

Cardiac effects of long-term exercise training and acute exhaustive exercise in rat models

PhD Doctoral Thesis

Attila Oláh, MD

Semmelweis University

Doctoral School of Basic Medicine



Tutors: Tamás Radovits, MD, PhD, assistant professor
László Gellér, MD, PhD, associate professor

Opponents: Tamás Csont, MD, PhD, associate professor
Tamás Ivanics, MD, PhD, associate professor

Head of Examination Commission:

Emil Monos, MD, DSc, professor emeritus

Members of Examination Commission:

Gábor Pavlik, MD, DSc, professor emeritus

Zsuzsanna Miklós, MD, PhD, assistant professor

Budapest
2015

Introduction

The role of regular physical activity is widely recognized in the prevention and treatment of several cardiovascular diseases, such as coronary heart disease and hypertension. Regular exercise reduces cardiovascular mortality approximately by 35% and all-cause of mortality by 33% and physical activity grants longer life for individuals. However, recently the possible harmful effects of high-intensity prolonged exercise were also emphasized and raised concerns about cardiovascular health consequences of such exercise. Recent American Heart Association Guidelines recommend that 150 minutes of moderate-intensity aerobic exercise or 60 minutes of vigorous-intensity physical activity weekly is necessary to promote and maintain health. This guideline also states that '[...] the shape of the dose–response curves, the possible points of maximal benefit, [...] remain unclear'.

Athlete's heart has been described as the complex structural, functional and electrical cardiac remodelling induced by long-term exercise training. Cardiac enlargement in athletes has been reported since the late 1890s and our understanding has gradually expanded in parallel with the dynamic development of new invasive and non-invasive tools. Regular intense physical activity induces cardiac hypertrophy, an important physiological adaptation, which includes reversible balanced increase of left ventricular and left atrial diameters, left ventricular wall thicknesses and cardiac mass effected by myocyte hypertrophy and increased capillary density by neo-angiogenesis to enhance myocardial performance. These structural alterations in physiological hypertrophy are associated with elongation of the diastolic phase due to resting bradycardia and increased metabolic efficiency as shown by enhanced glucose and fatty acid oxidation. There has been also great interest to reveal molecular mechanisms leading to these beneficial effects because inducing these molecular pathways may provide therapeutic intervention to prevent or treat heart failure.

It is well known that physiological hypertrophy, in contrast to pathological one, is associated with preserved or even enhanced cardiac performance with increased cardiac output under stress conditions. Several aspects of exercise-induced functional consequences were described during the last decades using advanced echocardiographic techniques and cardiac magnetic resonance imaging both in human subjects as well as in experimental animals. However, most of the parameters of these non-invasive investigations are dependent on cardiac loading conditions and the intrinsic myocardial mechanisms are still unknown. The harmless nature of physiological hypertrophy does not allow to perform invasive studies in humans for ethical reasons that can provide the most accurate and reliable, load-independent characterization of cardiac function. Therefore exercise models using animals are requisited for understanding training-induced alterations in myocardial mechanics. A number of animal models of exercise-

induced cardiac hypertrophy have been developed and several types of endurance exercise training have been recognized to effectively induce physiological cardiac hypertrophy in experimental animals, such as treadmill running, voluntary wheel running and swim training.

Although regular exercise training reduces cardiovascular risk, recent studies have documented elevations in cardioneurotic biomarkers consistent with cardiac damage after bouts of prolonged exercise in apparently healthy individuals without cardiovascular disease, which has raised concerns about cardiovascular health consequences of such exercise. Prolonged and intensive physical activity such as ironman triathlon, cross country biking or ultramarathon running are becoming increasingly popular. Thereafter numerous studies on human subjects and animals have reported elevated serum cardiac troponins -as highly specific and sensitive laboratory markers for biochemical detection of myocardial injury- after prolonged, exhaustive exercises that can exceed clinical cut-off value of myocardial infarction. Increased levels of reactive oxygen and nitrogen species, thus nitro-oxidative stress have also been demonstrated after excessive physical exercise in human studies. It is also well described that exhaustive exercise induced reactive oxygen species (ROS) generation leads to oxidative damage in rat myocardium. Excessive ROS formation causes cellular dysfunction, protein and lipid peroxidation and DNA injury. These processes can lead to irreversible cell damage and death, which have been implicated in a wide range of pathological cardiovascular conditions.

Cardiac functional effects of acute bouts of exhaustive exercise have been investigated in human studies using echocardiography and cardiac magnetic resonance imaging. The observed transient impairment in left ventricular function has been described as „exercise-induced cardiac fatigue”, however controversial data and study design differences result in uncertain conclusions. Animal experiments provide a much more controlled and integrated opportunity to investigate effects of exhaustive exercise, as well as the feasibility to directly measure a variety of oxidative stress biomarkers in biological tissues, such as myocardium. Appropriate, hemodynamically validated standards of in vivo experimental models of exhaustive exercise-induced cardiac injury are required for the reliable investigation of cardiac dysfunction after prolonged, strenuous exercise.

Pressure-volume (P-V) analysis is an useful approach for examining intact ventricular function independently from loading conditions. This analysis has been widely used in large animal studies and in humans since the mid-1980s. Advances of the last decades in the development and validation of miniature P-V catheters made it possible to use this approach for studies in small animals. Thus P-V analysis have become gold standard to characterize intact left ventricular function in experimental models of different pathophysiological conditions.

Aim of the work

Sports cardiology received considerable attention recent years. Numerous research groups published multiple articles focusing on long-term exercise training and acute exhaustive exercise induced alterations of the heart in human subjects and in experimental animals. However the detailed left ventricular (LV) functional aspects of athlete's heart and prolonged strenuous exercise-induced cardiac injury remained unclear.

The aims of the present study were:

1. Investigation of exercise training-induced changes of the LV in a rat model:

(i) Establishing the rat model of athlete's heart induced by swim training. Confirming physiological LV hypertrophy by imaging techniques, histology, molecular biology and biochemical measurements. Non-invasive investigation of morphological alterations of the LV and reversibility of the exercise-induced myocardial hypertrophy using echocardiography.

