

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

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24 **Abstract**

25 Long-term exercise training is associated with characteristic structural and functional
 26 changes of the myocardium, termed athlete's heart. Several research groups investigated
 27 exercise training induced left ventricular (LV) hypertrophy in animal models, however
 28 only sporadic data exists about detailed hemodynamics. We aimed to provide functional
 29 characterization of exercise-induced cardiac hypertrophy in a rat model using the in
 30 vivo method of LV pressure-volume (P-V) analysis. After inducing LV hypertrophy by
 31 swim training, we assessed LV morphometry by echocardiography and performed LV
 32 P-V analysis using a pressure-conductance microcatheter to investigate in vivo cardiac
 33 function. Echocardiography showed LV hypertrophy (LV mass index: 2.41 ± 0.09 vs.
 34 2.03 ± 0.08 g/kg, $p < 0.01$), which was confirmed by heart weight data and
 35 histomorphometry. Invasive hemodynamic measurements showed unaltered heart rate,
 36 arterial pressure and LV end-diastolic volume along with decreased LV end-systolic
 37 volume, thus increased stroke volume and ejection fraction (73.7 ± 0.8 vs. $64.1 \pm 1.5\%$,
 38 $p < 0.01$) in trained vs. untrained control rats. The P-V-loop-derived sensitive, load-
 39 independent contractility indexes, such as slope of end-systolic P-V relationship or
 40 preload recruitable stroke work (77.0 ± 6.8 vs. 54.3 ± 4.8 mmHg, $p = 0.01$) were found to be
 41 significantly increased. The observed improvement of ventriculoarterial coupling
 42 (0.37 ± 0.02 vs. 0.65 ± 0.08 , $p < 0.01$), along with increased LV stroke work and
 43 mechanical efficiency reflect improved mechanoenergetics of exercise-induced cardiac
 44 hypertrophy. Despite the significant hypertrophy, we observed unaltered LV stiffness
 45 (slope of end-diastolic P-V relationship: 0.043 ± 0.007 vs. 0.040 ± 0.006 mmHg/ μ l) and
 46 improved LV active relaxation (τ : 10.1 ± 0.6 vs. 11.9 ± 0.2 ms, $p < 0.01$). According to our

47 knowledge this is the first study, which provides characterization of functional changes
48 and hemodynamic relations in exercise-induced cardiac hypertrophy.

49

50 **Key words:** exercise-induced cardiac hypertrophy, pressure-volume analysis, systolic
51 function, diastolic function, cardiac mechanoenergetics

52

53 **Introduction**

54 Athlete's heart has been described as the complex structural, functional and
55 electrical cardiac remodelling induced by long-term exercise training (40). Exercise
56 training-induced cardiac hypertrophy is an important physiological adaption, which
57 include balanced increase of LV and left atrial diameters, cardiac mass and LV wall
58 thicknesses effected by myocyte hypertrophy and neo-angiogenesis. (10, 12, 25, 36, 37).

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

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

74 Several in vitro experimental studies from isolated rodent hearts (13, 26), papillary
75 muscles (31, 37) and isolated cardiomyocytes (23, 24, 50) have demonstrated positive

76 effect of exercise training on myocardial function. The major limitation of the
77 previously used invasive and noninvasive approaches to study cardiac function in small
78 animal models is that the hemodynamic parameters measured were not objective
79 enough (echocardiography) or were largely dependent on loading conditions. Therefore,
80 appropriate, hemodynamically validated standards of in vivo experimental models of
81 exercise training-induced cardiac hypertrophy are required for the reliable
82 investigations.

83 Pressure-volume (P-V) analysis is an useful approach for examining intact ventricular
84 function independently from loading conditions. This analysis has been widely used in
85 large animal studies and in humans since the mid-1980s (20). Advances of the last
86 decade in the development and validation of miniature P-V catheters have made it
87 possible to use this approach for studies in small animals (14, 33, 34, 35). The technique
88 of left ventricular (LV) P-V analysis in rats has been introduced only recently, and very
89 limited normative data are available.

