max} -EDV were unchanged in the detrained groups, suggesting similar contractility (Fig. 3).

Diastolic function. τ and dP/dt_{min} , which are parameters of active relaxation, were unaltered in the groups after the discontinuation of the training (Table 1 and Fig. 2).

Characteristic passive diastolic parameters as LVEDP and EDPVR were also not different in DEx animals compared with DCo rats (Table 1 and Fig. 3).

Cardiac mechanoenergetic data. SW, an important mechanoenergetic parameter to describe total LV mechanical work, was similar in DCo and DEx groups. Furthermore, PVA and mechanical efficiency were also comparable in detrained rats. Similar values of LV contractility (ESPVR) and E_a led to identical VAC values after the cessation of the training (Table 1 and Fig. 4).

DISCUSSION

According to our knowledge, this is the first study to comprehensively investigate functional reversibility of athlete's heart *in vivo* by providing a reliable characterization of all aspects of LV hemodynamics after an 8-wk-long detraining period in a rat model.

Long-term exercise is associated with characteristic morphological and functional adaptations of the myocardium resulting in a phenomenon known as athlete's heart. The

TABLE 1. Hemodynamic parameters in DCo and DEx rats.

	DCo (n = 8)	DEx (n = 8)	P
HR, bpm	247 ± 8	249 ± 11	0.888
MAP, mm Hg	82.4 ± 2.5	79.5 ± 2.4	0.419
LVESV, mm Hg	102.7 ± 2.5	101.0 ± 4.2	0.723
LVEDP, mm Hg	6.6 ± 0.9	7.6 ± 1.0	0.452
LVEDV, μ L	253.0 ± 12.7	250.4 ± 13.2	0.890
LVESV, μ L	109.1 ± 4.4	105.7 ± 5.8	0.642
SV, μ L	143.9 ± 9.6	144.8 ± 9.0	0.949
CO, mL·min ⁻¹	35.6 ± 2.6	35.9 ± 2.2	0.916
CI, (mL·min ⁻¹)/100 g BW	6.4 ± 0.4	6.7 ± 0.4	0.573
EF, %	56.6 ± 1.4	57.7 ± 1.3	0.563
TPR, mm Hg/(mL·min ⁻¹)	2.37 ± 0.12	2.25 ± 0.09	0.400
dP/dt_{max} , mm Hg·s ⁻¹	7084 ± 255	6903 ± 340	0.677
dP/dt_{min} , mm Hg·s ⁻¹	-7528 ± 216	-7323 ± 262	0.564
τ (Glantz), ms	11.3 ± 0.4	11.5 ± 0.3	0.760
ESPVR, mm Hg· μ L ⁻¹	1.66 ± 0.12	1.60 ± 0.06	0.655
EDPVR, mm Hg· μ L ⁻¹	0.029 ± 0.004	0.029 ± 0.004	0.914
PRSW, mm Hg	70.9 ± 2.4	69.5 ± 2.7	0.709
dP/dt_{max} -EDV, (mm Hg·s ⁻¹)· μ L ⁻¹	34.0 ± 1.9	34.2 ± 1.8	0.949
SW, mm Hg·mL ⁻¹	12.6 ± 0.9	12.3 ± 0.6	0.831
PVA, mm Hg·mL ⁻¹	19.5 ± 1.9	19.8 ± 1.1	0.856
Eff, %	69.4 ± 1.8	68.7 ± 1.2	0.742
E_a , mm Hg· μ L ⁻¹	0.70 ± 0.03	0.71 ± 0.09	0.796
VAC	0.45 ± 0.04	0.45 ± 0.03	0.907

Values are mean ± SEM. BW, body weight; 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; dP/dt_{max} -EDV, slope of dP/dt_{max} -end-diastolic volume relationship; Eff, mechanical efficiency.