(ii) Providing a detailed characterization of in vivo LV hemodynamics (systolic function, contractility, active relaxation, LV stiffness as well as mechanoenergetics) by using LV pressure-volume analysis for a deeper understanding of functional aspects of athlete's heart.

(iii) Correlating strain and strain rate values measured by non-invasive speckle-tracking echocardiography (STE) with sensitive, load-independent contractility parameters derived from pressure-volume analysis to prove its feasibility in experimental sports cardiology research.

2. In the rat model of exhaustive exercise-induced myocardial injury:

(i) Providing the first detailed in vivo description of LV hemodynamic alterations after an acute bout of exhaustive exercise using pressure-volume analysis to describe prolonged, strenuous exercise-induced LV dysfunction.

(ii) Determining key markers of cellular and molecular mechanisms leading to myocardial injury (nitro-oxidative stress, proapoptotic and profibrotic activation) as a consequence of excessive exercise.

Methods

I. Animals, experimental groups

Young adult male Wistar rats (m=275-375 g) were housed in a room with constant temperature of 22 ± 2 °C with a 12/12 h light-dark cycle and fed a standard laboratory rat diet.

Our experimental groups were:

1. Hemodynamic characterization of athlete's heart Animals underwent 12 week-long swim training program (rat model of athlete's heart). Echocardiographic measurements, histological and gene expression analyses were completed to confirm physiological LV hypertrophy and P-V analysis was performed to characterize exercise training-induced cardiac functional alterations.

2. Testing the reversible nature of exercise training-induced LV hypertrophy After completing the swim training protocol, these rats underwent an 8 week-long resting period. Echocardiography and histology were used to confirm the morphological reversibility of athlete's heart in our rat model.

3. Investigation of the correlation between strain values measured by STE and contractility parameters derived from P-V analysis After completion of the training program, STE and P-V analysis were performed to correlate sensitive contractility parameters with strain values.

4. Investigation of acute exhaustive exercise-induced cardiac changes Rats underwent the protocol of acute exhaustive exercise-induced injury (3 hours swimming with 5% workload followed by a 2 hour-long resting period). Blood plasma and myocardial samples were taken to detect acute exhaustive exercise-induced biochemical and histological alterations. Rats also underwent P-V analysis to characterize cardiac dysfunction after one bout of prolonged, strenuous exercise.

II. Animal models, exercise protocols

Rat model of athlete's heart

We designed a swimming apparatus specially planned for exercise training of rats and filled it with tap water maintained at 30-32 °C to allow individual swim training. Based on literature data and on the results of own preliminary pilot studies we provided a training plan to establish a rat model for inducing robust cardiac hypertrophy. Exercised rats swam for a total period of 12 weeks, 200 min session/day and 5 days a week. For adequate adaptation, the duration of swim training was limited to 15 min on the first day and increased gradually until the maximal swim duration (200 min) was reached. Untrained control rats were placed into the water for 5 min each day during the 12-week training program. For testing the reversibility of swim training-induced LV

hypertrophy, detrained rats remained sedentary for 8 weeks after completing the above described 12 week-long training program.

Rat model of acute exhaustive exercise-induced cardiac injury

We used our previously described swimming apparatus filled with water maintained at 30-32 °C to allow individual swim exercise. Attempting to minimize the general stress response, all rats were familiarized with swimming for 20 min 48 h before the experiments. Acute exercised rats were forced to swim for 3 h with a workload (5 % of body weight) attached to the tail. Control rats were taken into the water for 5 min. After completing the 3-h swimming exercise, rats were dried and monitored for a 2 h observation period. Investigations were performed after this resting period.

III. Echocardiography

Standard echocardiographic measurements

Rats were anesthetized with pentobarbital (60 mg/kg ip.). Animals were placed on controlled heating pads, and the core temperature was maintained at 37 °C. After shaving the anterior chest, transthoracic echocardiography was performed in the supine position. Two dimensional and M-mode echocardiographic images of long and short (mid-papillary muscle level) axis were recorded, using a 13 MHz linear transducer connected to an echocardiographic imaging unit. On two dimensional recordings of the short-axis at the mid-papillary level, LV anterior and posterior wall thickness in diastole and systole, left ventricular end-diastolic and end-systolic diameter were measured. End-diastolic (LVEDV) and end-systolic left ventricular volumes (LVESV) were estimated according to two different validated geometrical models: the single-plane ellipsoid model and the biplane ellipsoid model. The following parameters were derived from these measurements: fractional shortening (FS), stroke volume (SV), ejection fraction (EF). LV mass was calculated according to a cubic formula.

Speckle-tracking echocardiography

Loops of long- and short axis views of the LV dedicated for speckle tracking were acquired at least three times of each axis using a constant frame rate of 218 Hz by special software (EchoPAC). To calculate global longitudinal strain (GLS) and systolic strain rate (LSr) indices, 3-3 cardiac cycles from three different long axis loops were analyzed. To calculate global circumferential strain (GCS) and systolic strain rate (CSr), the same repetition of measures were performed using the short axis recordings. After manual delineation of the endocardial border on end-diastolic frame, the software automatically divided the region of interest to 6 segments and tracked them throughout the cardiac cycles. Ideally, for each parameter 54 segmental values were averaged.

IV. Hemodynamic measurements, left ventricular pressure-volume analysis

Rats were anesthetized, tracheotomized and intubated to facilitate breathing. Animals were placed on controlled heating pads, and the core temperature was maintained at 37 °C. A 2-Fr microtip pressure-conductance catheter was inserted into the right carotid artery and advanced into the ascending aorta. After stabilization mean arterial blood pressure (MAP) and heart rate (HR) were recorded. After that, the catheter was advanced into the LV. Signals were continuously recorded using a P-V conductance system connected to a data acquisition system and displayed by the LabChart5 software.