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

94

95 **Materials and methods**

96

97 **Ethical approval, animals**

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

105 Young adult (12 wk old, m=275-300 g) male Wistar rats (n=20) (Charles River,
106 Sulzfeld, Germany) were housed in a room with constant temperature of 22 ± 2 °C with
107 a 12/12h light-dark cycle and fed a standard laboratory rat diet ad libitum and free
108 access to water.

109

110 **Experimental groups**

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

117 For testing the reversibility of exercise-induced changes, additional series of rats
118 underwent the same 12-week training program followed by discontinuation of exercise

119 and an 8-week resting (detraining) (1) period (detrained exercise group, n=6), untrained
120 animals served as controls (detrained control group, n=6). Echocardiographic
121 assessments were performed after the detraining period and hearts were removed from
122 the animals and heart weight was measured immediately after sacrifice.

123

124 **Swim training protocol - rat model of exercise-induced cardiac hypertrophy**

125 We designed a swimming apparatus (Fig 1A) specially planned for exercise
126 training of rats. A 150-l water tank was divided into 6 lanes with a surface area of 20 x
127 25cm and a depth of 45cm per lane and filled with tap water maintained at 30-32°C to
128 allow individual swim training. The dimensions of the lanes were selected to avoid
129 floating of the rats by reclining to the walls. Based on literature data (11, 48) and on the
130 results of own preliminary pilot studies we provided a training plan to establish a rat
131 model for inducing robust cardiac hypertrophy. Exercised rats swam for a total period of
132 12 weeks, 200min session/day and 5 days a week (Fig. 1B). For adequate adaptation,
133 the duration of swim training was limited to 15min on the first day and increased by
134 15min every second training session until the maximal swim duration (200min) was
135 reached. Untrained control rats were placed into the water for 5min each day during the
136 12-week training program.

137

138 **Echocardiography**

139 Rats were anesthetized with pentobarbital (60mg/kg ip.). Animals were placed
140 on controlled heating pads, and the core temperature, measured via rectal probe, was
141 maintained at 37°C. After shaving of the anterior chest, transthoracic echocardiography
142 was performed in the supine position by one investigator blinded to the experimental

groups. Two dimensional and M-mode echocardiographic images of long and short (mid-papillary level) axis were recorded, using a 13MHz linear transducer (GE 12L-RS, GE Healthcare, USA), connected to an echocardiographic imaging unit (Vivid i, GE Healthcare, USA). Digital images were analyzed by an investigator in blinded fashion using an image analysis software (EchoPac, GE Healthcare, USA). On two dimensional recordings of the short-axis at the mid-papillary muscle level, LV anterior (AWT), posterior (PWT) and interventricular septal (IVST) wall thickness in diastole (index: d) and systole (index: s), left ventricular end-diastolic (LVEDD) and end-systolic diameter (LVESD) were measured. In addition, end-diastolic and end-systolic LV areas were planimetered from short and long axis two dimensional recordings. End-systole was defined as the time point of minimal left ventricular dimensions, and end-diastole as the time point of maximal dimensions. All values were averaged over three consecutive cycles.

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

167 **Hemodynamic measurements – left ventricular pressure-volume (P-V) analysis**

168 Rats were anesthetized with a mixture of ketamine (100mg/kg ip.) and xylazine
 169 (3mg/kg ip.), tracheotomized, and intubated to facilitate breathing. Animals were placed
 170 on controlled heating pads, and the core temperature, measured via rectal probe, was
 171 maintained at 37°C. A polyethylene catheter was inserted into the left external jugular
 172 vein for fluid administration. A 2-Fr microtip pressure-conductance catheter (SPR-838,
 173 Millar Instruments, Houston, TX) was inserted into the right carotid artery and
 174 advanced into the ascending aorta. After stabilization for 5 min, mean arterial blood
 175 pressure (MAP) and heart rate (HR) were recorded. After that, the catheter was
 176 advanced into the left ventricle under pressure control. After stabilization for 5 min,
 177 signals were continuously recorded at a sampling rate of 1,000 samples/s using a P-V
 178 conductance system (MPVS-Ultra, Millar Instruments, Houston, TX, USA) connected
 179 to the PowerLab 16/30 data acquisition system (AD Instruments, Colorado Springs, CO,
 180 USA), stored and displayed on a personal computer by the LabChart5 Software System
 181 (AD Instruments).

