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

PhD Doctoral Dissertation

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1. List of abbreviations

α-MHC - α-myosin heavy chain

ACTH - adrenocorticotropic hormone

AGE - advanced glycation end-products

Akt - protein kinase B

ANF - atrial natriuretic factor

AST - aspartate transaminase

β-MHC - β-myosin heavy chain

Bax – B-cell lymphoma 2 associated X protein

Bcl-2 - B-cell lymphoma 2

BNP - brain natriuretic peptide

BW - body weight

CI - cardiac index

CK - creatine kinase

CK-MB - myocardial band isoform of creatine kinase

cMRI - cardiac magnetic resonance imaging

CO - cardiac output

CSr - circumferential strain rate

cTn - cardiac troponin

cTnT - cardiac troponin T

DHE - dihydroethidium

dP/dt_{max} - maximal slope of systolic pressure increment

dP/dt_{max}-EDV - maximal slope of systolic pressure increment – end-diastolic volume relationship

dP/dt_{min} - maximal slope of diastolic pressure decrement

E/A - early-to-late diastolic filling rate

E_a - arterial elastance

ECG - electrocardiogram

ECLIA - electrochemiluminescence immunoassay

ECM - extracellular matrix

EDPVR - end-diastolic pressure-volume relationship

E_{es} - end-systolic elastance

EF - ejection fraction

Eff - mechanical efficiency

eNOS - endothelial nitric oxide synthase

ESPVR - end-systolic pressure-volume relationship
FAC - fractional area change
FS - fractional shortening
G6PD - glucose-6-phosphate dehydrogenase
GAPDH - glyceraldehyde-3-phosphate dehydrogenase
GCS - global circumferential strain
GLS - global longitudinal strain
GPX-1 - glutathione peroxydase-1
GSR - glutathione reductase
H_2O_2 - hydrogen peroxide
HCM - hypertrophic cardiomyopathy
HE - hematoxylin-eosin
HR - heart rate
HW - heart weight
IGF-1 - insulin-like growth factor 1
IGF-1R - insulin-like growth factor 1 receptor
IVST - interventricular septal thickness
LDH - lactate dehydrogenase
LSr - longitudinal strain rate
LV - left ventricle, left ventricular
LVAWT - left ventricular anterior wall thickness
LVEDD - left ventirular end-diastolic diameter
LVEDP - left ventricular end-diastolic pressure
LVEDV - left ventricular end-diastolic volume
LVESD - left ventricular end-systolic diameter
LVESP - left ventricular end-systolic pressure
LVESV - left ventricular end-systolic volume
LVPWT - left ventricular posterior wall thickness
MAP - mean arterial pressure
MAPK - mitogen-activated protein kinases
MMP - matrix metalloproteinase
MT - Masson’s trichrome
NADPH - nicotinamide adenine dinucleotide phosphate
$NO^{\cdot}$ - nitric oxide
NT - nitrotyrosine

NT-proBNP - N-terminal of the prohormone brain natriuretic peptide

$O_2^{-\cdot}$ - superoxide anion

$OH^{\cdot}$ - hydroxyl radical

$ONOO^-$ - peroxynitrite

PI3K - phosphatidylinositol-3-kinase

PRSW - preload recruitable stroke work

P-V - pressure-volume

PVA - pressure-volume area

ROS - reactive oxygen species

RV - right venticle, right ventricular

SCD - sudden cardiac death

SEM - standard error of the mean

SERCA2a - sarcoplasmatic reticulum Ca^{2+}-ATPase 2a

SOD-2 - superoxide dismutase 2

STE - speckle-tracking echocardiography

SV - stroke volume

SVI - stroke volume index

SW - stroke work

τ - time constant of left ventricular pressure decay

TBARS - thiobarbituric acid reactive substance

TDI - tissue Doppler imaging

TIMP - tissue inhibitor of metalloproteinases

TGF-β - transforming growth factor beta

TPR - total peripheral resistance

TUNEL - terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling

VAC - ventriculo-arterial coupling

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2. Introduction

It has been well documented that regular physical activity yields significant health benefits. Regular exercise reduces cardiovascular mortality approximately by 35% and all-cause of mortality by 33% and physical activity grants longer life for individuals (Nocon et al., 2008). By contrast, a lack of exercise can lead to many chronic diseases related to cardiovascular system (Roberts et al., 2013). Additional cardiovascular benefits of excercise include weight reduction, blood pressure reduction, increased insulin sensitivity and improved lipid profile (D'Silva and Sharma, 2014). However, recently the possible harmful effects of high-intensity prolonged exercise was also emphasized mainly because of increased vulnerability for atrial and ventricular arrhythmias and an increased risk of sudden cardiac death (La Gerche, 2007; La Gerche et Prior, 2013; Guasch and Nattel, 2013).

What remains unknown is precisely how much exercise is required to produce these beneficial effects and, more contraversially, how much exercise could cause harm? The first studies suggested that more intensive the physical activity is undertaken, the greater the cardiovascular benefits (Tanasescu et al., 2002; Schnohr et al., 2013; Sesso et al., 2000). Thus for many years 'the more, the better' concept has prevailed in evaluatuion of physical activity. Other data from large cohort studies have shown that increased exercise capacity, daily time of exercise and weekly run distances implies a large reduction in mortality until higher level of fitness (Kokkinos et al., 2008). After approaching this fitness level, the beneficial effect appeared to plateau: higher milage or more frequent running were not associated with better survival, thus a curvilinear relationship between mortality and level of fitness was supposed (Wen et al., 2011). Controversially, some publications have proposed that a U-shaped curve exists for optimal exercise dosage and extreme levels of vigorous physical activity for prolonged periods could have harmful effects on the heart (La Gerche, 2007, O'Keefe and Lavie, 2013). Recent American Heart Association Guidelines recommend that 150 minutes of moderate-intensity aerobic exercise or 60 minutes of vigorous-intensity physical activity is necessary to promote and maintain health (Haskell et al., 2007). This guideline also recognize that '[…] the shape of the dose–response curves, the possible points of maximal benefit, […] remain unclear'. Exercise training regimes planned to prepare for

prolonged races (i.e. marathon race) are several-fold greater than the recommendation, while the number of followers (also elite, trained as well as nonelite, amateur individuals) of extremely prolonged training and races has risen during the last decades and is still rising (Guasch and Nattel, 2013).

Hereinafter I present shortly the so far described cardiac effects of long-term exercise training and the acute, prolonged intense exercise as an introduction of my work.

2.1. Long-term exercise training-induced cardiac alterations, the athlete's heart

Regular exercise training is accompanied by structural, funcional and electrical alterations of the heart to enhance performance. The resulting phenotype, the cardiac enlargement was already recognized more than hundred years ago based on clinical examination, with the recognition of cardiac enlargement and bradycardia among highly trained athletes (Darling, 1899; Henschen, 1899). In the 20th century our understanding has gradually expanded in parallel with the dynamic development of new invasive and non-invasive tools for understanding the effects of physical activity on the heart. Initially, the chest x-ray and ECG demonstrated the enlargement of cardiac chambers and cardiac pathological examination were undertaken, which were followed by the use of modern imaging techniques, such as echocardiography and cardiac magnetic resonance imaging (cMRI) to deepen into the understanding of athlete's heart. Recently, numerous studies have been undertaken about athlete's heart in vivo as well as in vitro and the knowledge of these investigations were summarized in several expanded reviews (Pluim et al. 2000; Fagard, 2003, Maron and Pelliccia, 2006; Pavlik et al., 2010; Prior and La Gerche, 2012, Pavlik et al., 2013). Thus, during the last century there has been considerable interest on the constellation of exercise-induced cardiovascular alterations.

Investigations of athlete's heart is important for a number of essential reasons. First, to understand how the adaptation of the cardiovascular system contributes to improved athletic performance. The understanding of the mechanisms leading to enhanced function could be essential because pro-active therapeutic interventions -

based on the same stimuli and genes leading to physiological growth- may provide an additional strategy to prevent or treat heart failure (McMullen and Jennings, 2007).

The second is to enhance physical performance to optimise cardiac functional adaptation for elite athletes. This is important in terms of sports cardiology for elite, top-level athetes.

The third and maybe the most important is to allow the differentiation of normal athlete's heart from pathological cardiac states, especially from hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy. These are pathological conditions, which may share similar morphological features like exercise-induced hypertrophy, nevertheless these phenotypes are associated with increased risk of sudden cardiac death (Prior et La Gerche, 2012).

2.1.1. Structural alterations in athlete's heart

A fundamental component of exercise-induced cardiac remodeling is physiological cardiac hypertrophy, a process leading to increased cardiac myocyte size. During physical activity the loading conditions are altered in the cardiovascular system. Different forms of exercise impose different loads of the cardiovascular system. These differential effects of pressure and volume loading during training was first documented in the essential work by Morganroth, in which he identified cardiac morphological differences between different kind of sports (Morganroth et al., 1975). It was hypothesized that these adaptations reflect differential haemodynamic loading during training. According to this comparative work, athletes involved in dynamic, isotonic exercise (e.g. running and swimming) had increased left ventricular mass with paralelly increased cardiac dimensions because of chronic volume overload. Thus endurance-trained athletes are presumed to demonstrate eccentric left ventricular hypertrophy, characterized by an unchanged relationship between left ventricular (LV) wall thickness, LV diameter and as a consequence, unaltered relative wall thickness (ratio of wall thickness to diameter). Participation in sports with a high static demand (isometric exercise, e.g. weightlifting or wrestling) is associated with increased LV wall thickness, which is increased in relation to cavity size like in similar disease states with chronic pressure loads. Thus, strength-trained athletes were presumed to demonstrate concentric LV hypertrophy, which is characterized by increased relative wall thickness values.

Sports such as cycling and rowing are examples of combined endurance and strength exercise: endurance training while working against an elevated resistance. These athletes display the greatest degree of LV dilatation and wall thickening (Pelliccia et al., 1991; Pluim et al., 2000; Barbier et al., 2006). Since that, after a large amount of echocardiographic studies and meta-analyses, we know that the development of an endurance-trained heart and a strength-trained heart is not to be considered an absolute and dichotomous concept and the geometric pattern of athlete's heart is more complicated than expected by Morganroth (Pluim et al., 2010; Pavlik et al., 2010). Each sport can be classified by its dynamic (according to the estimated percent of maximal oxygen uptake) and static (according to estimated percent of maximal voluntary contraction) component, and the observed phyenotype of LV hypertrophy is dependent on these factors (Mitchell et al., 2005). Moreover, morphological manifestation is influenced by numerous other factors, such as age, gender, ethnicity and genetic factors (Pavlik et al., 2013).