After positioning and stabilization we registered baseline P-V loops. With the use of a P-V analysis program LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), the maximal slope of LV systolic pressure increment (dP/dt_{max}) and diastolic pressure decrement (dP/dt_{min}), time constant of LV pressure decay [τ ; according to the Weiss, as well as, the Glantz method], ejection fraction (EF) stroke work (SW) and pressure-volume area (PVA) were computed and calculated. LVEDV, LVESV, SV and cardiac output (CO) were calculated and corrected according to in vitro and in vivo volume calibrations. CO was normalized to body weight [cardiac index (CI)]. Total peripheral resistance (TPR) was calculated as MAP/CO . Arterial elastance (E_a) was calculated as $LVESP/SV$. Ventriculo-arterial coupling (VAC) was described by the quotient of E_a and ESPVR and mechanical efficiency (Eff) was calculated as the ratio of SW and PVA.

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

At the end of each experiment parallel conductance volume was calculated and volume calibration of the conductance system was performed by using cuvette wells. To remove erythrocytes from myocardial tissue an in vivo perfusion was performed through the apex of the heart by oxygenated Ringer solution. All animals were euthanized by exsanguination.

Thereafter the heart was quickly removed, heart weight was measured and LV myocardial tissue samples were collected immediately for histology (for fixation in 4 % buffered paraformaldehyde solution to embed in paraffin or snap-frozen in liquid nitrogen) and molecular biology (snap frozen in liquid nitrogen).

V. Biochemical measurements

Blood samples were collected from the inferior caval vein. The blood samples were centrifuged and prepared blood plasma samples were stored at -80 °C. Stress biomarkers [plasma adrenocorticotrophic hormone and cortisol levels] and cardioneurotic biomarkers [plasma cardiac troponin T (cTnT), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and creatinine (for assessing renal function)] were measured by automated clinical laboratory assays.

VI. Histology

Hematoxylin-eosin (HE) staining

From paraffin-embedded or snap frozen myocardial samples transverse transmural slices of the ventricles were sectioned. The sections were stained HE. Light microscopic examination was performed and digital images were captured (400x magnification).

In trained rats transverse transnuclear widths of randomly selected, longitudinally oriented cardiomyocytes were measured. The mean value of 100 LV cardiomyocytes represents each sample.

In acute exercised animals investigation of myocardial structure was performed to observe exhaustive exercise-induced myocardial injury.

Masson's trichrome (MT) staining

Transverse transmural slices of snap-frozen hearts were sectioned and stained with Masson's trichrome. The amount of myocardial collagen was determined by semiquantitative morphometry scoring of Masson's trichrome-stained sections as follows: (0) absent, (1) slight, (2) moderate and (3) intense. The mean value of twenty randomly selected visual fields (magnification 400x) of free LV wall represents each sample.

Dihydroethidium (DHE) staining

Hearts were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 °C. In situ detection of ROS was performed by using the oxidative fluorescent dye dihydroethidium (typically nuclear localization). Fresh frozen LV myocardial sections (16 µm) were incubated with 1µM DHE (in PBS; pH 7.4) at 37 °C for 30 min in a dark humidified chamber. Fluorescence in myocardial sections was visualized using a fluorescence microscope with a 590 nm long-pass filter after background corrections to saline treated negative control. Eight images (magnification 200x) were taken randomly from each of the slides and fluorescence area and intensity was analyzed using ImageJ image analysis software.

Nitrotyrosine (NT) staining

To demonstrate nitro-oxidative stress, tyrosine nitration was detected in LV myocardial sections by immunohistochemistry. Paraffin-embedded sections of myocardium were deparaffinized. After antigen retrieval slides were immunostained with a rabbit anti-NT antibody. Specific labeling was detected by incubation with a biotin-conjugated anti-rabbit goat antibody and amplified with an avidin-biotin peroxidase complex. Nickel-cobalt enhanced diaminobenzidine was used as chromogen. Five images (200x) were taken randomly from the free LV wall. NT positive area was calculated using conventional microscopy and ImageJ software.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining

Snap-frozen hearts were sectioned and TUNEL staining was performed using a colorimetric TUNEL system according to the manufacturers instruction. Sections were counterstained. Thirty visual fields of LV sections were randomly selected in each animal, and TUNEL-positive cells were counted. Data are expressed as mean number of apoptotic cells per field.

VII. Cardiac mRNA analysis

LV myocardial tissue samples were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 °C. LV tissue was homogenized, RNA was isolated from the ventricular samples. RNA purity was confirmed and then reverse transcription reaction was completed. Quantitative real-time PCR was performed in triplicates of each sample containing gene expression assay for the following targets: β -isoform of myosin heavy chain (β -MHC), transforming growth factor β (TGF- β), catalase, glucose-6-phosphate dehydrogenase (G6PD), glutathion peroxidase 1 (GPX-1), glutathione reductase (GSR), thioredoxin-1, superoxide dismutase 2 (SOD-2), endothelial nitric oxide synthase (eNOS), Bcl-2 associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-2 (TIMP-2), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1). Gene expression data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

VIII. Statistical analysis

All data are expressed as means \pm SEM. Normal distribution of variables was confirmed by Shapiro-Wilk test. An unpaired two sided Student t-test was used to compare parameters between exercised and untrained control, acute exercised and control rats as well as between detrained exercised and detrained control animals. Correlation relationships between P-V analysis derived contractility parameters and strain values by STE were calculated with Pearson correlation test. Differences were considered statistically significant when $p < 0.05$.

Results

I. Athlete's heart

Heart weight

Compared with untrained controls, exercise training was associated with markedly increased heart weight in exercised animals. The heart weight to body weight ratio showed an even more significant increase in the exercised group reflecting cardiac hypertrophy.

Echocardiographic parameters

At the end of the exercise training protocol, LV wall thickness values were significantly higher in the exercised rats compared with the control animals. Swim training was associated with significantly decreased LVESV along with unchanged LVEDV, thus SV, EF and FS were significantly increased in trained animals. LV mass was significantly higher in exercised animals versus controls confirming cardiac hypertrophy.

Histology

Exercise training resulted in a significant increase in mean cardiomyocyte width compared with untrained control rats, indicating cardiac hypertrophy at the microscopic level in exercised rats. The semiquantitative evaluation of Masson's trichrome staining indicated unaltered cardiac collagen content in the exercised group.