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

191 SW and TPR were normalized to body weight [cardiac index (CI), SW index (SWI) and
192 TRP index (TPRI)].

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

203 Arterial elastance (E_a) was calculated as LVESP/SV. Ventriculoarterial coupling (VAC)
204 was described by the quotient of E_a and E_{es} . According to Sunagawa and associates
205 mechanical efficiency (Eff) was calculated as the ratio of SW and PVA. (44).

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

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

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

217

218 **Histology**

219 Hearts were harvested immediately after sacrifice, snap-frozen in liquid nitrogen
220 and stored at -80°C. Transverse transmural slices of the ventricles were sectioned (5
221 µm) and processed conventionally for histological examination. The sections were
222 stained with hematoxyllin and eosin (HE) and Masson's trichrome. Light microscopic
223 examination was performed with a Zeiss microscope (Axio Observer.Z1, Carl Zeiss,
224 Jena, Germany) and digital images were captured using an imaging software (QCapture
225 Pro 6.0, QImaging, Canada). The transverse transnuclear widths of randomly selected,
226 longitudinally oriented cardiomyocytes were measured by a single investigator after
227 calibrating the system. The mean value of 100 LV cardiomyocytes represents each
228 sample. The amount of myocardial collagen was determined by semiquantitative
229 morphometry scoring of Masson's trichrome-stained sections by one blinded observer as
230 follows: (0) absent, (1) slight, (2) moderate and (3) intense. The mean value of twenty
231 randomly selected visual fields (magnification 40x) of free LV wall represents each
232 sample.

233

234 **Biochemical measurements**

235 After completing the haemodynamic measurements blood samples from the
236 inferior caval vein were collected in tubes pre-rinsed with EDTA. The blood samples
237 were centrifuged at 3000g for 15min at 4°C. Plasma adrenocorticotrophic hormone
238 (ACTH) and cortisol levels were determined by electrochemiluminescence

239 immunoassay (ECLIA) using commercial kits (Elecsys ACTH Kit and Elecsys Cortisol
240 Test, Cobas, Roche Diagnostics, Mannheim, Germany).

241

242 **Cardiac mRNA analysis**

243 LV myocardial tissue samples were harvested immediately after sacrifice, snap-
244 frozen in liquid nitrogen and stored at -80°C. LV tissue was homogenized in RLT
245 buffer, RNA was isolated from the ventricular samples using the RNeasy Fibrous Tissue
246 Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and
247 quantified by measuring optical density (OD) at 260nm. RNA purity was ensured by
248 obtaining a 260/280nm OD ratio approximately 2.0. Reverse transcription reaction (1µg
249 total RNA of each sample) was completed using the QuantiTect Reverse Transcription
250 Kit (Qiagen). Quantitative real-time PCR was performed with the StepOnePlus™ Real-
251 Time PCR System (Applied Biosystems, Foster City, USA) in triplicates of each sample
252 in a volume of 10µl in each well containing cDNA (1µl), TaqMan® Universal PCR
253 MasterMix (5µl) and a TaqMan® Gene Expression Assay for the following targets
254 (0.5µl): β-isoform of myosin heavy chain (β-MHC, assay ID: Rn00568328_m1) and
255 transforming growth factor β (TGF-β, assay ID: Rn00572010_m1), all purchased from
256 Applied Biosystems. Gene expression data were normalized to glyceraldehyde-3-
257 phosphate dehydrogenase (GAPDH; reference gene; assay ID: Rn01775763_g1) and
258 expression levels were calculated using the C_T comparative method ($2^{-\Delta CT}$). All results
259 are expressed as values normalized to a positive calibrator (a pool of cDNA's from all
260 samples of the control group).