The observation of training-induced structural changes of RV by echocardiography was not so clear for a long time because RV geometry and its location in the chest made it difficult to investigate. Thus the effect of exercise training on the right ventricle has been observed more recently, due to the technical development in cardiac imaging with the appearence of cMRI. An increased RV volume, mass and stroke volume have all been observed in athletes, largely based on measurements using cMRI. The balanced hypertrophy of the RV, similar as in the LV, has been described in endurance athletes (Scharhag et al. 2002; Scharf et al. 2010). However, recent works suggest that the RV undergoes remodelling process and dilate slightly more than the LV after endurance training (La Gerche et al., 2011).

In addition to the findings in ventricle morphological alterations, studies have shown atrial enlargement in trained athlete's heart, which predispose athletes to an increased risk of subsequent atrial fibrillation (Grimsmo et al., 2010; Wilhelm et al., 2012).

Detraining has been defined as the partial or complete loss of training-induced anatomical, physiological and performance adaptations, as a consequence of training reduction or cessation (Mujika and Padilla, 2001). Clinical studies have reported regression of the LV morphological changes characteristic of the athlete's heart after

long-term detraining (Pelliccia et al., 2002), which property can be used to differentiate it from pathological conditions (Mitchell et al., 2013).

2.1.2. Functional consequences of exercise training

While structural changes within the LV has been very clearly and consistently demonstrated, the evidence regarding functional change has been less consistent (La Gerche et al., 2009). These studies demonstrate consistency in the fact, that athlete's heart, in contrast to pathological hypertrophy, have preserved or even enhanced systolic and diastolic function (Utomi et al., 2013). These findings are based mainly on non-invasive functional studies that have used echocardiography, while a small subset have used cMRI.

Echocardiography has contributed most to our current understanding of cardiac morphology and function in athletes because of its low cost, widespread availablity and lack of ionizing radiation. LV systolic function has most often been studied by using echocardiography and expressed as fractional shortening of the LV internal dimension or ejection fraction. The meta-analyses on these conventional indices of LV systolic function were not different between athletes at rest and matched control subjects (Fagard et al. 1996, Pluim et al. 2000). cMRI studies also did not show any difference in systolic fuction between athletes and untrained individuals during resting conditions (Pluim et al., 1998; Scharf et al., 2010). However, the parameters used in human studies reflect chamber mechanics rather than intrinsic myocardial mechanics. Tissue Doppler and speckle-tracking imaging permits a more detailed and more accurate assessment of LV systolic function. Normal or even supernormal cardiac systolic and diastolic function was found in investigations using these methods, which can be used for the differential diagnosis of athlete's heart from pathological conditions (D'Andrea et al., 2006; Richand et al., 2007, Simzek et al., 2013). The evaluation of contractility in humans is complicated and all of these above mentioned systolic parameters are influenced by cardiac loading conditions and heart rate. Whereas the harmless nature of physiological hypertrophy does not allow to perform invasive studies that can provide the most accurate and reliable, load-independent characterization of LV function.

LV diastolic function is commonly assessed by studying the pattern of ventricular filling through the mitral valve by Doppler echocardiography (Nishimura et al., 1989).

The ratio of the transmitral Doppler peak flow velocity during atrial contraction (A) to the peak flow velocity during rapid LV filling (E) was found to be normal or slightly enhanced in all type of sports (Fagard, 1997; D'Ascenzi et al., 2011). In some studies, however, the A wave was proportionately lower than the E wave. According to Pavlik et al. observations in young ages show no difference in early-to-late diastolic filling rate (E/A) ratio, while in older age E/A is definitely higher in athletic than in non-athletic subjects (Pavlik et al., 2010). Thus, it seems that regular physical training attenuates the age-associated impairment of diastolic function. These results should be interpreted with some caution, because E/A ratio is not only related to LV compliance but can be also influenced by other factors such as heart rate and loading conditions (Harrison et al., 1991). It is possible that the higher E/A is only a consequence of the training induced bradycardia and not an independent training effect. In general, studies of diastolic filling have confirmed that the structural remodelling seen as part of athlete's heart is not associated with impairment of diastolic filling as observed in other kinds of LV hypertrophy and further examination is needed to observe load-independent parameters.

Right ventricular myocardial function has been described by echocardiography in athletes compared to healthy controls and patients with hypertrophic cardiomyopathy (HCM), and in these investigations improved systolic function (higher RV systolic velocities as well as strain values) was observed along with unaltered fractional area change (FAC, calculated as the ratio of area change in one cardiac cycle and end-diastolic area) in resting conditions (D'Andrea et al., 2003; D'Andrea et al., 2009). Cardiac magnetic resonance imaging (cMRI) has excellent spatial resolution and the image quality is not influenced by body habitus. These features permit the very precise assessment of cardiac chamber size, particulary for the irregularly-shaped right ventricle (RV), where it is considered as gold standard. Studies using cMRI showed a balanced hypertrophy and unaltered ejection fraction in the right ventricle (Scharhag et al., 2002).

2.1.3. Molecular pathways related to physiological hypertrophy

There has been great interest to reveal molecular mechanisms leading to enhanced function in physiological hypertrophy. All of these studies are in agreement with the fact that the insulin-like growth factor 1 (IGF-1)- phosphatidylinositol-3-kinase (PI3K)-

protein kinase B (Akt) pathway plays a key role in mediating physiological cardiac growth (Ellison et al., 2012). It is well recognised that IGF-1 is released during development and in response to exercise training (Neri Serneri et al., 2001). IGF-1 produced also within the myocardium acts via the IGF-1 receptor (IGF-1R), a transmembrane receptor tyrosine kinase. IGF-1R transgenic mice displayed a greater hypertrophy with enhanced systolic function (McMullen et al., 2004), whereas cardiac selective knockout of IGF-1R showed a resistant phenotype against exercise-induced cardiac changes (Kim et al., 2008). Activation of IGF-1R leads to activation of PI3K(p110α), a lipid kinase composed of separate regulatory and catalytic subunits that phosphorylates lipids to form phosphatidyl-inositol 3,4,5-trisphosphate (PIP_3), which acts as a second messenger to cause downstream signaling events, such as phosphorylation and activation of Akt. Transgenic and knockout mice studies have confirmed that PI3K(p110α) plays also a key part in exercise-induced hypertrophy and is also important for protecting the heart against pathological insults (McMullen et al., 2003; Lin et al., 2010). Akt, a serine/threonine kinase is a well characterized target of PI3K activity and it is well recognized that Akt1 is the predominant Akt isoform in the myocardium (Fujio et al., 2000). Akt1 is required for physiological heart growth as proved by an investigation revealing that Akt1 knockout mice showed a blunted hypertrophic response to exercise training (DeBosch et al., 2006). In the heart, the IGF1-PI3K pathway induces physiological hypertrophy with preserved or enhanced cardiac function, thus this pathway is important for maintaining cardiac function and its antifibrotic and anti-apoptotic effects can provide protection for the heart against pathological insults (McMullen, 2008). The gp130-JAK-STAT pathway and thyroid hormone receptor signaling contribute to the development of cardiac hypertrophy and postnatal heart growth, respectively, and have been associated with the IGF1-PI3K-Akt physiological hypertrophy pathway, although the exact mechanisms are still unclear (Bernardo et al., 2010). The specific activation of these pathways could be a potential target for the treatment of cardiovascular diseases, such as heart failure.

2.1.4. Other characteristic changes of exercise-induced cardiac hypertrophy

In addition to structural and functional remodeling, it is recognised that there are electrical alterations in the athlete’s heart in response to training, resulting in distinct

changes in the ECG. Autonomous regulation of the physically trained subjects is characterized by an enhanced parasympathetic and a decreased sympathetic activity resulting in bradycardia during resting conditions. Resting bradycardia is the most common and characteristic electrical feature of the athlete's heart: with this alteration the duration of diastole is elongated in athletic heart, while duration of systole remains relatively unaltered (Pavlik et al., 1999). This is more advantageous as coronary circulation occurs only in diastole, thus there will be a better blood supply of the myocardium between two cardiac contractions. Exercise-induced remodeling can result in other, well-described electrical alterations of the myocardium. It is really important for clinicians to be able to identify those changes, which are resulted from intense physical training and do not carry an increased risk of adverse cardiac outcomes (Pellicia et al., 2000). The European Society of Cardiology recently published guidelines for interpretation of the 12 lead ECG in the athlete. It provides classification of ECG abnormalities, which are considered as commonly related to training (such as sinus bradycardia, first-degree atriventricular block, incomplete right bundle branch block, signs of early repolarisation and isolated QRS voltage criteria for left ventricular hypertrophy) and those which are uncommon and should prompt examination for underlying cardiac pathology (Corrado et al. 2010).

Beside elongation of the diastolic phase as a consequence of bradycardia, an increased capillary density also contributes to the better blood supply of the myocardium and this improvement seems to be associated with training in younger ages (Tomanek, 1994). Athlete's heart has also been characterized with a more effective metabolism. Positron emission tomography demonstrated, that trained athletes are able to perform the similar physical performance with lower myocardial perfusion, thus the heart needs less oxygen to provide the same performance (Laaksonen et al., 2007). The lower rates of glycolysis along with balanced enhancement in glucose and fatty acid oxidation suggests a protective phenotype (Burcelle et al., 2004).

2.1.5. Cardiac arrhytmogenic remodeling, sudden cardiac death

Despite the evident benefits of alterations in the heart of an athlete, numerous observational studies have raised concerns that high-level exercise training may be associated with increased risk of cardiac arrhythmia and even primary cardiac arrest.

Estimated incidence of sudden cardiac death (SCD) in young athletes varies in a wide range, from 1:25,000 to 1:300,000, mainly because of differences in the population studied (D'Silva and Sharma, 2014). The highest volume study, a long-term registry of italian athletes indicated 1.9 deaths/100,000 person yearly, with a 2.4-fold risk compared to untrained controls (Corrado et al., 2006). In athletes aged above 35 years atheromatous coronary artery is obviously the leading cause of SCD. In contrast, in young athletes suffered from SCD, hypertrophic cardiomyopathy, congenital anomalies in coronary arteries and arrhythmogenic right ventricular cardiomyopathy were found as most common underlying causes. Only about 2 percent of young athletes who die suddenly have normal cardiac structure at autopsy, and no definitive cause of death can be established (Maron, 2003). As most SCDs in young athletes occur during or immediately after exercise, it is hypothesized that exercise-driven mechanisms, such as dehydration, hyperpyrexia or electrolyte imbalances can trigger the operation of underlying arrhythmogenic substrate (Corrado et al., 2006). Several clinical studies suggested that long-term high level exercise might be associated with an increased risk of ventricular tachyarrhytmias, which have been shown to originate from the RV (Biffi et al., 2002; Heidbuchel et al., 2003). It is also observed that long-term endurance training may promote atrial enlargement and increased risk of atrial fibrillation in large epidemiological studies (Molina et al., 2008; Aizer et al., 2009). Confirming these concerns, an animal model of long-term intensive exercise-induced hypertrophy by Benito et al. showed an increase in ventricular arrhythmia vulnerability and atrial fibrillation incidence in exercised rats, while an increased interstitial collagen deposition was observed in the right ventricle and left atrium with a reversible character (Benito et al., 2011). Further investigations are needed to evaluate the relevance of these findings about cardiac arrhytmogenic remodeling in athletes.