Markers of stress and pathological hypertrophy

Myocardial gene expression levels of β -MHC and TGF- β were unaltered after exercise training, indicating absence of pathological hypertrophy and remodeling, respectively. Unaltered plasma stress biomarker concentrations provide evidence against the influence of stress by swimming training.

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 1.). dP/dt_{\max} as a classical contractility parameter showed only an increasing tendency in trained rats, without reaching the level of statistical significance. Decreased τ was also detected in exercised rats, suggesting improved active relaxation in trained animals. Figure 1. shows representative original steady-state P-V loops obtained from exercised and untrained control rats. The wider baseline P-V loops in exercise-induced hypertrophy reflect increased stroke volume along with unaltered LVEDV and decreased LVESV. EF, CO, CI and SW increased significantly, suggesting increased systolic performance in trained rats. Decreased TPR and E_a were detected in exercised rats (Table 1.).

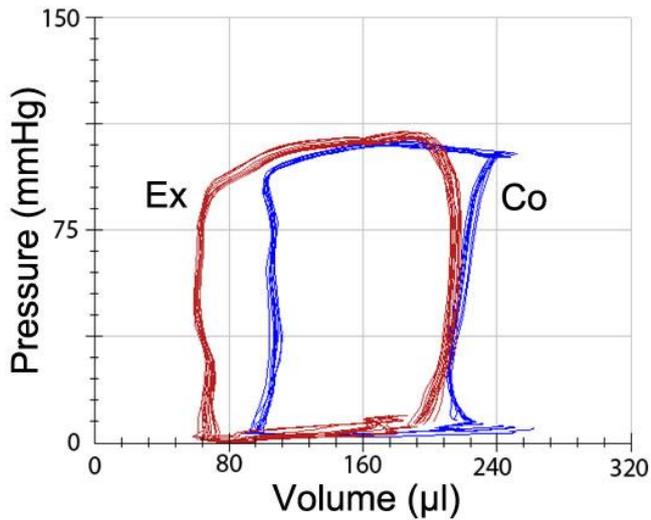


Figure 1. Steady-state left ventricular (LV) pressure-volume (P-V) loops

Original recordings of LV steady-state P-V loops from one representative rat from the exercised (Ex) and control (Co) groups. The wider P-V loop indicates increased stroke volume along with unaltered end-diastolic volume and decreased end-systolic volume, thus increased ejection fraction in exercised rats compared with sedentary controls.

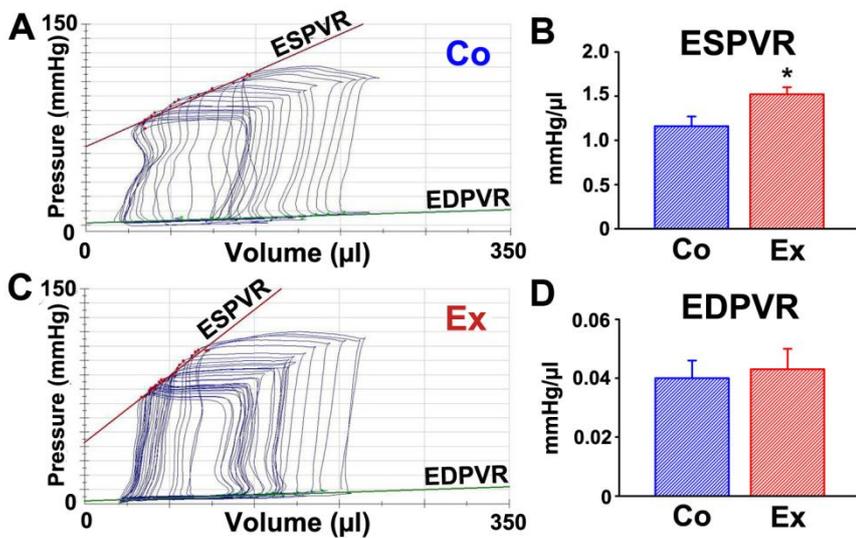
Table 1. Hemodynamic parameters in untrained control and exercised rats

| | Control | Exercised |
|--|----------------|-------------------|
| Heart rate, beats/min | 235±7 | 236±7 |
| Mean arterial pressure, mmHg | 81.8±2.5 | 83.4±2.4 |
| LV end-systolic pressure, mmHg | 95.1±3.2 | 91.5±2.4 |
| LV end-diastolic pressure, mmHg | 8.2±0.8 | 8.5±0.5 |
| Maximal dP/dt, mmHg/s | 7295±189 | 7668±196 |
| Minimal dP/dt, mmHg/s | 6771±396 | 6878±470 |
| τ (Weiss), ms | 10.7±0.2 | 9.6±0.3* |
| τ (Glanz), ms | 11.9±0.2 | 10.1±0.6* |
| LV end-diastolic volume, μ l | 206.0±8.0 | 209.4±11.9 |
| LV end-systolic volume, μ l | 99.5±6.7 | 75.4±5.0* |
| Stroke volume, μ l | 145.4±7.7 | 175.4±8.5* |
| Cardiac output, ml/min | 32.3±1.9 | 44.7±3.7* |
| Cardiac index, (ml/min)/kg body weight | 66.5±4.0 | 100.0±8.0* |
| Ejection fraction, % | 64.1±1.5 | 73.7±0.8* |
| Total peripheral resistance, mmHg/(ml/min) | 2.63±0.20 | 1.98±0.19* |
| Arterial elastance, mmHg/ μ l | 0.69±0.05 | 0.53±0.03* |
| Stroke work, mmHg·ml | 11.1±0.8 | 15.1±0.9* |
| Pressure-volume area, mmHg·ml | 25.9±0.9 | 28.3±2.6 |
| Mechanical efficiency, % | 44.6±2.1 | 53.0±0.02* |
| Ventriculoarterial coupling | 0.65±0.08 | 0.37±0.02* |

Values are means \pm SEM. LV, left ventricular. * p <0.05 vs. controls.