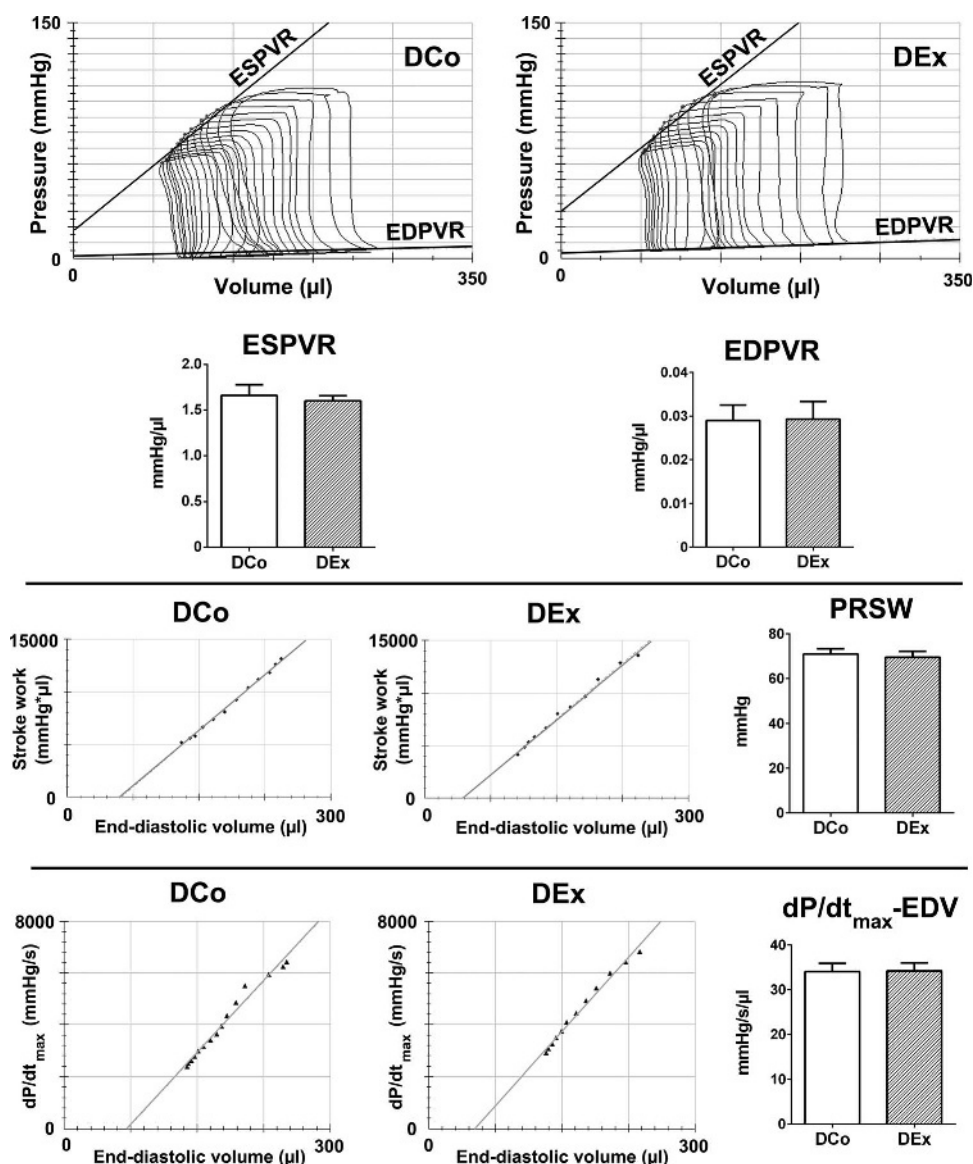


FIGURE 3—Load-independent parameters derived from LV P–V analysis at different preloads during transient occlusion of vena cava inferior. *Upper panel:* original recordings of LV P–V loops recorded at different preloads. Slope of ESPVR (index of LV contractility) and slope of EDPVR (index of LV stiffness) in one representative animal from DCo and DEx groups. These parameters were unchanged in DEx rats compared with DCo animals after detraining. *Mid and lower panel:* PRSW (the slope of the relationship between SW and end-diastolic volume) and maximal slope of the systolic pressure increment ($dP/dt_{\max}$)—end-diastolic volume relationship ($dP/dt_{\max}$ -EDV) from one representative rat from the DCo and DEx groups, respectively. These load-independent contractility indices were unaltered after the detraining period, reflecting similar inotropic state in detrained animals.

principal adaptations include ventricular wall thickening and alterations in cavity dimensions by cardiomyocyte hypertrophy and hyperplasia, without pathological features in the myocardium (e.g., interstitial collagen deposition), resulting in an increased LV mass (8,40). Hearts of highly trained endurance athletes show significant LV cavity enlargement and proportionally increased wall thickness, thus unaltered relative wall thickness values (20,31), whereas in animal experiments, echocardiographic measurements confirm significantly increased LV wall thickness (in both regions) along with variable alterations in LV cavity dimensions after long-term exercise training (1,3,34,40). Accordingly, anterior and

posterior wall thickness values increased significantly in the DEx group, and consequently LV mass and LV mass index values were markedly increased after 12 wk of swim training in DEx animals (Fig. 1A). In accordance with our previous results (34), conventional echocardiographic systolic parameters (FS and EF) were increased in exercised rats at week 12 and completely regressed to control values after the detraining period (Fig. 1A).