2.1.6. Differences between physiological and pathological cardiac hypertrophy

In response to an increased load, the heart must work harder than under normal conditions. To counterbalance the chronic increase in wall stress, cardiomyocytes enlarge leading to an increase in size and mass, which is called cardiac hypertrophy (Zak et al., 1979; Cooper, 1987). Pathological and physiological cardiac hypertrophy are caused by different stimuli, and are associated with distinct structural, functional and

molecular phenotypes (McMullen and Jennings, 2007). Both types of hypertrophy can be subclassified as concentric and eccentric based on changes in shape that are dependent on the initiating stimulus. This type of structural classification of athlete's heart has been already discussed above. A pathological stimulus causing presure overload (e.g. hypertension, aortic stenosis) produces an increase in systolic wall stress that results in concentric hypertrophy, while volume overload of the heart (e.g. mitral regurgitation, arteriovenosus fistulas) leads to an increase in diastolic wall stress resulting in eccentric hypertrophy (Grossman et al., 1975).

The balanced increase of myocardial mass in hypertrophy following long-term exercise training results in preserved or enhanced cardiac function with increased cardiac output under stress condition (Ellison et al., 2012). In contrast, pathological hypertrophy due to pressure or volume overload is initially a compensatory mechanism, which may eventually decompensate, leading to left ventricle dilatation and heart failure with an increased risk of SCD (Zak et al., 1979; Opie et al., 2006).

Animal studies have demonstrated that physiological and pathological cardiac hypertrophy have distinct structural and molecular bases in the myocardium (Iemitsu et al., 2001; McMullen et al., 2003). In contrast to physiological heart growth, which is stimulated by IGF-1 and acts on the IGF-1R-PI3K-Akt pathway, the main triggers for pathological hypertrophy are the release of endothelin-1, angiotensin II and norepinephrine. These prohypertrophic hormones act by binding to G_q protein-coupled receptors and act on a fundamentally distinct pathway, where the main downstream effectors are calcineurin, protein kinase C and mitogen-activated protein kinases (MAPK) (Weeks and McMullen, 2011). In response to pathological stimuli, after loss of cardiomyocytes because of apoptosis and necrosis, extracellular matrix proteins accumulate disproportionately and this fibrotic replacement excessively leads to mechanical stiffness. In contrast, a normal network of collagen fibres surrounding cardiac myocytes could be observed in exercise-induced hypertrophy without apoptosis. Models of pathological cardiac hypertrophy are associated with upregulation of fetal genes, including atrial natriuretic factor (ANF) and β-myosin heavy chain (β-MHC), which does not occur in models of physiological hypertrophy (McMullen et al., 2003). Gene expression of proteins associated with contractile function, like α-myosin heavy chain (α-MHC) and sarcoplasmatic reticulum Ca^{2+}-ATPase 2a (SERCA2a) are down-

regulated in pathological conditions, in contrast to physiological hypertrophy (Bernardo et al., 2010).

As mentioned before, exercise-induced cardiac hypertrophy is characterized by enhanced fatty acid and glucose oxidation. In contrast, pathological hypertrophy is associated with a decreases in fatty acid oxidation and increases in glucose metabolism (Christe and Rodgers, 1994). In case of progression to heart failure glucose metabolism decreases reducing the overall ability of the heart to generate sufficient energy (Neubauer, 2007).

Reversibility is a distinct feature between physiological and pathological hypertrophy. The most actual question for physicians in the field of cardiology is the differentiation of physiological LV hypertrophy from HCM, the leading cause of exercise related SCD in young athletes (Maron, 2003). The observed morphological alterations can overlap between the heart of highly trained athletes and structurally mild HCM (Basavarajaiah et al., 2008). Thus, the differentiation between the two entities may be challenging for even the most experienced cardiologist. Systematic evalutation containing a detailed physical and family history, 12-lead ECG and echocardiography and additional investigation with exercise testing, cMRI as well as screening for casual genetic mutations may be necessary for clear differentation (Rawlins et al., 2009). In doubtful cases the reversible nature of the observed hypertrophy after discontinuation of training can provide the definitive diagnosis.

2.1.7. Animal models of athlete's heart

Because of research ethics and technical difficulties in humans, exercise models using animals are requisited for the future development of exercise physiology. The broader goals of animal models are to deepen our knowledge in exercise physiology and to uncover the molecular and cellular mechanisms (Seo et al., 2014). The use of animals in laboratory studies has been recommended by many researchers because of their homogeneity (genetically, physiologically) compared to human subjects and the tight control of exercise duration. Despite the number of advantages in animal exercise models, some limitations are also present. The tissue response to mechanical stresses and therapeutic treament may not be same to humans because of differences in the size and the movement patterns of the musculoskeletal system (Hasenfuss, 1998).

Animal exercise models have been developed to simulate the physical activities of humans. Indeed, there are no golden standard exercise modes or protocols for modeling athlete's heart since in addition to exercise mode the intensity, the timing and the duration are the critical determinants of physiological responses and various final outcomes and also enviromental factors (temperature), sex, age and strain affect the outcome (Wang et al., 2008). Additionally, a large amount of variation in animal studies was observed even between the same exercise modalities, because of the different apparatuses used with various training protocols. Thus animal exercise protocols should be well-designed to achieve research goals.

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 (Wang et al., 2008).

Treadmill running is a widely used model because its relative simplicity and effectiveness. Moreover, both exercise intensity and duration are clearly controllable and several animals can be trained simultaneously. It can be performed either in a continuous manner with a fixed or progressively increased speed, or in an interval running mode. The disadvantages of treadmill running are the possibility of causing stress or limb injury and the need for expensive treadmill apparatus (Wang et al., 2008).

Forced exercise may be recognized as a type of stress, voluntary exercise is the most effective intervention in lessening this stress response. When compared to other types of exercise, spontaneous, voluntary wheel running has the strong advantage of voluntariness, that the exercise can be accomplished with minimal intervention by the investigator, thus it is comfortable for investigating long-term effects of increased physical activity. However, this type of exercise is not suitable for studies that require precise timing for training to achieve certain cardiac hypertrophy, since the investigator cannot easily regulate the duration or intensity of exercise sessions (Seo et al., 2014).

Regular swimming training appears to be effective for inducing relevant cardiac hypertrophy. Swimming exercise involves an inexpensive device as compared to treadmill running and the duration and load of exercise can be controlled to a greater extent than with voluntary wheel running. Rodents have the innate ability to swim, the animals already possess self-motivation, thus swimming exercise can be perfomed after

a short period of familiarization. Moreover, a relatively large number of animals can be exercised simultaneously and there is a negligible chance an animal to suffer injuries. Unlike with the treadmill or wheel running, sedentary animals should be placed in the water to exclude the effect of the water itself (Wang et al., 2010; Seo et al., 2014).

2.2. Acute exhaustive exercise-induced cardiac changes

Ultraendurance athletes routinely complete repeated bouts of prolonged, strenuous exercise. Thus, it was not surprising that the first scientific papers concerning the cardiovascular consequences of prolonged exercise were published in this extreme group of athletes (Saltin and Stenberg, 1964). This kind of intensive physical activity such as ironman triathlon, cross country biking or ultramarathon and 24-h running races are becoming more popular. Despite the increased prevalence, these extremely prolonged exercise efforts tend to be limited to the exceptionally well trained and prepared individuals.

Similar changes have been noted after marathon races, where non-professional, ”amateur” runners can participate, hence even greater scientific and media interest was observed (Neilan et al., 2006c; Fortescue et al., 2007). Articles in the media such as „Runners who don’t train well can have marathon miseries” (The Boston Globe, April 17, 2006) and ‘‘Ironman athletes put hearts at risk of fatal damage, experts warn’’ (The Times, January 22, 2007) have been published about an awkward paradox for both the scientist and endurance athlete. The number of participants in marathon-races have strongly increased for the last 20 years, resulting in more than 500,000 runners finished a marathon in 2012 (Guasch and Nattel, 2013). To date, numerous research groups have been investigated the short- and long-term consequences of exhaustive exercise regarding cardiovascular health.

2.2.1. Myocardial biomarkers of cardiac damage

The first reports of post-exercise elevations in serum concentrations of myocardial band isoform of creatine kinase (CK-MB) after the completion of endurance events led to the recognition that such activities could result in cardiac injury (Siegel et al., 1981). Elevations in serum CK-MB after prolonged exercise is not enough specific for the detection of myocardial damage, as CK-MB levels could be increased in the skeletal muscle of distant runners (Apple et al., 1984).

Cardiac troponins, originated from myocardial troponin complex, as a part of myocardial sarcomeric unit, are highly specific and sensitive markers of myocardial insult and are considered as the gold standard for biochemical detection of myocardial cell damage (Alpert et al., 2000). Numerous reports on animals (Chen et al., 2000. Nie

et al., 2010) and humans (Koller et al., 1999; Rifai et al., 1999; Shave et al., 2007; Nie et al., 2008; Scharhag et al. 2008) reported cardiac troponin (cTn) elevation in serum after prolonged exercise that can transiently exceed clinical cut-off value for myocardial infarction. Troponin levels from participants in marathon-distance (Mousavi et al., 2009), ultra-distance running races (Shave et al., 2002), triathlons (Tulloh et al., 2006) and dedicated cycling events (Neumayr et al., 2005) have each been studied and according to a recent meta-analysis detectable troponin values occurred in approximately one-half of participants (Shave et al., 2007). Interestingly, incidence of cTn detection was growing as duration shortened; specifically, there was a higher occurrence of post-exercise cTn elevation in marathon-type events in contrast to ultra-marathon competitions. This inverse relationship between event duration and troponin elevation might be because shorter races are generally performed at higher exercise intensities. For unclear reasons the prevalence and absolute serum concentrations of the cTn increases vary considerably. Possible explanations include differences in the fitness levels of participants, the type or duration of exercise and the timing of the postexercise sample. Although numerous studies have reported troponin-release after exercise, there is no consensus regarding the aetiology, mechanisms and clinical relevance of exercise-induced troponin release. Increased membrane permeability and/or myocardial cell necrosis propagated by increased coronary artery shear stress, myocardial stretch, oxidative stress and ischaemia have been proposed as possible explanations (Shave et al., 2010).