Functional indices derived from P-V analysis at different preloads. Figure 2. shows representative original P-V loops registrated during transient occlusion of the inferior vena cava in exercised and untrained control animals with overall results of EDPVR and ESPVR. As shown, ESPVR was steeper in exercised than in control animals, suggesting increased contractility in trained hearts. EDPVR did not differ between the groups indicating unchanged LV stiffness in exercised rats.

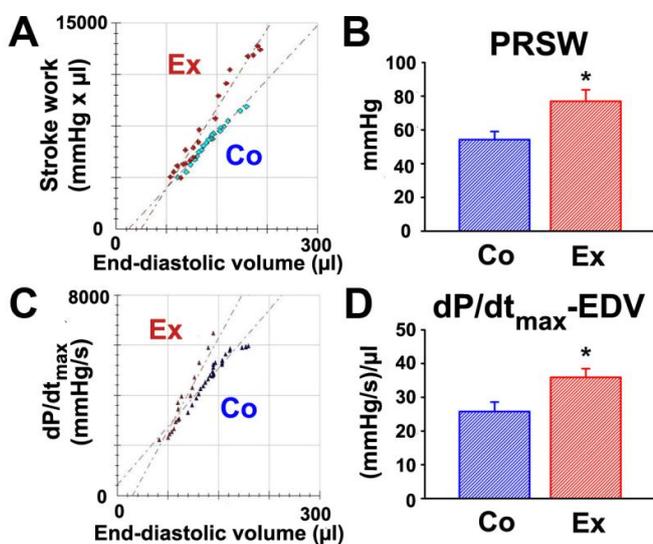
Figure 2. Left ventricular end-systolic pressure-volume (P-V) relationship (ESPVR) and end-diastolic P-V relationship (EDPVR)



ESPVR and EDPVR in one representative animal from the exercised (Ex; A) and control (Co; C) groups. Original recordings of representative P-V loops were obtained at different preloads during vena cava occlusion and showed increased slope of ESPVR (B) in exercised rats compared with sedentary controls. The slope of EDPVR did not differ between the groups (D). * $p < 0.05$ vs. Co.

Figure 3.A shows PRSW in a representative exercised and control animal. The slope was steeper in trained rats than in controls. The overall PRSW values were significantly increased in exercised rats suggesting improved contractile state of the myocardium (Fig. 3.B). We also determined the relation between dP/dt_{max} and EDV. dP/dt_{max} is known as a classical contractility parameter, but it is dependent on changes in preload. Analysis of dP/dt_{max} -EDV allowed us to compare dP/dt_{max} values of exercised and control rats at a given EDV. The slope of this relation was steeper and the overall slope values were significantly elevated in trained animals than in untrained animals, indicating increased contractility in exercise-induced hypertrophy (Fig. 3.C and D).

Figure 3. Pressure-volume loop derived left ventricular contractility parameters



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

Cardiac mechanoenergetics. P-V analysis revealed a significant increase in contractility and a decrease of E_a . Subsequently, VAC was significantly decreased in trained rats suggesting optimized ventriculo-arterial interaction. SW (the effective mechanical work of LV) was increased in exercised rats and PVA (which is supposed to be proportional to the energy demand of LV performance) did not differ between the groups. Consequently Eff was increased in trained rats indicating improved LV mechanoenergetics (Table 1.).

II. Reversibility of exercise-induced cardiac hypertrophy

Heart weight did not differ between the detrained exercised and detrained control groups. The mean cardiomyocyte width regressed to control levels after discontinuation of the training. No differences could be observed between the detrained exercised and detrained control groups regarding morphological and functional echocardiographic parameters, which suggests the complete reversibility of exercised-induced changes.

III. Investigation of the correlation between strain values measured by speckle-tracking echocardiography and contractility parameters derived from pressure-volume analysis

Morphological markers of left ventricular hypertrophy

Echocardiographic morphological data showed increased wall thickness values both in anterior and posterior region in exercised animals compared to controls. The calculated LV mass values indicated LV hypertrophy after completion of the exercise training protocol. These data were underpinned by post mortem measured heart weight data, which showed increased heart weight values.

Contractility indices derived from pressure-volume analysis at different preloads

Sensitive, load-independent contractility indices, such as ESPVR, PRSW as well as dP/dt_{\max} -EDV have been showed to be steeper and the overall slope values were markedly increased in exercised animals compared to controls indicating improved LV contractility.

Strain parameters derived from speckle-tracking analysis

In line with contractility indices, exercised rats showed supernormal systolic function. Both longitudinal and circumferential strain and systolic strain rate were significantly increased compared to untrained rats (Fig. 4.).