Several studies presented the morphological reversibility of long-term exercise-induced LV hypertrophy after cessation of physical training (13,21,29,31); however, no specified detraining time has been defined to observe complete regression

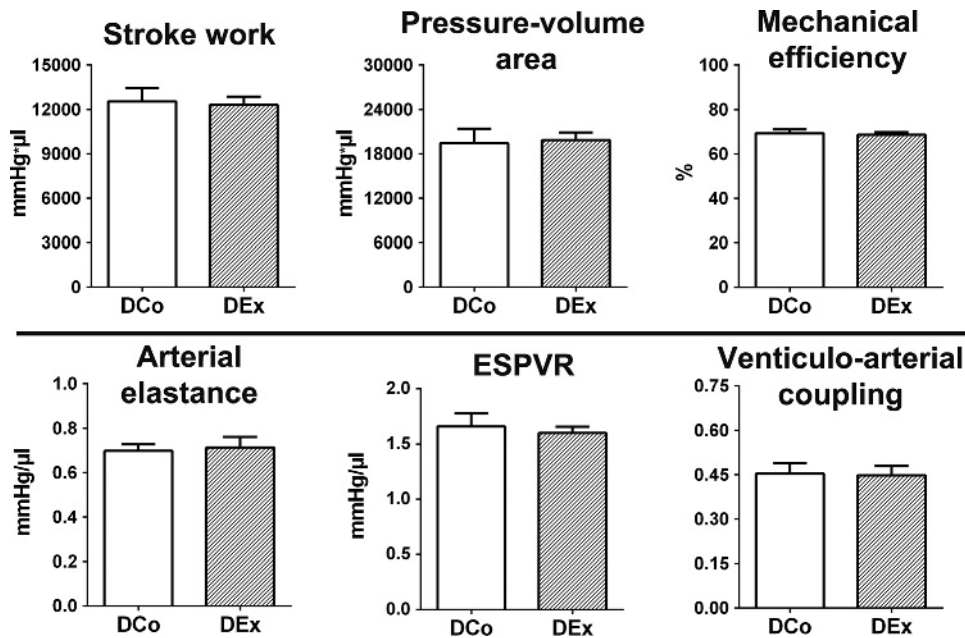


FIGURE 4—Cardiac mechanoenergetics. Similar values of LV SW and PVA led to an unaltered mechanical efficiency after cessation of training in DEx rats compared with DCo ones. Unaltered E_a and LV contractility (ESPVR) resulted in similar VAC in DEx and DCo rats.

of physiological cardiac mass enlargement. Structural reversibility appears to be especially meaningful when cardiac dimensions of physiological and pathological hypertrophy (i.e., hypertrophic cardiomyopathy) overlap (“gray zone”) and a short-term deconditioning period can solve the differential diagnostic question (20). In human studies of cardiac morphological parameters, as LV wall thickness, LV cavity enlargement already decreased after 2 months of cessation of training (22,29). Certain experimental observations found complete regression of the analyzed data after 2 wk of inactivity (3), compared with other research groups where total reversibility only appeared after a longer period of exercise cessation (13,39). Although most of the research groups showed complete regression of exercise-induced LV structural alterations, Pelliccia et al. (31) demonstrated incomplete reduction regarding to cavity dimensions in a part of the investigated athletes, even after many years of deconditioning. This might be explained by increased body weight and recreational physical activity during the follow-up period. Because of this variability in period needed for morphological regression and according to our previous echocardiographic follow-up, we examined cardiac functional alterations after 8 wk of detraining to assuredly detect the regression of athlete's heart morphology.