Increased myocardial wall stress caused by volume or pressure overload elevate blood concentration of brain natriuretic peptide (BNP) and its cleaved inactive fragment NT-proBNP, due to myocyte stretch. As a marker of cardiac dysfunction, BNP and NT-proBNP can be a helpful tool in cardiovascular diagnostics and risk stratification. As a counter-regulatory hormone, BNP reduces myocardial wall stress by an increase in natriuresis, vasodilation and sympathoinhibitory effects as an opponent of the renin-angiotensin system (Panagopoulou et al., 2013). Physical exercise can acutely elevate serum or plasma BNP and NT-proBNP concentrations (Scharhag et al., 2008). After prolonged and strenuous endurance exercise, BNP and NT-proBNP levels were increased both in healthy elite and nonelite athletes above the upper reference limit (Neilan et al., 2006a; Ohba et al., 2001). The degree of BNP and NT-proBNP elevation

has been related to endurance exercise duration (Scharhag et al., 2005), analogous to the time-dependent increase in BNP expression in stretched cardiomyocytes in vitro (Wiese et al., 2000). A feasible explanation for the exercise associated increase in BNP and NT-proBNP can be derived from the physiological significance of the active hormone BNP, which reduces preload and afterload to diminish myocardial wall stress.

It is important to note that exercise-associated elevations of cTn and NT-proBNP typically decrease significantly within 24 h after exercise and usually reach normal values within this period (Herrmann et al., 2003). Although biomarker elevation can be detected also in hihgly trained elite athletes, a reverse relation between exercise-associated elevations and prerace endurance training could be observed (Neilan et al. 2006a).

The appearance of such biomarkers of myocardial damage in healthy subjects participating in ultraendurance races associated with prolonged, strenuous exercise has raised concerns about the cardiovascular consequences of such exercise and the theory that extreme exercise can induce harmful processes in the heart was widespread reported (La Gerche and Prior, 2007; Dangardt, 2013). The clinical consequences of elevations in biomarkers following an acute bout of exercise are dubious because the alterations reported are quite small and transitory (George et al., 2008). Therefore the impact of long-term prolonged, strenuous exercise should receive more attention. We should also mention that methodological variation as well as differences in exercise mode, duration, training status, age and gender in studies that have examined the possibility of postexercise myocardial injury, making it difficulty to study the factors involved or the mechanisms responsible for postexercise cardiac troponin and NT-proBNP elevation (Shave et al., 2007).

2.2.2. Myocardial dysfunction after prolonged exercise

The concept that prolonged exercise can lead to a depression in left ventricular function was first presented by Saltin and Stenberg in the mid-1900's (Saltin and Stenberg, 1964). Since then there has been enormous interest to observe prolonged strenuous exercise-induced functional changes and these alterations have been called as "exercise-induced cardiac fatigue" (Douglas et al., 1987). Over 50 studies have been published so far about this phenomenon, but the findings have often been inconsistent

because of differences in research design, subject heterogenity and mode of assessment of cardiac function (Oxborough 2010).

Despite the known limitations in the two-dimensional (2D) assessment of global left ventricular systolic function, numerous studies have utilized this modality before and after an acute bout of exercise. Short duration exercise seems to have insignificant impact on LV function and as a consequence of increased preload and sympathetic activity, ejection fraction have been shown to be either unchanged or improved (Neilan et al., 2006b). The impact of prolonged exercise on LV systolic function is considerably controversial with no alteration in EF after ultra-long duration intensive physical activity (Hassan et al., 2006), while other studies reported clearly reduced EF after brief prolonged exercise (Vanoverchelde et al., 1991). This disparity could be a consequence of the differences in training status, exercise intensity and duration and the small sample size involved in one study. Recently several substantial meta-analyses have been reported for unified conclusions (Middleton et al., 2006; Oxborough et al., 2010). An overall reduction in the EF immediately following prolonged endurance exercise was observed, suggesting a transient impairment of LV systolic function, which returns to baseline following a 24-48 h recovery period (Whyte et al., 2000). The subgroup analyses showed that exercise-induced cardiac fatique is dependent on the duration of exertion in elite athletes as the systolic impairment was observed only in the ultralong duration (i.e. ironman and ultraendurance events, 640-1440 min) group, but not in moderate (i.e. half marathon races, 60-150 min) or long duration (i.e. marathon races, 166-430 min) group. (Whyte et al., 2000). However, the mean alteration of EF was significantly related to the decrease in the LV diastolic diameter, suggesting that postrace reduction of preload influence this parameter. As EF is a load-independent systolic parameter, more evidence is needed to prove systolic LV dysfunction after prolonged exercise. It is important to note that an untrained subgroup was defined in the moderate duration group. These data suggest that exercise-induced cardiac fatigue could concern professional athletes in ultra-endurance races and untrained individuals participating in moderate or long duration competitions (Whyte et al., 2000).

Tissue Doppler imaging (TDI)-derived systolic velocities appears to be unchanged after marathon race (George et al., 2006). Myocardial strain may be a more representative parameter of contraction and relaxation. TDI derived strain and strain rate

showed a reduction in both LV and RV systolic and diastolic parameters after completion of a marathon race (Neilan et al., 2006c). Speckle-tracking echocardiography (STE) offers a more unique and reproducible assessment of myocardial contractility with less dependency on loading factors (Marwick, 2006). The few works using STE demonstrated individual heterogenity between myocardial segments and plane, thus no consistent consequence could be concluded (La Gerche et al., 2008; George et al., 2009; Scott et al., 2009).

Regarding LV diastolic function, E/A has been a widely used index to observe diastolic changes after races. An immediate postexercise E/A ratio reduction was observed due to a drop in E and rise in A waves, reflecting altered diastolic filling dynamics. This impairment was independent of exercise duration and did not correlated with changes in loading conditions. The few available data suggest that E/A ratio returns to baseline following a 24-h recovery period (Whyte et al., 2000; Shave et al., 2004), suggesting minimal clinical impact of this phenomenon. In accordance, color flow Doppler investigation observed a decrease in postexercise early diastolic flow propagation velocity, thus a decreased E value (Middleton et al., 2006). Findings from tissue velocities during diastole are consistent in demonstrating a reduced early diastolic LV myocardial velocity (E') and E'/A' ratio (George et al., 2005). These findings were complemented by pulmonary venous Doppler measurements which demonstrated a reduction in atrial filling during diastole. Therefore, the interpretation of these results is complex: impaired E/A ratio could reflect a true impairment in myocardial relaxation or a reduction in ventricular filling secondary to decreased preload (Oxborough et al., 2010). More recently STE after marathon races revealed that strain indices of diastolic function from radial, circumferential and longitudinal planes were significantly reduced, suggesting a global reduction of LV diastolic function (Dawson et al., 2008). Load-independent indices of active relaxation would improve our knowledge about exercise-induced cardiac fatigue.

Alterations in LV function after an acute bout of prolonged exercise are normally transient, with resumption of normal function typically observed after 24-48 h of recovery (Shave 2004).

The assessment of right ventricular volumes with 2D echocardiography, is extremely complicated because of the geometry, location and the trabeculation of RV.

Therefore FAC was used to measure RV systolic function after strenuous exercise, however the results were controversial. Studies also demonstrated increased RV FAC (Douglas et. al, 1990), as well as reduced RV FAC (La Gerche et al., 2008) after prolonged exercise. La Gerche et al. also observed a reduction in RV systolic tissue velocity after a triathlon which complemented their findings from 2D echocardiography. MRI is maybe the most suitable noninvasive investigation for examining RV. function. Mousavi et al. validated RV systolic and diastolic dysfuncion detected by echocardiography by cMRI in a small group after completion of a marathon race (Mousavi et al, 2009). They reported decreased RV ejection fraction, which was likely due to exercise-induced pulmonary hypertension (thus increased RV afterload), which normalized after one week. Surprisingly, RV diastolic dysfunction had not completely normalized after one week follow-up. A more recent cMRI study showed transient RV dilation and dysfunction after intense exercise (La Gerche et al., 2012). The observed fibrosis in RV is in line with the hypothesis that repetitive insults eventually lead to RV dilatation and chronic dysfunction, providing a substrate for ventricular arrhythmogenesis (La Gerche et al, 2008). This is in line with the observation that athletes diagnosed with ventricular arrhythmia had RV abnormalities which served as an arrhytmogenic focus (Heidbuchel et al., 2003; Ector et al., 2007). Marked RV dysfunction and dilation with subsequent fibrosis as a consequence of repeated long-term exercise sessions could play a key role in the origination of complex ventricular arrhythmias, thus in causing sudden cardiac death of athletes.

A number of investigators have coupled biochemical and functional testing after endurance events. These data suggest that there is a correlation between cardiac biomarkers and post-race diastolic dysfunction (Neilan et al., 2006c) as well as with RV dysfunction (Mousavi et al. 2009).

2.2.3. Acute exercise and oxidative stress

Oxidative stress is a condition, in which the delicate balance between production of pro-oxidant free radicals and their subsequent amelioration via the antioxidant defense system becomes skewed in favor of free radical generation. Production or formation of free radicals in vivo is primarily initiated by the consumption of molecular oxygen (Halliwell and Cross, 1994). Reactive oxygen species (ROS) are oxygen-based chemical species with high reactivity. They include free radicals, such as superoxide ($O_2^{\cdot-}$) and hydroxyl radical ($^{\cdot}OH$) and nonradicals capable of generating free radicals, such as hydrogen peroxide (H_2O_2). The antioxidant defense system of the body serves to protect the cells from excess ROS production and is composed of endogenous (superoxide dismutases, catalase, glutathione peroxidase) and exogenous (ascorbate, bioflavinoids, carotenoids) antioxidants. Oxidative stress is defined as an excess production of ROS relative to the levels of antioxidants.