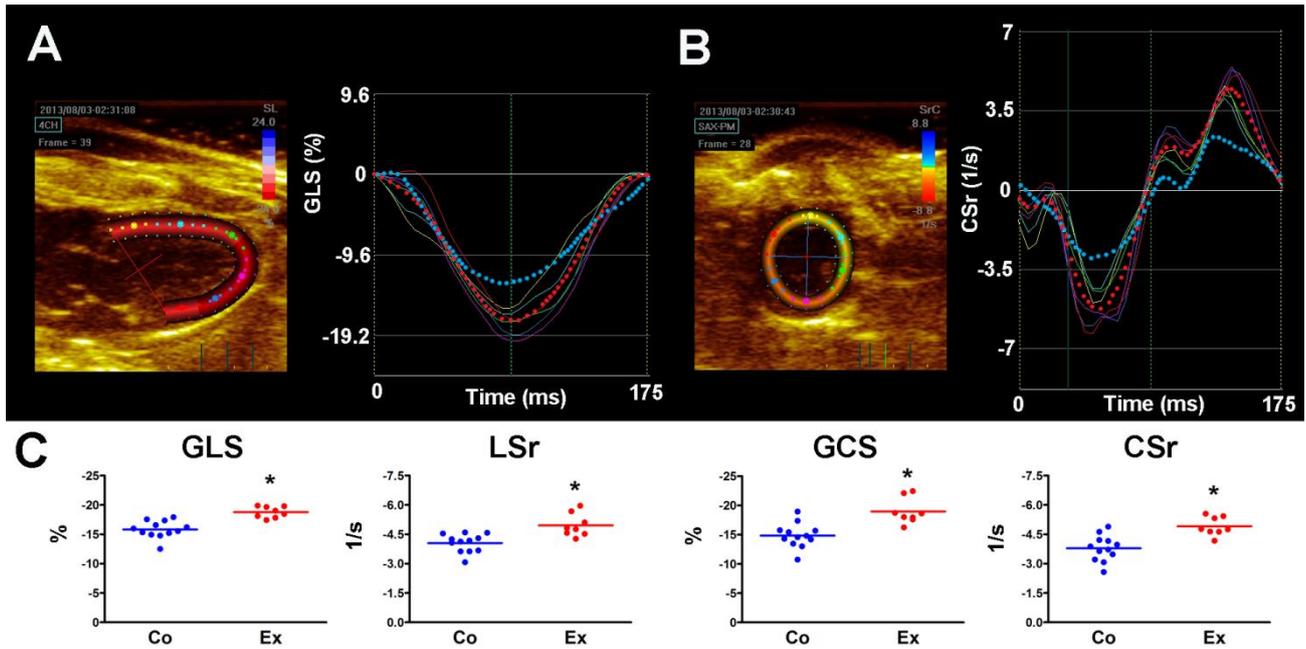


Figure 4. Layout and results of the speckle tracking analysis. Representative original recordings of an exercised rat. Each continuous curve represents a given segment with the same colour on the echocardiographic image. Average values of the 6 segments are delineated with the red dotted line and compared to an original recording from a control rat (blue dotted line). (A) Determination of longitudinal strain on a long-axis image. The negative peak of the averaged curve represents the global longitudinal strain (GLS) (B) Determination of circumferential strain rate on a mid-papillary short-axis view. The peak negative value of the averaged curve is the systolic strain rate (CSr). As depicted by the dot plots (C), all measured strain and strain rate indices (GLS, LSr - longitudinal systolic strain rate, GCS – global circumferential strain, CSr) were improved in the exercised group (Ex) compared to the control group (Co) in line with the invasive contractility parameters. Horizontal lines represent mean values. *: $p < 0.05$ vs. Co

Correlations between contractility and strain parameters

ESPVR correlated with GLS ($r = -0.71$, $p < 0.001$), LSr ($r = -0.53$, $p = 0.016$), but robustly with GCS ($r = -0.83$, $p < 0.001$) and CSr ($r = -0.75$, $p < 0.001$). PRSW and dP/dt_{\max} -EDV showed also strong or moderate correlation with strain and strain rate values.

IV. Cardiac effects of acute, exhaustive exercise

Biochemical parameters

When compared to the control group, serum cTnT concentrations increased significantly after exhaustive exercise. Serum CK and LDH enzyme activity levels as well as that of AST were also markedly increased after exhaustive exercise. Serum creatinine did not differ between the groups.

Histology

In contrast to control myocardium, sporadic fragmentation of myocardial fibers, leukocyte infiltration, tissue edema and cytoplasmic eosinophilia could be observed in the LV myocardium of acute exercised rats (Fig. 5.).

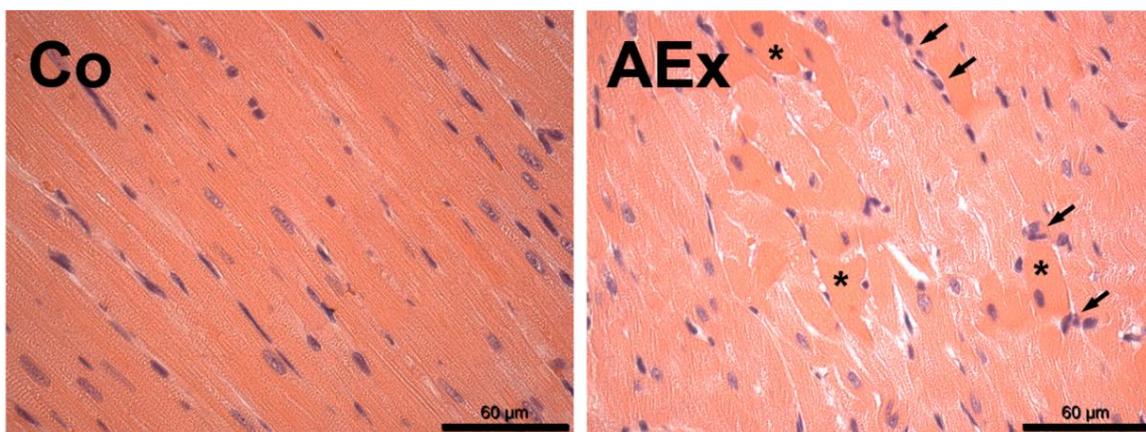


Figure 5. Histological investigation of hematoxylin-eosin (HE) stained left ventricular (LV) myocardium of control (Co) and acute exercised (AEx) rats

HE stained LV tissue sections showed sporadic fragmentation of myocardial fibers, interstitial edema, cytoplasmic eosinophilia (see star symbol) and leukocyte infiltration (see arrows) - signs of myocardial injury - in the LV myocardium of acute exercised rats compared to intact myocardium of control animals (magnification 400x, scale bar 60 µm).

The red fluorescence signal intensity of DHE-stained LV myocardial sections was markedly increased after exhaustive exercise suggesting a robust generation of ROS. Nitrotyrosine-staining showed increased tyrosine nitration in the myocardium of rats underwent excessive exercise. The number of TUNEL-positive cardiomyocyte nuclei were significantly increased in the exercised group.

Gene expression analysis

Myocardial gene expression analysis showed a significant increase of endogenous antioxidants G6PD, GSR, thioredoxin-1, SOD-2, while catalase and GPX-1 had a strong tendency towards higher expression values without reaching the level of statistical significance in rats after exhaustive exercise. Myocardial expression of eNOS was increased in the exercised group.

Myocardial gene expression of proapoptotic mediator Bax significantly increased, while the antiapoptotic mediator Bcl-2 expression significantly decreased which led to a markedly significant augmentation of Bax/Bcl-2 ratio.