Despite the marked differences after the 12-wk-long training period, the anatomical adaptations of the heart returned to control levels after a deconditioning period, resulting in equal values in detrained groups (Fig. 1A). Increased cardiac (especially LV) mass is due to LV remodeling in athlete's heart. Latest studies reveal that not only hypertrophy of the preexisting myocytes can be observed in response to exercise-induced workload increment, and also newly formed cardiomyocytes and neoangiogenesis are produced in trained

animals by the stimulation of endogenous cardiac stem cells toward the cardiomyocyte and endothelial differentiation (40). Consistently, there is a significant increment in cardiomyocyte diameters and heart weight values after a long-term endurance exercise period (2,34). However, this activation is rapidly lost because of detraining, and loss of hypertrophic stimuli after cessation of exercise can revert the exercise-induced cellular and histological adaptations to levels of control animals (39). Also, in our study, we found equal heart weight and heart weight-to-body weight ratio after the 8-wk-long detraining period, and even the cardiomyocyte diameters of DEx rats showed no difference compared with DCo animals (Fig. 1B and C). Therefore, our data revealed that cessation of exercise for 8 wk leads to complete reversion of exercise-induced structural adaptations.

According to previous human and experimental investigations, long-term intense training might be associated with myocardial fibrotic infiltrates and, thus, cardiac arrhythmogenic remodeling, principally as a result of right ventricular dilatation and diffuse interstitial collagen deposition (18). This potential substrate for cardiac arrhythmias has been characterized to be completely reversible after detraining (2). Consistently, we experienced unaltered LV collagen content after the evaluation of picrosirius red staining in both detrained groups (Fig. 1D). These data might support the point of view that exercise training is not associated with arrhythmogenic remodeling in the LV myocardium (17).

Baseline P–V relations. Although structural changes within the LV have been clearly demonstrated, the evidence of exercise- and detraining-induced functional alterations has been less consistent.

Long-term exercise training results in an improved cardiovascular capacity to adapt to the increased circulatory demand

during exercise sessions. As an autonomic adaptation, endurance training is associated with lower resting and submaximal HR leading to elongated diastolic phase (30). Loss of resting bradycardia has been observed after 2 wk of detraining as a consequence of reverted exercise-induced autonomic alterations (9). Accordingly, during P–V analysis, HR and pressure values (MAP, LVESP, and LVEDP) did not show differences between DEx and DCo groups (Table 1 and Fig. 2). The other main adaptation of the heart to improve cardiovascular performance is increased LV SV. According to most human studies, SV increment is associated with a slight LV dilatation (increased end-diastolic volume) after intense endurance training period (2,35). In our rat model, we previously described unaltered LVEDV in exercised rats along with significantly decreased LVESV, thus resulting in significantly increased SV and resting EF after the 12-wk-long swim training period (34). These chamber volume alterations equalized completely after cessation of swimming (Table 1). After detraining, the exercise-induced adaptations regress corresponding to those of the DCo as shown by the representative baseline P–V loops (Fig. 2). According to our results, detraining leads to reductions in SV in elite endurance athletes (22).

Myocardial systolic properties. Most functional studies have used noninvasive investigations, such as echocardiography and cardiac magnetic resonance imaging to describe exercise-induced alterations in cardiac systolic mechanics (33,36). Traditional parameters of LV systolic performance (EF, CO, CI, and $dP/dt_{\max}$) did not differ between the groups (Table 1 and Fig. 2). A static or even reduced resting EF can be detected as a secondary effect of elevated cardiac preload and consequent LV dilatation among elite athletes. These classic parameters are not sensitive enough to describe the inotropic state of the myocardium as they are dependent on many factors altered by regular exercise training, such as cardiac loading conditions or HR. Thus, it is not unexpected that these parameters could not show enhancement in systolic performance of athlete's heart properly (33,36). Our research group recently reported that novel imaging modalities, such as speckle-tracking echocardiography, might provide a promising noninvasive technique to characterize systolic function in exercise-induced hypertrophy more independently from loading conditions (16); however, to date, there are only sporadic data on its clinical application.

Intrinsic contractile state plays a determinant role in myocardial performance. To the best of our knowledge, only two *in vitro* experimental studies investigated the effect of exercise deconditioning on myocardial contractility. According to these investigations, physical training induced increment in inotropic capacities of isolated rodent cardiomyocytes (15), and papillary muscles (3) regressed after 2–4 wk of detraining of the animals and alterations of myofilaments. Ca^{2+} sensitivity was associated with these observations. By means of preload-reducing maneuver, P–V analysis provides the opportunity to obtain sensitive contractility parameters, which may properly indicate the systolic

performance of the entire LV myocardium *in vivo* (28). Foremost, ESPVR has been used to describe alterations in cardiac inotropic status. However, this index can be influenced by chamber geometry and other factors; therefore, we determined PRSW and $dP/dt_{\max}$ -EDV, two even more precise contractility parameters. These load-independent inotropic indices (ESPVR, PRSW, and $dP/dt_{\max}$ -EDV) did not reveal difference between DCo and DEx groups (Table 1 and Fig. 3), which reflects the complete reversibility of systolic enhancement observed in exercise-induced cardiac hypertrophy (34).