Exercise-induced oxidative stress was recognized long time ago (Davies et al., 1982). Because of difficulties in measuring free radical production directly, most human studies have used indirect markers to demonstrate exercise-induced oxidative stress. Most often used markers of lipid peroxidation (e.g. thiobarbituric acid reactive substance (TBARS) levels, oxidative modifications to DNA (8-oxo-7,8-dihydroxy-2'-deoxyguanosine), protein oxidation (plasma protein carbonyls), antioxidant markers of the glutathione system (ratio of reduced and oxidized glutathione) as well as plasma levels of antioxidants and antioxidant enzymes reflect increased oxidative stress after prolonged exercise (Vollaard et al., 2005). Even moderate exercise may increase ROS production exceeding the capacity of antioxidant defence (Alessio, 1993). Exhaustive exercise-induced nitro-oxidative stress was demonstrated by increased nitrotyrosine levels in serum and urine after ultra-endurance race (Radák et al., 2003). Regular endurance exercise training causes adaptation, such as elevated antioxidant enzyme activity, to reduce systemic oxidative stress following an acute bout of exhaustive exercise (Radak et al., 2001; Miyazaki et al., 2001).

Although xanthine oxidase and NADPH-oxidase enzyme systems in cardiomyocytes, as well as infiltrating neutrophil granulocytes can generate free radicals in the myocardium, the primary source of ROS is the electron transport chain of mitochondria (Ji, 1999). Cardiac muscle has a high oxygen uptake even at resting

conditions. During heavy physical exercise oxygen uptake from the blood by the heart is markedly increased. During maximal exercise, whole-body oxygen consumption increases up to 20-fold, for which the myocardium and the skeletal muscle are responsible. High oxygen uptake and utilization in cardiac mitochondria to provide sufficient energy during exercise may lead to increased formation of free radicals, especially superoxide anions. Thus, this increased oxygen metabolism can lead to increased oxidative stress in the myocardium during physical exercise (Frankiewicz-Jozko et al., 1996). Furthermore, superoxide anions can interact with nitric oxide (NO) spontaneously to form toxic peroxynitrite ($ONOO^-$) (Pacher et al., 2007). NO is necessary for normal cardiac physiology in the regulation of cardiac function including coronary vasodilatation and modulation of cardiac contractile function (Takimoto and Kass, 2007). Therefore superoxide anions effect not only directly but also by inactivation of cytoprotective NO and formation of the reactive oxidant peroxynitrite.

Regarding oxidative status exercise has double-edge effects on myocardium. On the one hand, it results in increased formation of free radicals, on the other hand it may also induce antioxidant enzymes to minimize the effects of oxidative stress due to exercise (Gul et al., 2006). Heart is equipped with all the major antioxidant enzymes such as superoxide dismutases, catalase as well as glutathione peroxidase and reductase. It has been well demonstrated that acute bouts of exercise activates Nrf2, a primary transcriptional regulator of major antioxidants, which results in alterations of the transcription of antioxidant genes such as catalase, glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxydase-1 (GPX1), glutathione reductase (Muthusamy et al, 2012). This repeated exposure can lead to a favorable adaptation, an improved myocadial antioxidant defense system in trained heart, which can reduce potential damage from future acute bouts of exercise. This theory is underpinned by studies that shows reduced myocardial oxidative stress after preconditioning by exercise training (Gul et al., 2006; Okudan et al., 2012)

At physiological circumstances, ROS can act as second messengers in several cellular functions, because stimulation of DNA-synthesis and induction of growth-related genes are associated with free radicals (Grieve et al., 2004). This role of ROS appears to be essential for normal cell proliferation and growth.

Numerous experimental studies have demonstrated marked oxidative stress directly and indirectly in the myocardium after exhaustive exercise (Davies et al., 1982; Gul et al., 2006; Nie et al., 2010; Okudan et al., 2012). Under pathophysiological conditions, markedly elevated levels of ROS can be harmful to all cellular macromolecules, such as lipids, proteins and DNA and can lead to irreversible cell damage and death, which have been implicated in a wide range of pathological cardiovascular conditions such as coronary atherosclerosis and heart failure (Halliwell and Gutteridge, 1984). Specifically, in the myocardium excess ROS can cause remodeling, including contractile dysfunction by modifying proteins central to excitation-contraction coupling and structural alterations (Takimoto and Kass, 2007). Moreover, ROS activate a broad variety of hypertrophy signaling kinases and transcription factors and mediate apoptosis (Tsutsui et al., 2011). A recently published experimental study showed exhaustive-exercise induced apoptosis in LV myocardium, though the explanation of data is difficult because of the small sample size investigated and marked variability (Huang et al., 2009). ROS can stimulate cardiac fibroblast proliferation and activate matrix metalloproteinases (MMPs), playing a central role in physiological and pathological tissue remodeling processes. Activation of the MMP system [increased expression of MMPs or downregulation of their endogenous inhibitors, the tissue inhibitor of metalloproteinases (TIMPs)] might influence the structural properties of the myocardium by increased matrix turnover (Kandasamy et al., 2010). Although induction of apoptosis and activation of MMP system in skeletal muscle after exhaustive exercise are well documented (Koskinen et al., 2001; Phaneuf and Leeuwenburgh, 2001), limited information is available about these processes in the myocardium.

2.2.4. Animal models of acute exhaustive exercise induced cardiac injury

Animal experiments provide a much more controlled and integrated opportunity to investigate effects of exhaustive exercise, as well as the feasibility of directly measuring of a variety of oxidative stress biomarkers in biological tissues, such as myocardium. Briefly, treadmill and swimming acute exercise protocols are widespread to. Treadmill exhaustion protocols often use inclination and high final speed for exhaustion. In these protocols, exhaustion was defined as the animal is unable to upright itself when placed on its back (Gul et al., 2006; Lin et al., 2006; Huang et al., 2009).

There are two approaches of acute swimming exercise used by experimental researchers. The first perspective is to force rats to swim until exhaustion, which was determined by the inability of the rat to remain at the surface of water (Okudan et al., 2012; Zheng et al., 2012). The marked variability of swimming time until exhaustion makes the interpretation of data difficult. According to the other view an equal exertion imposed on animals is more suitable to investigate exhaustive exercise-induced alterations. Animals unable to complete the protocol should be removed from the investigation. To provide certain and effective exhaustion, these swimming protocols use workload attached to the animal (Chen et al., 2000; Nie et al., 2010).

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3. 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 LV functional aspects of athlete's heart 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 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.

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4. Methods

4.1. Animals, experimental groups

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

Young adult male Wistar rats (n=102,; m=275-375 g; from Charles River, Sulzfeld, Germany and Toxi-Coop, Dunakeszi, Hungary) were housed in a room with constant termperature of 22±2 ^{o}C with a 12/12 h light-dark cycle and fed a standard laboratory rat diet ad libitum and free access to water.

The detailed description of our research projects and experimental groups are summarized in Table 1.

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Table 1. Research projects and experimental groups

Project	Animals	Experimental groups	Protocol	Anesthesia	Measurements
1. Hemodynamic characterization of athlete's heart	Young adult (m=275-300 g) male Wistar rats (Charles River, Sulzfeld, Germany)	Exercised group (n=9) Control group (n=11)	12 week-long swim training	pentobarbital (60 mg/kg ip.) for echocardiography ketamine (100 mg/kg ip.) and xylazine (3 mg/kg ip.) for pressure-volume analysis	echocardiography pressure-volume analysis histology (HE, MT) gene expression analysis (markers of pathological hypertrophy) stress biomarkers
2. Testing the reversible nature of exercise training-induced LV hypertrophy	Young adult (m=275-300 g) male Wistar rats (Charles River)	Detrained exercised group (n=6) Detrained control group (n=6)	12 week-long swim training + 8 week-long resting period	pentobarbital (60 mg/kg ip.)	echocardiography histology (HE)
3. Investigation of the correlation between strain values measured by speckle-tracking echocardiography and contractility parameters derived from pressure-volume analysis	Young adult (m=275-300 g) male Wistar rats (Toxi-Coop, Dunakeszi, Hungary)	Exercised group (n=10) Control group (n=12)	12 week-long swim training	pentobarbital (60 mg/kg ip.)	echocardiography pressure-volume analysis
4. Investigation of acute exhaustive exercise-induced changes	Young adult (m=325-375 g) male Wistar rats (Toxi-Coop)	Acute exercised group (n=12) Control group (n=12)	3 hours swimming (5% workload) + 2 hours resting period	ketamine (100 mg/kg ip.) and xylazine (3 mg/kg ip.)	cardionecrotic biomarkers histology (HE, NT, DHE, TUNEL) gene expression analysis (oxidative stress, apoptosis, ECM turnover)
		Acute exercised group (n=10) Control group (n=10)		pentobarbital (60 mg/kg ip.)	pressure-volume analysis

HE: hematoxylin-eosin; MT: Masson's trichrome; NT: nitrotyrosine; DHE: dihydroethidium; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; ECM: extracellular matrix

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4.2. Animal models, exercise protocols

4.2.1. Rat model of athlete's heart

We designed a swimming apparatus (Fig. 1.) specially planned for exercise training of rats. A 150-l water tank was divided into 6 lanes with a surface area of 20x25 cm and a depth of 45 cm per lane and filled with tap water maintained at 30-32 ^{o}C to allow individual swim training. The dimensions of the lanes were selected to avoid floating of the rats by reclining to the walls. Based on literature data (Evangelista et al., 2003) 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 by 15 min every second training session until the maximal swim duration (200 min) was reached (Fig. 2.). Untrained control rats were placed into the water for 5 min each day during the 12-week training program to eliminate the possible impact of stress related to contacting the water. For testing the reversibility of swim training-induced LV hypertrophy, detrained rats remained sedentery for 8 weeks after completing the above described 12 week-long training program.

Figure 1. Our swimming apparatus designed for swim training and exhaustive exercise

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Figure 2. Training plan

Following gradual acclimatization (Day 0-35), exercised rats swam 200 min, five times a week. After completion of the training program, echocardiographic examination and pressure-volume (P-V) analysis were performed.

4.2.2. Rat model of acute exhaustive exercise-induced cardiac injury

We used our previosly described swimming apparatus (Fig. 1.) filled with tap water maintained at 30-32 ^{o}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, as previously described (Nie et al., 2010). Animals unable to complete the exercise protocol (n=4) were rescued from the water and excluded from the investigation. 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, the duration of which was chosen according to previous literature data (Nie et al., 2010). In order to eliminate diurnal effects, the experiments were performed at the same time of the day.