MMP-2 and MMP-9 expression values were both increased after intense exercise. TIMP-2 did not differ between the groups, while TIMP-1 was significantly upregulated in exercised rats leading to increased MMP-2/TIMP-2 and decreased MMP-9/TIMP-1 ratio. Myocardial gene expression of TGF- β 1 showed a strong tendency toward higher values after exercise, however without reaching the level of statistical significance.

Hemodynamic parameters

Baseline hemodynamic data. Figure 6. shows representative original steady-state P-V loops obtained from acute exercised and control rats. MAP, HR, LVESP, LVEDP, τ , dP/dt_{max} and dP/dt_{min} were not different in acute exercised animals compared to the control group. The decreased width of baseline P-V loops after exhaustive swimming reflects reduced SV along with unaltered LVEDV and increased LVESV. EF, CO, CI and SW decreased significantly suggesting deteriorated systolic performance in rats after exhaustive exercise. TPR and E_a were increased in rats after intense exercise.

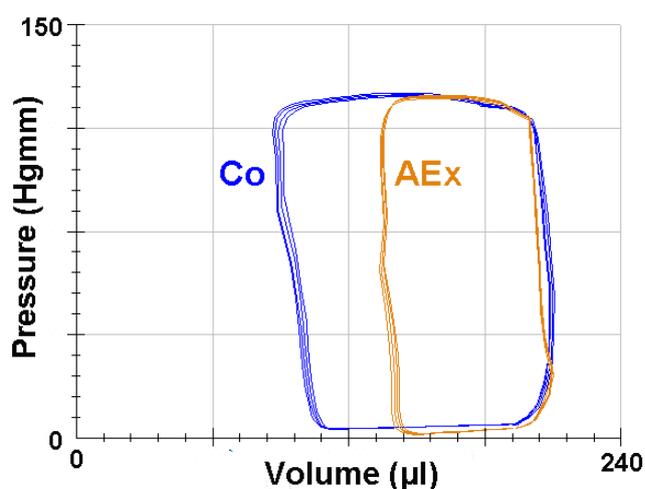


Figure 6. Steady-state left ventricular (LV) pressure-volume (P-V) loops after exhaustive exercise

Original recordings of steady-state P-V loops in representative rats from control (Co) and acute exercised (AEx) groups. The decreased width of P-V loops in exercised animals indicate reduced stroke volume as a result of unaltered end-diastolic volume and increased end-systolic volume, thus decreased ejection fraction.

Systolic and diastolic functional indexes derived from P-V analysis at different preloads. Figure 7. shows representative original P-V loops recorded during transient occlusion of the inferior vena cava in exercised and control animals. The ESPVR was less steep in exhaustive exercised than in control animals suggesting decreased contractility. EDPVR did not differ between the groups indicating unchanged LV stiffness after strenuous exercise. PRSW were significantly decreased after intense exercise indicating impaired contractility. The linear relation of dP/dt_{max} and EDV was less steep after exhaustive exercise than in controls. The overall dP/dt_{max} -EDV slope values were significantly lower in acute exercised rats (Fig. 7). All of these data indicate impaired contractility after acute exercise.