Diastolic function. Ventricular diastolic function can be divided into two main components: early diastolic function, characterizing the process of active relaxation, and myocardial stiffness as a feature of passive ventricular filling. Energy-dependent cardiac early diastolic parameters are substantial regarding investigations of LV hypertrophy, as this parameter has been able to distinguish athlete's heart from compensated pathological LV hypertrophy (6,27). Previous investigations in athletes revealed that despite the pronounced myocardial hypertrophy, LV filling was found to be normal or even enhanced (30). Moreover, exercise-induced hypertrophy has been characterized by improved active relaxation, which might reflect an improved energetic state of the myocardium (5,34). According to our data, neither load-dependent ($dP/dt_{\min}$) nor load-independent (τ) indices of early diastolic function did differ between the detrained groups, suggesting that exercise-induced enhancement of early diastolic phase reverted completely after the resting period of 8 wk (Table 1 and Fig. 2).

The load-independent index of LV stiffness, EDPVR may be reflective of changes in myocardial interstitial material properties (e.g., fibrosis and edema) and chamber structural attributes. Pathological cardiac hypertrophy is associated with collagen deposition, whereas physiological LV remodeling develops without interstitial fibrosis (2,23). Accordingly, unaltered chamber stiffness was described both *in vivo* and *in vitro* (19,34). In our model, deconditioning did not alter the collagen content of the myocardium and parameters characterizing LV stiffness (LVEDP and slope of EDPVR), thus reverse remodeling during the detraining period might not be associated with myocardial fibrotic processes (Fig. 1D and Table 1).

These data are in accordance with human echocardiographic studies, which showed regression of enhancement in diastolic function after cessation of exercise using less specific and precise diastolic indices in young and adult individuals (i.e., E/A ratio) (26,32).

Cardiac mechanoenergetics. Besides ventricular diastolic and systolic functional enhancement, regular intensive exercise is able to induce metabolic and energetic adaptations in the heart. Physiological hypertrophy has been characterized with a more effective metabolism, as the heart of athletes needs less oxygen consumption to provide similar mechanical performance than control ones, which can be related to a protective, balanced enhancement in glucose and fatty acid

oxidation (4,14). In correspondence to these observations, *in vivo* investigations demonstrated exercise training-induced improvement in LV mechanical efficiency both in pathological conditions and in healthy subjects by increasing SW along with similar energy consumption (34,37). The relation between total LV mechanical work (SW) and total mechanical energy of cardiac contraction (PVA) can be described by P–V analysis (38). After the 8-wk-long detraining period, these cardiac mechanoenergetic values reverted completely in DEx rats and equalized with DCo animals resulting in similar mechanical efficiency (Table 1 and Fig. 4).

Moreover, the cardiovascular system of trained experimental animals and endurance athletes can be characterized by an optimized coupling between the LV and the arterial system as consequence of increased contractility along with decreased E_a (11,34). VAC, an important determinant of net cardiovascular performance that describes the matching between the LV and the arterial system, was also similar to control values, further assuming unchanged mechanoenergetic status after long-term cessation of the training (Table 1 and Fig. 4).

Limitations. The interpretation of results from the present study is limited to young male rats. The possible influence of gender, age, or species should be assessed in future studies.

P–V loop analysis allows the measurement of LV performance in detail, independently from loading conditions. However, the invasive hemodynamic measurements need to be performed in anesthetized animals and under this condition the findings on HR could not be fully appropriate.