261

262 **Statistical analysis**

263 Statistical analysis was performed on a personal computer with a commercially
264 available software (Origin 7G; OriginLab, Northampton, MA, USA). All data are
265 expressed as means $\pm$ SEM. An unpaired two sided Student's t-test was used to compare
266 parameters of exercised and control rats. Differences were considered statistically
267 significant when $p < 0.05$.
268

269 **Results**

270 **Body weight and heart weight**

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

279 **Histology**

280 Exercise training resulted in a significant increase in cardiomyocyte width
 281 compared with untrained control rats, indicating cardiac hypertrophy (Fig 2C-E). The
 282 semiquantitative evaluation of Masson's trichrome staining indicated unaltered cardiac
 283 collagen content in the exercised group (Fig.2F-H). Figure 2 shows representative
 284 photomicrographs of LV myocardium in exercised and control rats.

286 **Markers of stress and pathological hypertrophy**

287 Plasma ACTH (172.1 ± 32.3 pg/ml exercised vs. 211.8 ± 58.4 pg/ml untrained
 288 control, $p=0.5470$) and cortisol (87.4 ± 10.1 nmol/l exercised vs. 95.6 ± 11.4 nmol/l
 289 untrained control, $p=0.6000$) concentrations did not differ between the exercised and
 290 control rats.
 291 Myocardial gene expressions of β -MHC (relative expression: 0.85 ± 0.11 exercised vs.
 292 0.93 ± 0.04 untrained control, $p=0.5516$) and TGF- β (relative expression: 0.84 ± 0.08

exercised vs. 0.84 ± 0.02 untrained control, $p=0.9867$) were unaltered in exercised rats compared with untrained controls, indicating absence of pathological hypertrophy and remodeling, respectively.

Echocardiographic parameters

The morphological and functional echocardiographic results are shown in Table 1. At the end of the exercise training protocol, the LV wall thickness values were significantly higher in the exercised rats - except the septal thickness which showed only a strong tendency towards higher values – compared with the control animals. Figure 3 shows representative long- and short-axis two-dimensional end-systolic snapshots in exercised and control rats. Irrespectively of the geometrical model used for volume calculation, swim training was associated with significantly decreased LVESV along with unchanged LVEDV, thus EF was significantly increased in trained animals. The SV and SVI values showed a marked and significant increase using the single plain and the biplane model respectively in exercised rats compared with untrained control rats. The LV mass and the LV mass index were significantly higher in exercised animals versus controls indicating robust cardiac hypertrophy. The FS was increased in exercised animals, suggesting increased systolic performance.

Hemodynamic parameters

Baseline hemodynamic data. HR, MAP, LVESP, LVEDP and $dp/dt_{\min}$ were not different in exercised animals compared with the control group (Table 2). The $dp/dt_{\max}$ as a classic contractility parameter showed only an increasing tendency in trained rats, without reaching the level of statistical significance. Decreased τ was also detected in

317 exercised rats, suggesting improved active relaxation in trained animals. Figure 4A
 318 shows representative original steady-state PV loops obtained from exercised and
 319 untrained control rats. The wider baseline P-V loops in exercise-induced hypertrophy
 320 reflect increased stroke volume along with unaltered LVEDV and decreased LVESV.
 321 The EF increased significantly, suggesting increased systolic performance in trained
 322 rats. CO, CI, SW, SWI and also the LV maximal power were increased in swimming
 323 animals.

324

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

332 Figure 5 shows PRSW (the slope of the linear relation between SW and EDV, a
 333 sensitive contractility parameter) in a representative exercised and control animal. The
 334 slope was steeper in trained rats than in control rats, indicating increased systolic
 335 performance. Compared with the corresponding control animals the overall PRSW
 336 values were significantly higher in exercised rats (Fig. 5, Table 2).

337 We also determined the relation between dp/dt_{max} and EDV. dp/dt_{max} is a classic
 338 contractility parameter, but it is dependent on changes in preload. Analysis of dp/dt_{max} -
 339 EDV allowed us to compare dp/dt_{max} values of exercised and control rats at a given
 340 EDV. As Figure 5 shows, the slope of this relation was steeper in trained animals than

341 in untrained animals, indicating increased contractility in exercise-induced hypertrophy.
 342 The overall $dP/dt_{\max}$ -EDV slope values were significantly higher in exercised rats (Fig.
 343 5, Table 2).