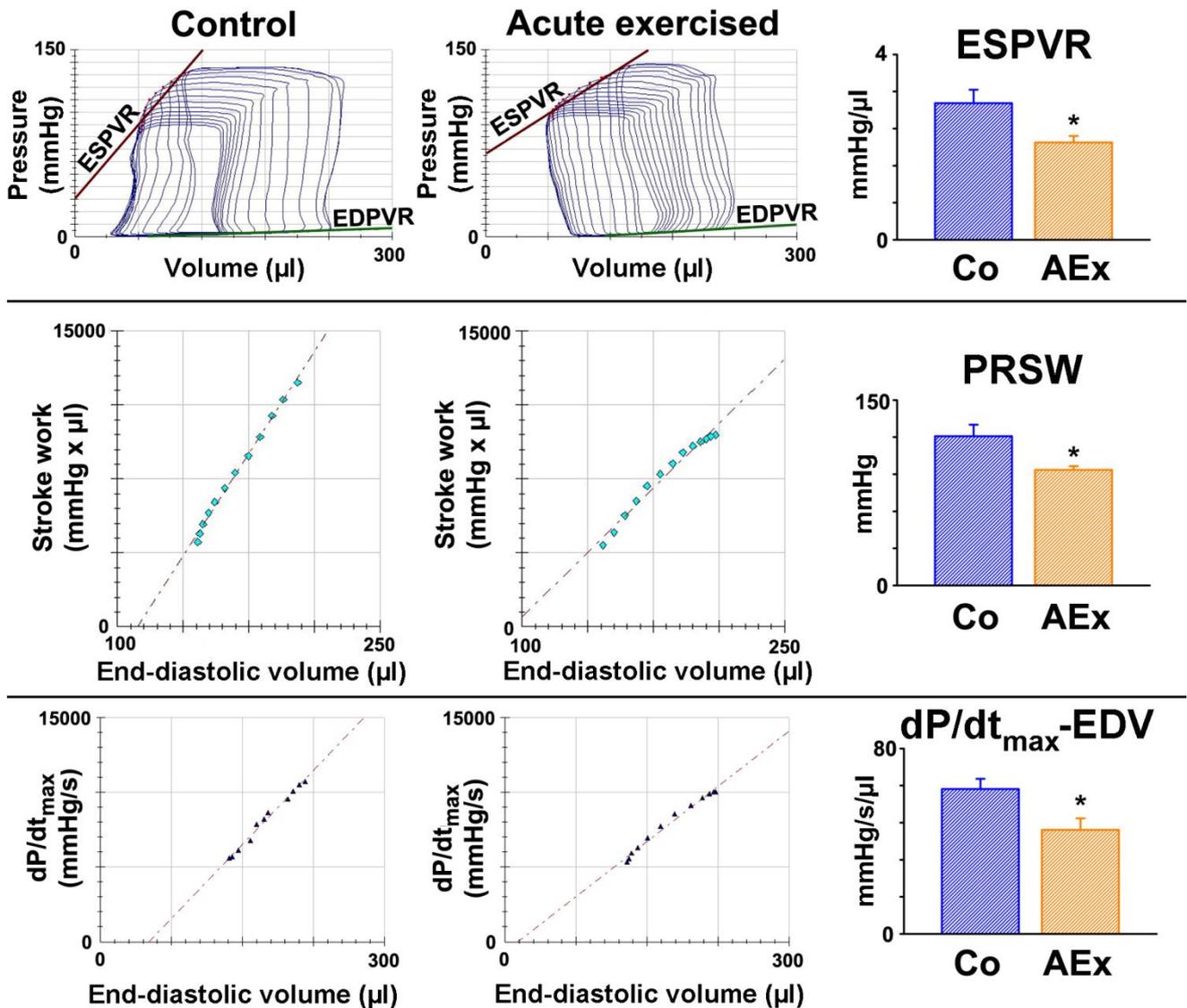


Figure 7. Pressure-volume (P-V) loop derived load independent left ventricular contractility parameters after acute exhaustive exercise

The slope of end-systolic P-V relationship (ESPVR) (upper panel); preload recruitable stroke work (PRSW), the slope of the relationship between stroke work (SW) and end-diastolic volume (EDV) (mid panel); and maximal slope of the systolic pressure increment (dP/dt_{max}) - EDV relationship (dP/dt_{max} -EDV) (lower panel) in representative rats from control (Co) and acute exercised (AEx) groups. As seen on the bar graphs, all of these contractility parameters are decreased in the AEx group, suggesting reduced systolic performance after exhaustive exercise. * $p < 0.05$ vs. Co.

Cardiac mechanoenergetics. P-V analysis revealed a significant decrease in contractility and an increase of E_a . Subsequently, VAC was significantly increased in exhaustively exercised rats suggesting contractility-afterload mismatch. Despite the decreased SW in exercised rats, PVA did not differ between the groups. As a consequence, mechanical efficiency of LV work was also impaired in exercised rats compared to the controls. All of these data suggest deterioration of LV mechanoenergetics after prolonged, strenuous exercise.

Conclusions

In the first project we provided the first in vivo investigation to characterize left ventricular hemodynamic alterations in exercise-induced cardiac hypertrophy by pressure-volume analysis. We confirmed exercise-induced reversible physiological cardiac hypertrophy in our rat model. Based on reliable load-independent indices of left ventricular performance, in the present study we demonstrated systolic (improved contractility) and diastolic (improved active relaxation and unchanged myocardial stiffness) functional amelioration in exercised animals. Improved mechanoenergetics were shown by increased mechanical efficiency and optimized ventriculo-arterial coupling.

In additional experiments both pressure-volume analysis and speckle-tracking echocardiography demonstrated increased systolic function in our rat model of athlete's heart. Speckle tracking derived indices were in close relationship with invasive load-independent measures of cardiac contractility. Correlations between pressure-volume analysis and strain parameters are promising in terms of widespread use of non-invasive speckle-tracking echocardiography during consecutive evaluation of physiological myocardial hypertrophy in small animal models.

Exhaustive exercise-induced cardiac injury and nitro-oxidative stress in our rat model and for the first time we described in vivo left ventricular dysfunction in detail by using the pressure-conductance catheter system. Based on reliable load-independent indices we demonstrated systolic functional deterioration (reduced contractility) in exhaustive exercised animals along with unchanged diastolic function and impaired mechanoenergetics (decreased efficiency, ventriculo-arterial mismatch). Elevations in cardiac enzymes, sporadic cardiac injuries and possible impaired myocardial function along with the activation of proapoptotic and profibrotic activity raise the question whether prolonged endurance exercise could induce persistent myocardial damage and dysfunction.

List of publications

Publications related to the dissertation:

Radovits T*, **Oláh A***, Lux Á, Németh BT, Hidi L, Birtalan E, Kellermayer D, Mátyás C, Szabó G, Merkely B. (2013) Rat model of exercise-induced cardiac hypertrophy: hemodynamic characterization using left ventricular pressure-volume analysis. *Am J Physiol Heart Circ Physiol*, 305: H124-134.

IF: 4.012

*equal contribution

Oláh A, Németh BT, Mátyás C, Horváth EM, Hidi L, Birtalan E, Kellermayer D, Ruppert M, Merkely G, Szabó G, Merkely B, Radovits T. (2015) Cardiac effects of acute exhaustive exercise in a rat model. *Int J Cardiol*, 182: 258-266.

IF: 6.175

Kovacs A*, **Oláh A***, Lux Á, Mátyás C, Németh BT, Kellermayer D, Ruppert M, Torok M, Szabo L, Meltzer A, Assabiny A, Birtalan E, Merkely B, Radovits T. (2015) Strain and strain rate by speckle tracking echocardiography correlate with pressure-volume loop derived contractility indices in a rat model of athlete's heart. *Am J Physiol Heart Circ Physiol*, 308: H743-748.

IF: 4.012

*equal contribution

Oláh A, Lux Á, Németh BT, Hidi L, Birtalan E, Kellermayer D, Mátyás C, Ruppert M, Merkely G, Szabó G, Merkely B, Radovits T. (2013) A sportszív részletes hemodinamikai jellemzése bal kamrai nyomás-térfogat analízis segítségével. *Cardiol Hung*, 43: 224-232.

Publications not related to the dissertation:

Radovits T, Korkmaz S, Mátyás C, **Oláh A**, Németh BT, Páli S, Hirschberg K, Zubarevich A, Gwanmesia PN, Li S, Loganathan S, Barnucz E, Merkely B, Szabó G. (2015) An altered pattern of myocardial histopathological and molecular changes underlies the different characteristics of type-1 and type-2 diabetic cardiac dysfunction. *J Diabetes Res*, 2015: 728741.
IF: 3.536

Radovits T, Mátyás C, **Oláh A**, Kökény G, Barnucz E, Szabó G, Merkely B. (2012) A foszfodiészteráz-5-gátló vardenafil hatásai diabéteszes kardiovaszkuláris diszfunkcióra. *Cardiol Hung*, 42: 272-279.