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4.3. Echocardiography

4.3.1. Standard echocardiographic measurements

Rats were anesthetized with pentobarbital (60 mg/kg ip.). Animals were placed on controlled heating pads, and the core temperature, measured via rectal probe, was maintained at 37 ^{o}C. After shaving the anterior chest, transthoracic echocardiography was performed in the supine position by one investigator blinded to the experimental groups (Fig. 3). Two dimensional and M-mode echocardiographic images of long and short (mid-papillary muscle level) axis were recorded, using a 13 MHz linear transducer (GE 12L-RS, GE Healthcare, USA), connected to an echocardiographic imaging unit (Vivid i, GE Healthcare). Digital images were analyzed by a blinded investigator using an image analysis software (EchoPac, GE Healthcare). On two dimensional recordings of the short-axis at the mid-papillary 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-sysolic 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 (Reffelmann and Kloner, 2003). Fractional shortening (FS) was calculated as ((LVEDD-LVESD)/LVEDD)x100. End-diastolic (LVEDV) and end-sysolic left ventricular volumes (LVESV) were estimated according to two different validated geometrical models as previously described: the single-plane ellipsoid model and the biplane ellipsoid model (van de Weijer et al., 2012). Stroke volume (SV) was calculated as LVEDV-LVESV. Ejection fraction (EF) was determined as (SV/LVEDV)x100. LV mass was calculated according to a cubic formula, suggested by Devereux et al.: LVmass=(((LVEDD+AWTd+PWTd)^3-LVEDD^3) x 1.04) x 0.8+0.14 (Devereux et al., 1986). To calculate LV mass index and SV index (SVI), we normalized the LV mass and SV values to the body weight of the animal.

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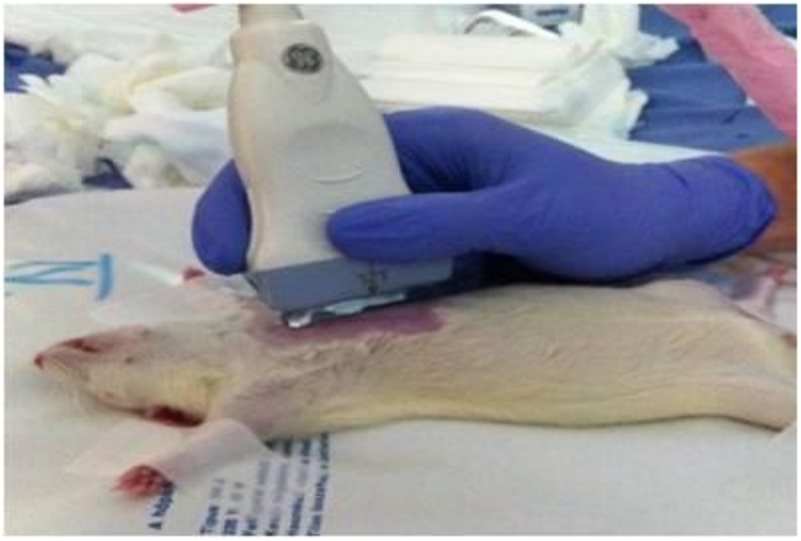

Figure 3. Echocardiographic examination using a 13 MHz linear transducer

4.3.2. Speckle-tracking echocardiography

Strain is a dimensionless measure of relative deformation which enables to characterize different directions of myocardial function both on global and regional levels. The novel method of STE allows to quantify strain and its temporal derivative, strain rate resulting in promising new parameters of systolic and diastolic function (Blessberger et Binder, 2010). 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. Speckle-tracking analysis was done by a blinded operator with remarkable expertise on the software environment (EchoPAC v113). 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. In case of low tracking quality, the tracing was manually corrected and analyzed again by the software. Acceptance or rejection of a certain segment to be included in statistical analysis was guided by the software's recommendation. Ideally, for each parameter (3x3x6=) 54 segmental values were averaged. Animals with less than 36 segmental values (due to technically suboptimal tracking quality despite the aforementioned efforts) were excluded from the study.

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Based on these criteria, 8 trained and 12 control rats were eligible to be included in the statistical analysis.

4.4. 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, measured via rectal probe, was maintained at 37 ^{o}C. A median laparotomy was performed to secure access to the inferior caval vein. A polyethylene catheter was inserted into the left external jugular vein for fluid administration. A 2-Fr microtip pressure-conductance catheter (SPR-838, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the ascending aorta. After stabilization for 5 min, mean arterial blood pressure (MAP) and heart rate (HR) were recorded. After that, the catheter was advanced into the LV under pressure control. After stabilization for 5 min, signals were continuously recorded at a sampling rate of 1,000 samples/s using a P-V conductance system (MPVS-Ultra, Millar Instruments, Houston, TX, USA) connected to the PowerLab 16/30 data acquisition system (AD Instruments, Colorado Springs, CO, USA), stored and displayed on a personal computer by the LabChart5 Software System (AD Instruments).

After positioning the catheter we registered baseline P-V loops (Fig. 4.). With the use of a special P-V analysis program (PVAN, Millar Instruments), LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), the maximal slope of LV systolic pressure increment (dP/dt_{max}) and diastolic pressure decrement (dP/dt_{min}), time constant of LV pressure decay [τ; according to the Weiss method and Glantz method (Pacher et al., 2008)], ejection fraction (EF) stroke work (SW) and pressure-volume area (PVA) and LV maximal power were computed and calculated. Stroke volume (SV) and cardiac output (CO) were calculated and corrected according to in vitro and in vivo volume calibrations using PVAN software. Total peripheral resistance (TPR) was calculated by the following equation: TPR=MAP/CO. To exclude the influence of body weight differences, CO was normalized to body weight cardiac index (CI).

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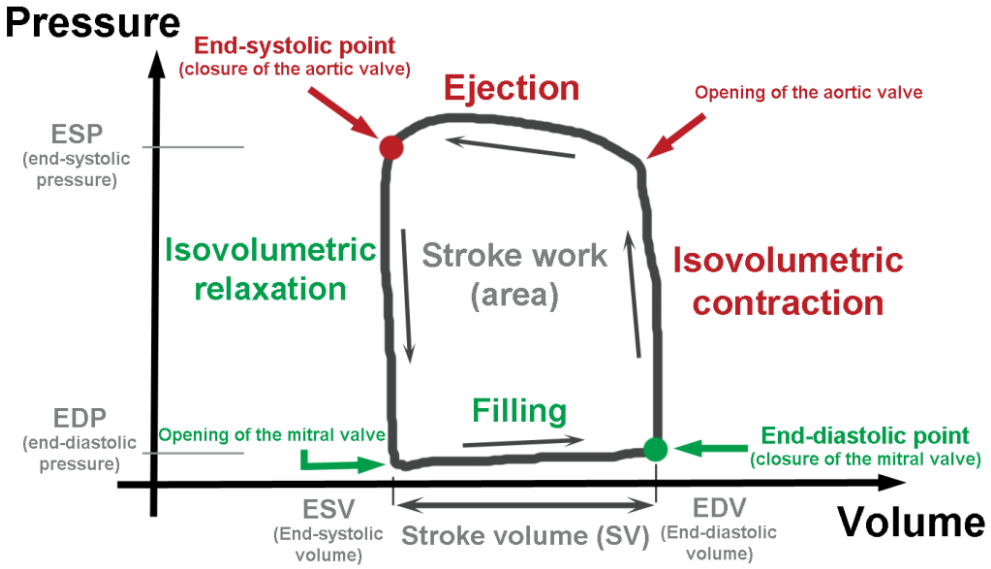

Figure 4. Schematic figure of a baseline pressure-volume loop recorded during one cardiac cycle by left ventricular pressure-volume analysis.

In addition to the above parameters, P-V loops recorded at different preloads can be used to derive other useful systolic function indexes that are less influenced by loading conditions and cardiac mass (Kass, 1995; Pacher et al., 2008). Therefore, LV P-V relations were measured by transiently compressing the inferior vena cava (reducing preload) under the diaphragm with a cotton-tipped applicator (Fig. 5.). The slope of the LV end-systolic P-V relationship [ESPVR; according to the parabolic curvilinear model (Kass et al., 1989)], 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 indexes of LV contractility. The slope of the LV end-diastolic P-V relationship (EDPVR) was calculated as a reliable index of LV stiffness (Kass, 1995).

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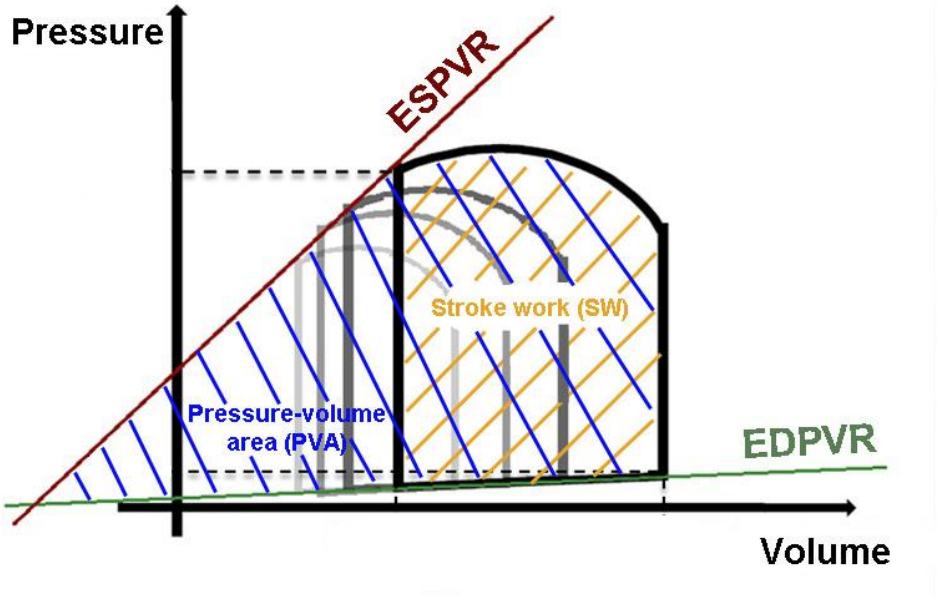

Figure 5. Schematic figure of a pressure-volume (P-V) loops recorded during transient occlusion of inferior vena cava

The fading gray loops represent the P-V relations while reducing preload. The line connecting the end-systolic points of the loops defines the end-systolic P-V relationship (ESPVR) and the slope value of this line is a sensitive contractility index. The slope of end-diastolic P-V relationship (EDPVR) is an indicator of LV stiffness. Mechanical efficiency is referred as an important mechanoenergetic parameter, and can be calculated as the ratio of stroke work (SW; the area encircled by the baseline P-V loop) and the P-V area (PVA; the area within the ESPVR line, the EDPVR line and the baseline P-V loop).

Arterial elastance (E_a) was calculated as LVESP/SV. LV end-systolic elastance (E_{es}) is defined as the slope of ESPVR. Ventriculo-arterial coupling (VAC) was described by the quotient of E_a and E_{es}. According to Sunagawa and associates mechanical efficiency (Eff) was calculated as the ratio of SW and PVA. (Fig. 5., Sunagawa et al., 1983).