344

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

352

353 **Reversibility of exercise-induced cardiac hypertrophy**

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

360

361 **Discussion**

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

368 The adaptation of myocardial tissue that accompany exercise training in
369 experimental animal models depends on factors, such as modality, intensity, duration
370 and frequency of the physical activity (47). According to literature data and results of
371 our own previous pilot studies we established a rat model of robust cardiac hypertrophy
372 induced by swim training. Although the duration of swim exercise varies considerably
373 in previously published works, it generally induces hypertrophy of the myocardium by
374 as much as 15% (15, 30, 49, 50). Our present data on rats that underwent a 12-week
375 intensive (200min/day) swim exercise training program showed a more robust cardiac
376 hypertrophy as indicated by heart weight and calculated LV mass index values (Fig. 2).
377 According to the results of echocardiography measurements we observed significantly
378 increased LV wall thickness and LV mass index values in exercised animals compared
379 with controls, which is in line with previous studies (2, 47). The histomorphometry of
380 LV myocardium showed increased cardiomyocyte diameter (Fig. 2E) in exercise-
381 induced cardiac hypertrophy. This finding is consistent with the features described in
382 isolated cardiomyocytes (47, 50) as well as in LV myocardium (30) derived from
383 animals underwent endurance training.

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

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

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

407 **Systolic function and cardiac contractility**

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

412 We found in our rat model that exercise training was associated with markedly
413 increased $dP/dt_{\max}$ and significantly increased EF (Table 2). Although $dP/dt_{\max}$ has been
414 widely used as a cardiac contractility parameter, it is well recognized that it is
415 dependent on loading conditions, especially on changes in preload (16, 19). Classical
416 echocardiographic parameters of cardiac contractility, FS and EF are also known to be
417 influenced by both preload and afterload and therefore can not reliably be used to assess
418 contractile function in models where loading is altered. The method of simultaneous LV
419 pressure and volume analysis by the miniaturized pressure-conductance catheters
420 allowed us to calculate highly sensitive and reliable load-independent indexes of LV
421 contractility.

422 Historically, ESPVR (E_{es}) has been proposed as a fairly load-insensitive index of
423 ventricular contractility. According to present results, E_{es} was significantly increased in
424 exercised animals, indicating an improved contractile state of the LV myocardium (Fig.
425 4 and Table 2). However, because this relation can be altered not only by changes in the
426 inotropic state but also by changes in chamber geometry and other diastolic factors, we
427 also calculated other parameters (16). PRSW represent the slope of the linear relation
428 between SW and EDV. It has been described as a parameter independent of chamber
429 size and mass, and it is sensitive to the cardiac contractile function of the ventricle.

430 Although it integrates data from the entire cardiac cycle, it is influenced most of all by
431 the systole (16). PRSW was also significantly increased in exercised rats compared with
432 control rats (Fig. 5, Table 2). A precious investigation (27) has demonstrated that the
433 slope of the relation between $dp/dt_{\max}$ and EDV, another P-V loop-derived index,
434 represent a sensitive but less load-dependent parameter of LV contractility. This
435 sensitive contractility index was also augmented in exercised animals compared with
436 untrained controls (Fig. 5, Table 2).

437 **Diastolic function**

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

445 Improved ventricular relaxation has been observed in exercised animals (as indicated by
446 decreased τ ; Table 2). Ventricular relaxation is an ATP-dependent active process,
447 depends mostly on Ca^{2+} uptake by the sarcoplasmatic reticulum, during the first third of
448 diastole. The detected functional differences might reflect improved Ca^{2+} handlings as
449 suggested by Kemi et al. (24) and improved energetic state of the myocardium.