REFERENCES

1. Baggish AL, Yared K, Wang F, et al. The impact of endurance exercise training on left ventricular systolic mechanics. *Am J Physiol Heart Circ Physiol*. 2008;295:H1109–16.
2. Benito B, Gay-Jordi G, Serrano-Mollar A, et al. Cardiac arrhythmogenic remodeling in a rat model of long-term intensive exercise training. *Circulation*. 2011;123:13–22.
3. Bocalini DS, Carvalho EV, de Sousa AF, Levy RF, Tucci PJ. Exercise training-induced enhancement in myocardial mechanics is lost after 2 weeks of detraining in rats. *Eur J Appl Physiol*. 2010;109:909–14.
4. Burelle Y, Wambolt RB, Grist M, et al. Regular exercise is associated with a protective metabolic phenotype in the rat heart. *Am J Physiol Heart Circ Physiol*. 2004;287:H1055–63.
5. Colan SD, Sanders SP, MacPherson D, Borow KM. Left ventricular diastolic function in elite athletes with physiologic cardiac hypertrophy. *J Am Coll Cardiol*. 1985;6:545–9.
6. D'Andrea A, D'Andrea L, Caso P, Scherillo M, Zeppilli P, Calabrò 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*. 2006;23:149–57.
7. Ehsani AA, Hagberg JM, Hickson RC. Rapid changes in left ventricular dimensions and mass in response to physical conditioning and deconditioning. *Am J Cardiol*. 1978;42:52–6.
8. Ellison GM, Waring CD, Vicinanza C, Torella D. Physiological cardiac remodelling in response to endurance exercise training: cellular and molecular mechanisms. *Heart*. 2012;98:5–10.
9. Evangelista FS, Martuchi SED, Negrao CE, Brum PC. Loss of resting bradycardia with detraining is associated with intrinsic heart rate changes. *Braz J Med Biol Res*. 2005;38:1141–6.
10. Fiuzza-Luces C, Garatachea N, Berger NA, Lucia A. Exercise is the real polypill. *Physiology (Bethesda)*. 2013;28:330–58.
11. Florescu M, Stoicescu C, Magda S, et al. “Supranormal” cardiac function in athletes related to better arterial and endothelial function. *Echocardiography*. 2010;27:659–67.
12. Giada F, Bertaglia E, De Piccoli B, et al. Cardiovascular adaptations to endurance training and detraining in young and older athletes. *Int J Cardiol*. 1998;65:149–55.
13. Guasch E, Benito B, Qi X, et al. Atrial fibrillation promotion by endurance exercise: demonstration and mechanistic exploration in an animal model. *J Am Coll Cardiol*. 2013;62:68–77.
14. Hafstad AD, Boardman NT, Lund J, et al. High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart. *J Appl Physiol*. 2011;111:1235–41.
15. Kemi OJ, Haram PM, Wisløff U, Ellingsen Ø. Aerobic fitness is associated with cardiomyocyte contractile capacity and endothelial function in exercise training and detraining. *Circulation*. 2004;109:2897–904.
16. Kovács A, Oláh A, Lux Á, et al. 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*. 2015;308:H743–8.
17. La Gerche A. Can intense endurance exercise cause myocardial damage and fibrosis? *Curr Sports Med Rep*. 2013;12:63–9.

CONCLUSIONS

We confirmed the complete morphological and functional reversibility of exercise training-induced cardiac hypertrophy after an 8-wk-long detraining period in a rat model. According to our results, morphological properties of athlete's heart developed in 12 wk during the training program and regressed completely after cessation of exercise for 8 wk. On the basis of reliable load-independent indices of LV performance, we demonstrated equal values of systolic (contractility) and diastolic (active relaxation) as well as mechanoenergetic (mechanical efficiency and VAC) parameters in detrained animals after the discontinuation of swim training, suggesting complete regression of exercise training-induced functional enhancement after detraining. According to our data, deconditioning-induced reversion of physiological hypertrophy did not alter myocardial collagen content and ventricular stiffness.

This project was supported by a grant from the National Research, Development and Innovation Office (NKFIH) of Hungary (K 120277). This work was also supported by a grant from the National Development Agency of Hungary (TÁMOP-4.2.2/B-10/1-2010-0013), by the UNKP-16-4 New National Excellence Program of the Ministry of Human Capacities of Hungary (to T. R.) and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (to T. R.). The expert technical assistance of Henriett Biró, Tímea Fischinger, Viktória Gregor, Dóra Juhász, and Gábor Fritz is gratefully acknowledged. A. O. and D. K. contributed equally to this work. B. M. and T. R. contributed equally to this work.