At the end of each experiment, 100 µl of hypertonic saline were injected intravenously, and from the shift of P-V relations, parallel conductance volume was calculated by the software and used for the correction of the cardiac mass volume. The volume calibration of the conductance system was performed as previously described (Kass, 1995). Briefly, nine cylindrical holes in a block 1 cm deep and with known

diameters ranging from 2 to 11 mm were filled with fresh heparinized whole rat blood and the conductance values were measured. In this calibration, the linear volume-conductance regression of the absolute volume in each cylinder versus the raw signal acquired by the conductance catheter was used as the volume calibration formula.

4.5. Blood and tissue sample collection

In anesthethized animals, blood samples were collected from the inferior caval vein in tubes pre-rinsed with EDTA. The blood samples were centrifuged at 3000 g for 15 min at 4 ^{o}C and prepared blood plasma samples were aliquoted and stored at -80 ^{o}C.

To remove erythrocytes from myocardial tissue an in vivo perfusion was performed. After opening the thoracic cavity and dissecting the inferior caval vein in the thorax, a total volume of 40 ml oxygenated Ringer solution (37 ^{o}C) was infused into the LV through the apex of the heart with a speed of 8 ml/min. The effluent perfusate was removed from the thorax by gauze bandages. All animals were euthanized by exsanguination.

Thereafter the heart was quickly removed and placed into cold (4 ^{o}C) Ringer solution. Heart weight was measured and LV myocardial tissue samples were collected immediately for histology (for fixation in 4 % buffered paraformaldehyde solution or snap-frozen in liquid nitrogen) and molecular biology (snap frozen in liquid nitrogen).

4.6. Biochemical measurements

4.6.1. Stress biomarkers

Plasma adrenocorticotropic hormone (ACTH) and cortisol levels were determined by electrochemiluminescence immunoassay (ECLIA) using commercial kits (Elecsys ACTH Kit and Elecsys Cortisol Test, Roche Diagnostics, Mannheim, Germany).

4.6.2. Cardionecrotic biomarkers

Plasma creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and creatinine (for assessing renal function) were measured by automated clinical laboratory assays on a Cobas Integra 400 (Roche Diagnostics, Mannheim, Germany) autoanalyzer. Plasma cardiac troponin T (cTnT) was assessed by

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a human immunoassay (Elecsys Troponin T STAT, Roche Diagnostics) that was validated and approved for reliable application in rats (O'Brien et al., 1998).

4.7. Histology

4.7.1. Hematoxylin-eosin (HE) staining

In trained rats (model of athlete's heart), hearts were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 ^{o}C.

In acute exercised animals tissue samples were fixed in buffered paraformaldehyde solution (4 %) and embedded in paraffin.

Transverse transmural slices of the ventricles were sectioned (5 μm) and processed conventionally for histological examination. The sections were stained with hematoxylin and eosin (HE). Light microscopic examination was performed with a Zeiss microscope (Axio Observer.Z1, Carl Zeiss, Jena, Germany) and digital images were captured using an imaging software (QCapture Pro 6.0, QImaging, Canada) with a magnification of 400x.

In trained rats transverse transnuclear widths of randomly selected, longitudinally oriented cardiomyocytes were measured by a single investigator after calibrating the system. The mean value of 100 LV cardiomyocytes represents each sample.

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

4.7.2. Masson's trichrome (MT) staining

Hearts were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 ^{o}C. Transverse transmural slices of the ventricles were sectioned (5 μm) and processed conventionally for histological examination. The sections were stained with Masson's trichrome. The amount of myocardial collagen was determined by semiquantitative morphometry scoring of Masson's trichrome-stained sections by one blinded observer as follows: (0) absent, (1) slight, (2) moderate and (3) intense. The mean value of twenty randomly selected visual fields (magnification 400x) of free LV wall represents each sample.

4.7.3. Dihydroethidium (DHE) staining

Hearts were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 ^{o}C. In situ detection of ROS was performed by using the oxidative fluorescent dye dihydroethidium (DHE; Sigma-Aldrich, St. Louis, MO, USA). DHE is freely permeable to cell membranes and emits a red fluorescent signal when oxidized by ROS to ethidium, which is intercalating into DNA (typically nuclear localization). Fresh frozen LV myocardial sections (16 μm) were incubated with 1μM DHE (in PBS; pH 7.4) at 37 ^{o}C for 30 min in a dark humidified chamber (Csont et al., 2007). Fluorescence in myocardial sections was visualized using a fluorescence microscope (Axio Observer.Z1, Carl Zeiss, Jena, Germany) 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 (NIH, Bethesda, MD, USA) image analysis software.

4.7.4. Nitrotyrosine (NT) staining

To demonstrate nitro-oxidative stress, tyrosine nitration was detected in LV myocardial sections by immunohistochemistry (Masszi et al., 2013). Heart tissue samples were fixed in buffered paraformaldehyde solution (4 %) and embedded in paraffin. Paraffin-embedded sections of myocardium were deparaffinized. After antigen retrieval (0.1 M citrate buffer, pH 3.0, heating in microwave oven for 15 min) and quenching endogenous peroxidase with 0.3 % H_2O_2 in 100 % methanol for 15 min, slides were immunostained with a rabbit anti-nitrotyrosine antibody (1:200, Millipore, Bedford, MA, USA) overnight at 4 ^{o}C. Specific labeling was detected by incubation for 30min at room temperature with a biotin-conjugated anti-rabbit goat antibody (Vector Laboratories, Burlingame, CA, USA) and amplified with an avidin-biotin peroxidase complex (Vector Laboratories). Nickel-cobalt enhanced diaminobenzidine (Vector Laboratories) was used as chromogen. Five images of LV wall at a magnification of 200x were taken randomly from each section. Nitrotyrosine positive area was calculated using conventional microscopy and the ImageJ software. After background subtraction, eye controlled auto-threshold have been determined to detect NT positive areas. The fractional area (NT positive area to total area ratio) was determined.

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

Hearts were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 ^{o}C. Apoptosis in cardiomyocytes was determined with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique. TUNEL staining was performed using DeadEnd™ Colorimetric TUNEL System (Promega, Madison, WI, USA) according to the manufacturers instruction. Sections were counterstained by the red colored Nuclear Fast Red (Sigma-Aldrich, St. Louis, MO, USA). 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.

4.8. Cardiac mRNA analysis

LV myocardial tissue samples were harvested immediately after sacrifice, snap-frozen in liquid nitrogen and stored at -80 ^{o}C. LV tissue was homogenized in a lysis buffer (RLT buffer, Qiagen, Hilden, Germany), RNA was isolated from the ventricular samples using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions and quantified by measuring optical density (OD) at 260 nm. RNA purity was ensured by obtaining a 260/280 nm OD ratio approximately 2.0. Reverse transcription reaction (1 μg total RNA of each sample) was completed using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed with the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA) in triplicates of each sample in a volume of 10 μl in each well containing cDNA (1 μl), TaqMan® Universal PCR MasterMix (5 μl) and a TaqMan® Gene Expression Assay for the following targets (0.5 μl): β-isoform of myosin heavy chain (β-MHC, assay ID: Rn00568328_m1), transforming growth factor β (TGF-β, assay ID: Rn00572010_m1), catalase (assay ID: Rn00560930_m1), glucose-6-phosphate dehydrogenase (G6PD; assay ID: Rn00566576_m1), gluthathion peroxidase 1 (GPX-1, Rn00577994_g1), glutathione reductase (GSR, assay ID: Rn01482159_m1), thioredoxin-1 (assay ID: Rn00587437_m1), superoxide dismutase 2 (SOD-2, assay ID: Rn00690587_g1), endothelial nitric oxide synthase (eNOS, assay ID: Rn02132634_s1), Bcl-2 associated X protein (Bax, assay ID: Rn02532082_g1), B-cell lymphoma 2 (Bcl-

2, assay ID: Rn99999125_m1), matrix metalloproteinase-2 (MMP-2, assay ID: Rn01538170_m1), tissue inhibitor of metalloproteinase-2 (TIMP-2, assay ID: Rn00573232_m1), matrix metalloproteinase-9 (MMP-9, assay ID: Rn00579162_m1), tissue inhibitor of metalloproteinase-1 (TIMP-1, assay ID: Rn00587558_m1), all purchased from Applied Biosystems. Gene expression data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; reference gene; assay ID: Rn01775763_g1) and expression levels were calculated using the C_T comparative method ($2^{-\Delta CT}$). All results are expressed as values normalized to a positive calibrator (a pool of cDNA's from all samples of the control group).

4.9. Statistical analysis

Statistical analysis was performed on a personal computer with a commercially available software (Origin 7G; OriginLab, Northampton, MA, USA). Normal distribution of variables was confirmed by Shapiro-Wilk test. All data are expressed as mean ± SEM. An unpaired two sided Student t-test was used to compare parameters between exercised and untrained control rats as well as between detrained exercised and detrained control rats. Relationships between P-V analysis derived contractility parameters and strain values by STE were calculated with Pearson correlation test. An unpaired two-sided Student's t-test was used to compare parameters of acute exercised and control rats after confirming the normal distribution of data. Differences were considered statistically significant when $p<0.05$.

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5. Results

5.1. Athlete's heart

5.1.1. Body weight and heart weight

Exercise training was associated with decreased body weight gain of rats during the study period (+129±8 g exercised vs. +206±17 g control, p=0.0349), which resulted in decreased body weight of exercised rats after completion of training protocol compared with control animals. Heart weight measured immediately after the hemodynamic measurements was markedly increased in exercised animals. The heart weight to body weight ratio showed an even more significant increase in the exercised group reflecting cardiac hypertrophy (Table 2.).

Table 2. Body and heart weight data in control and exercised rats after completion of the exercise training period

	Control (n=11)	Exercised (n=9)	p
Body weight (BW), g	490±14	409±7*	0.0005
Heart weight (HW), g	1.41±0.03	1.73±0.08*	0.0005
HW/BW, %	0.29±0.01	0.42±0.02*	<0.0001

Values are means ± SEM. *p<0.05 vs. controls.

5.1.2. Histology

Exercise training resulted in a significant increase in mean cardiomyocyte width compared with untrained control rats, indicating cardiac hypertrophy in exercised rats (Fig. 6.). The semiquantitative evaluation of Masson's trichrome staining indicated unaltered cardiac collagen content in the exercised group. Figure 6. shows representative photomicrographs of LV myocardium in exercised and control rats.