450 **Mechanoenergetics**

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

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

473 **Limitations**

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

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

493

494 **Conclusions**

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

503

504

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508

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514

515 **Disclosures**

516 The authors have no conflict of interest to disclose.

517

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715

716 **Figure captions**

717 **Figure 1. Swimming apparatus and training plan**

718 Our swimming apparatus (A) ensured six lanes for parallel swimming training. The
719 training plan of exercised animals (B) included five weeks of adaptation phase and
720 seven weeks of intense exercise training, five days a week, 200 minutes per daily
721 swimming session.

722

723 **Figure 2. Evaluation of physiological cardiac hypertrophy**

724 The increased heart weight values (A) and heart weight to body weight ratio (B) in
725 trained rats showed robust cardiac hypertrophy. Representative photomicrographs of
726 LV myocardium after hematoxylin and eosin (C and D) as well as Masson's trichrome
727 staining (F and G). Longitudinally oriented cardiomyocytes are displayed for control
728 (C) and exercised (D) animals. An increase of left ventricular cardiomyocyte width was
729 observed in exercised rats compared with untrained controls (E). Masson's trichrome
730 stained myocardium showed low, physiological amounts of blue collagen staining both
731 in the control (F) and exercised (G) groups. Additionally, semiquantitative scoring
732 values indicated no difference between the groups (H). Magnification: 400x, scale bar
733 60µm. *:p<0.05

734

735 **Figure 3. Echocardiographic assessment**

736 Representative two-dimensional echocardiographic images at end-systole (long-axis: A
 737 and B; short-axis: C and D) and M-mode recordings (E and F). The images demonstrate
 738 increased wall thickness values and decreased left ventricular end-systolic dimensions
 739 (LVESD) along with unaltered left ventricular end-diastolic dimensions (LVEDD) in
 740 exercised rats (B, D and F) compared with untrained controls (A, C and E).

741

742 **Figure 4. Left ventricular pressure-volume loops**

743 Original recordings of steady-state pressure-volume (P-V) loops (A) obtained with the
 744 Millar P-V conductance catheter system from one representative rat from the exercised
 745 (Ex) and control (Co) groups. The wider P-V loop indicates increased stroke volume
 746 along with unaltered end-diastolic volume and decreased end-systolic volume, thus
 747 increased ejection fraction in exercised rats compared with untrained controls. End-
 748 systolic P-V relationship (ESPVR) and end-diastolic P-V relationship (EDPVR) in one
 749 representative animal from the exercised (B) and control (D) groups. Original
 750 recordings of representative P-V loops were obtained with a P-V conductance catheter
 751 system at different preloads during vena cava occlusion and showed increased slope of
 752 ESPVR (C) in exercised rats compared with untrained controls. The steeper ESPVR
 753 indicated increased contractile function. The slope of EDPVR did not differ between the
 754 groups indicating unaltered diastolic stiffness (E). *:p<0.05

755

756 **Figure 5. Pressure-volume loop derived left ventricular contractility parameters**

757 Preload recruitable stroke work (PRSW), the slope of the relationship between stroke
 758 work (SW) and end-diastolic volume (EDV) (A); and maximal slope of the systolic
 759 pressure increment ($dP/dt_{\max}$) - EDV relationship (C) in one representative rat from the
 760 exercised (Ex) and control (Co) groups. Note that for both relationships, slope values
 761 are increased in exercised rats compared with untrained controls (B and D), suggesting
 762 increased systolic performance in exercised animals. * $p < 0.05$

763 **Figure 6. Cardiac mechanoenergetics**

764 The increased end-systolic elastance (E_{es} ; A) and the decreased arterial elastance (E_a ; B)
 765 resulted in improved ventriculoarterial coupling ($VAC = E_a/E_{es}$; C) in exercised rats
 766 compared with untrained controls. The increased stroke work (SW; D) along with
 767 unaltered pressure-volume area (PVA; E) led to an amelioration of mechanical
 768 efficiency ($Eff = SW/PVA$; F) in trained rats. Note that all of these parameters suggest
 769 improved LV mechanoenergetics in exercise-induced cardiac hypertrophy. * $p < 0.05$