The authors declare that they have no conflict of interest. The present study does not constitute endorsement by the American College of Sports Medicine. Hereby, we confirm that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

18. La Gerche A, Burns AT, Mooney DJ, et al. Exercise-induced right ventricular dysfunction and structural remodelling in endurance athletes. *Eur Heart J*. 2012;33:998–1006.
19. Libonati JR. Cardiac remodeling and function following exercise and angiotensin II receptor antagonism. *Eur J Appl Physiol*. 2012;112:3149–54.
20. Maron BJ, Pelliccia A. The heart of trained athletes: cardiac remodeling and the risks of sports, including sudden death. *Circulation*. 2006;114:1633–44.
21. Maron BJ, Pelliccia A, Spataro A, Granata M. Reduction in left ventricular wall thickness after deconditioning in highly trained Olympic athletes. *Br Heart J*. 1993;69:125–8.
22. Martin WH, Coyle EF, Bloomfield SA, Ehsani AA. Effects of physical deconditioning after intense endurance training on left ventricular dimensions and stroke volume. *J Am Coll Cardiol*. 1986;7:982–9.
23. McMullen JR, Jennings GL. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. *Clin Exp Pharmacol Physiol*. 2007;34:255–62.
24. Moker EA, Bateman LA, Kraus WE, Pescatello LS. The relationship between the blood pressure responses to exercise following training and detraining periods. *PLoS One*. 2014;9:e105755.
25. Mujika I, Padilla S. Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus. *Sports Med*. 2000;30:79–87.
26. Obert P, Mandigout S, Vinet A, N'Guyen LD, Stecken F, Courteix D. Effect of aerobic training and detraining on left ventricular dimensions and diastolic function in prepubertal boys and girls. *Int J Sports Med*. 2001;22:90–6.
27. Oláh A, Németh BT, Mátyás C, et al. Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics. *Am J Physiol Heart Circ Physiol*. 2016;310:H587–97.
28. Pacher P, Nagayama T, Mukhopadhyay P, Bátkai S, Kass DA. Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. *Nat Protoc*. 2008;3:1422–34.
29. Pavlik G, Bachl N, Wollein W. Effect of training and detraining on the resting echocardiographic parameters in runners and cyclists. *J Sports Cardiol*. 1986;3:35–45.
30. Pavlik G, Major Z, Varga-Pintér B, Jeserich M, Kneffel Z. The athlete's heart: part I (review). *Acta Physiol Hung*. 2010;97:337–53.
31. Pelliccia A, Maron BJ, De Luca R, Di Paolo FM, Spataro A, Culasso F. Remodeling of left ventricular hypertrophy in elite athletes after long-term deconditioning. *Circulation*. 2002;105:944–9.
32. Pelliccia A, Maron MS, Maron BJ. Assessment of left ventricular hypertrophy in a trained athlete: differential diagnosis of physiologic athlete's heart from pathologic hypertrophy. *Prog Cardiovasc Dis*. 2012;54:387–96.
33. Pluim BM, Zwinderman AH, van der Laarse A, van der Wall EE. The athlete's heart. A meta-analysis of cardiac structure and function. *Circulation*. 2000;101:336–44.
34. Radovits T, Oláh A, Lux Á, et al. Rat model of exercise-induced cardiac hypertrophy: hemodynamic characterization using left ventricular pressure–volume analysis. *Am J Physiol Heart Circ Physiol*. 2013;305:H124–34.
35. 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*. 2002;40:1856–63.
36. Spence AL, Naylor LH, Carter HH, et al. A prospective randomised longitudinal MRI study of left ventricular adaptation to endurance and resistance exercise training in humans. *J Physiol*. 2011;589:5443–52.
37. Stolen KQ, Kempainen J, Ukkonen H, et al. Exercise training improves biventricular oxidative metabolism and left ventricular efficiency in patients with dilated cardiomyopathy. *J Am Coll Cardiol*. 2003;41:460–7.
38. Suga H. Cardiac mechanics and energetics—from Emax to PVA. *Front Med Biol Eng*. 1990;2:3–22.
39. Waring CD, Henning BJ, Smith AJ, Nadal Ginard B, Torella D, Ellison GM. Cardiac adaptations from 4 weeks of intensity-controlled vigorous exercise are lost after a similar period of detraining. *Physiol Rep*. 2015;3:e12302.
40. Waring CD, Vicinanza C, Papalamprou A, et al. The adult heart responds to increased workload with physiologic hypertrophy, cardiac stem cell activation, and new myocyte formation. *Eur Heart J*. 2014;35:2722–31.
41. Weiner RB, Baggish AL. Exercise-induced cardiac remodeling. *Prog Cardiovasc Dis*. 2012;54:380–6.