770

771

772

773 **Tables**774 **Table 1. Echocardiography parameters in untrained control and exercised rats**

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

775

776 Values are means ± SEM. LVAWTd and LVAWTs, left ventricular (LV) anterior wall
777 thickness at diastole and systole, respectively; LVPWTd and LVPWTs, left ventricular
778 (LV) posterior wall thickness at diastole and systole, respectively; LVIVSTd and
779 LVIVSTs, left ventricular (LV) interventricular septal wall thickness at diastole and
780 systole, respectively; LVEDD, LV end-diastolic dimension; LVESD LV end-systolic

781 dimension; FS, LV fractional shortening; LVEDVsp and LVEDVbp, LV end-diastolic
782 volume calculated using single-plane ellipsoid (sp) and biplane ellipsoid (bp),
783 respectively; LVESVsp and LVESVbp, LV end-systolic volume calculated using sp and
784 bp model, respectively; SVsp and SVbp, LV stroke volume (SV) calculated using sp
785 and bp model, respectively; SVIsp and SVIbp, LV SV index calculated using sp and bp
786 model, respectively; EFsp and EF bp, LV ejection fraction using sp and bp model,
787 respectively; BW: body weight. *:p<0.05 vs. untrained controls.

788

789 **Table 2. Hemodynamic parameters in untrained control and exercised rats**

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

790

791 Values are means $\pm$ SEM. Hemodynamic parameters were measured by the Millar
 792 pressure-volume conductance catheter system. HR, heart rate; MAP, mean arterial
 793 pressure; LVSP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diastolic
 794 pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; SV,

795 stroke volume; CO, cardiac output; CI, cardiac index; EF, ejection fraction; SW, stroke
796 work; SWI, SW index; $dP/dt_{\max}$ and $dP/dt_{\min}$ maximal slope of the systolic pressure
797 increment and the diastolic pressure decrement, respectively; τ , time constant of LV
798 pressure decay; TPR, total peripheral resistance; TPRI, TPR index; E_{es} , slope of the
799 end-systolic pressure-volume relationship; EDPVR, end-diastolic pressure-volume
800 relationship; PRSW, preload recruitable SW; EDV, end-diastolic volume; E_a , arterial
801 elastance; PVA, pressure-volume area; VAC, ventriculoarterial coupling; Eff,
802 mechanical efficiency; BW, body weight. *:p<0.05 vs. untrained controls.

803

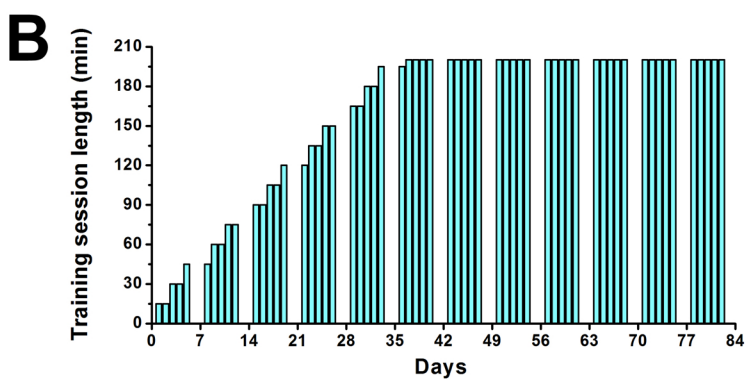
804 **Table 3. Cardiac morphometric and echocardiography parameters in detrained**
 805 **control and detrained exercised rats**

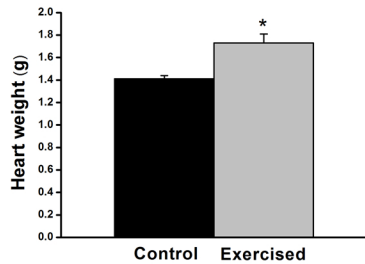
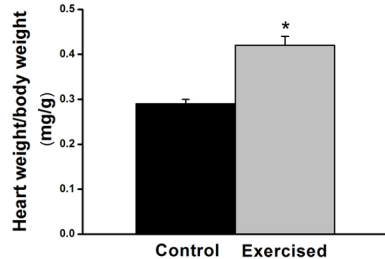
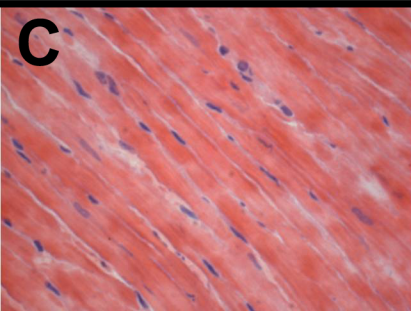
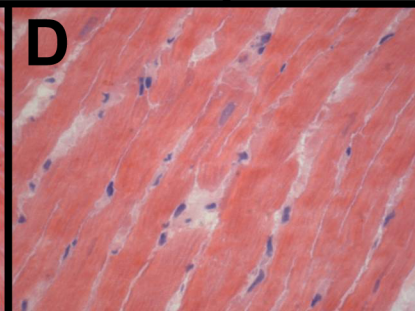
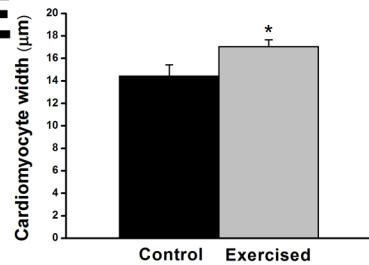
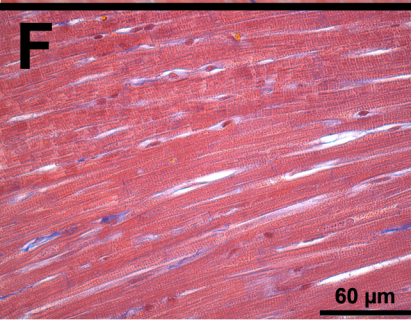
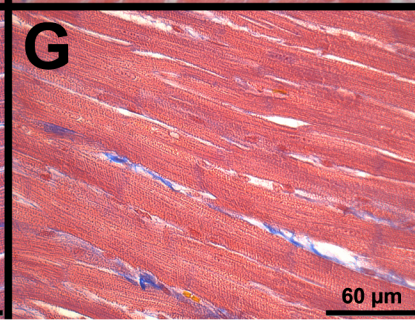
	Detrained control (n=5)	Detrained exercised (n=6)	p value versus Detrained control group
HW, g	1.58±0.13	1.50±0.03	0.5228
BW, g	524±37	561±10	0.3170
Cardiomyocyte width, μm	15.13±0.12	15.31±0.35	0.6368
LVAWTd, mm	2.26±0.12	2.28±0.03	0.8080
LVAWTs, mm	3.24±0.19	3.37±0.03	0.4857
LVPWTd, mm	2.03±0.13	2.10±0.03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

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807 Values are means $\pm$ SEM. BW, body weight; HW, heart weight; LVAWTd and
 808 LVAWTs, left ventricular (LV) anterior wall thickness at diastole and systole,
 809 respectively; LVPWTd and LVPWTs, left ventricular (LV) posterior wall thickness at

810 diastole and systole, respectively; LVIVSTd and LVIVSTs, left ventricular (LV)
811 interventricular septal wall thickness at diastole and systole, respectively; LVEDD, LV
812 end-diastolic dimension; LVESD LV end-systolic dimension; FS, LV fractional
813 shortening; LVEDVsp and LVEDVbp, LV end-diastolic volume calculated using
814 single-plane ellipsoid (sp) and biplane ellipsoid (bp), respectively; LVESVsp and
815 LVESVbp, LV end-systolic volume calculated using sp and bp model, respectively;
816 SVsp and SVbp, LV stroke volume (SV) calculated using sp and bp model,
817 respectively; SVIsp and SVIbp, LV SV index calculated using sp and bp model,
818 respectively; EFsp and EF bp, LV ejection fraction using sp and bp model, respectively;
819 BW: body weight. *:p<0.05 vs. untrained controls.



A**B****C****D****E****F****G****H**