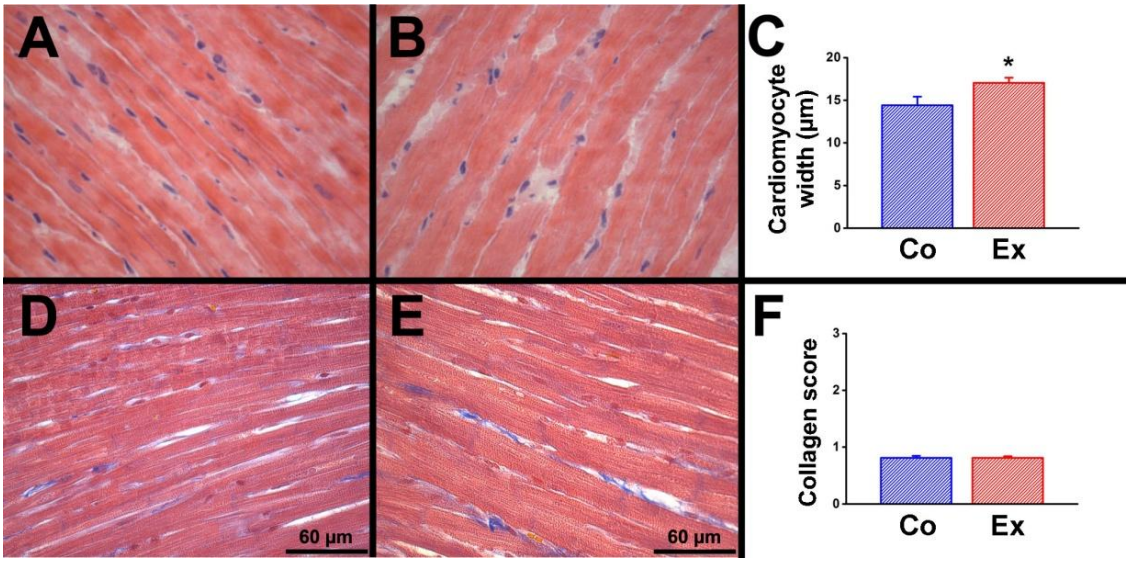

Figure 6. Histological evaluation of exercise-induced hypertrophy

Representative photomicrographs of left ventricular (LV) myocardium after hematoxylin-eosin (A and B) as well as Masson's trichrome staining (D and E). Longitudinally oriented cardiomyocytes are displayed for control (Co, A) and exercised (Ex, B) animals. An increase of LV cardiomyocyte width was observed in exercised rats compared with sedentary controls (C). Masson's trichrome stained myocardium showed only slight blue collagen staining both in the control (D) and exercised (E) groups and semiquantitative score values indicated no difference between the groups (F). Magnification: 40x, scale bar 60 µm. *p<0.05 vs. Co.

5.1.3. Markers of stress and pathological hypertrophy

Plasma ACTH and cortisol concentrations did not differ between the exercised and control rats (Table 3.).

Table 3. Plasma stress biomarkers

	Control (n=11)	Exercised (n=9)	p
ACTH, pg/ml	211.8±58.4	172.1±32.3	0.5470
Cortisol, nmol/l	95.6±11.4	87.4±10.1	0.6000

ACTH: adrenocorticotropic hormone. Values are means ± SEM.

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Myocardial gene expressions of β-MHC and TGF-β were unaltered in exercised rats compared with untrained controls, indicating absence of pathological hypertrophy and remodeling, respectively (Fig. 7.).

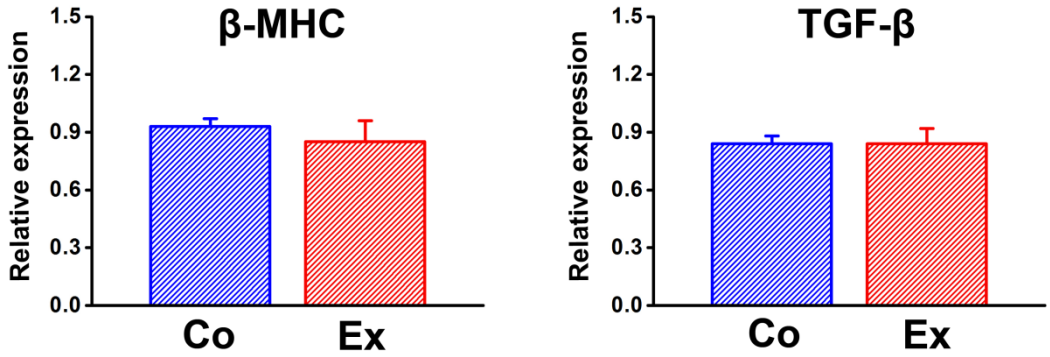

Figure 7. mRNA analysis of pathological hypertrophy markers

Gene expression of β-isoform of myosin heavy chain (β-MHC) and transforming growth factor β (TGF-β) did not differ between the control (Co) and exercised (Ex) groups.

5.1.4. Echocardiographic parameters

The morphological and functional echocardiographic results are shown in Table 4. At the end of the exercise training portocol, LV wall thickness values were significantly higher in the exercised rats compared with the control animals. Figure 8. shows representative long- and short-axis two-dimensional end-sytolic 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-plane and the biplane model, respectively, in exercised rats compared with untrained control rats. The LV mass and the LV mass index were significantly higher in exercised animals versus controls indicating robust cardiac hypertrophy. The FS was increased in exercised animals, suggesting increased systolic performance.

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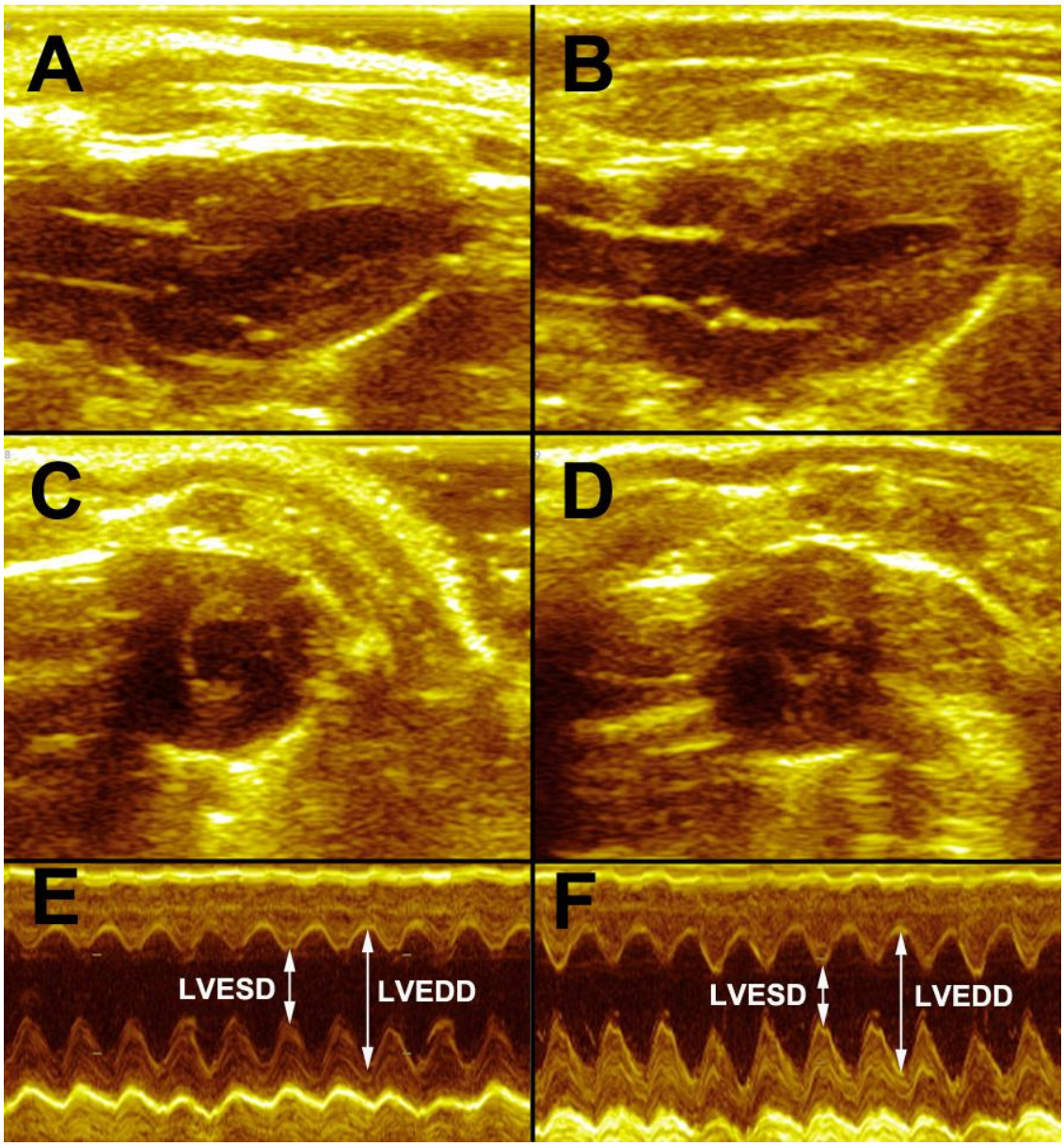

Figure 8. Representative images of echocardiographic assessment

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

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Table 4. Echocardiographic parameters in exercised and sedentary control rats

	Control (n=6)	Exercised (n=6)	p
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
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/kg BW	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/kg BW	640.9±18.3	802.6±52.4*	0.0118
EFbp, %	62.5±1.9	72.6±2.3*	0.0109

Values are means ± SEM. LVAWTd and LVAWTs, left ventricular (LV) anterior wall thickness at diastole and systole, respectively; LVPWTd and LVPWTs, LV posterior wall thickness at diastole and systole, respectively; LVIVSTd and LVIVSTs, LV interventricular septal wall thickness at diastole and systole, respectively; LVEDD, LV end-diastolic dimension; LVESD LV end-systolic dimension; FS, LV fractional shortening; BW, body weight; LVEDVsp and LVEDVbp, LV end-diastolic volume calculated using single-plane ellipsoid (sp) and biplane ellipsoid (bp), respectively; LVESV, LV end-systolic volume; SV, stroke volume; SVI, SV index; EF, ejection fraction. *$p<0.05$ vs. controls.

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5.1.5. Hemodynamic parameters

Baseline hemodynamic data. Figure 9. shows representative LV pressure relations and dP/dt curves. HR, MAP, LVESP, LVEDP and dP/dt_{min} were not different in exercised animals compared with the control group (Table 5.). The 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 10. 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 increased significantly, suggesting increased systolic performance in trained rats. CO, CI, SW and also LV maximal power were increased in swimming animals. Decreased TPR was detected in exercised rats (Table 5.).

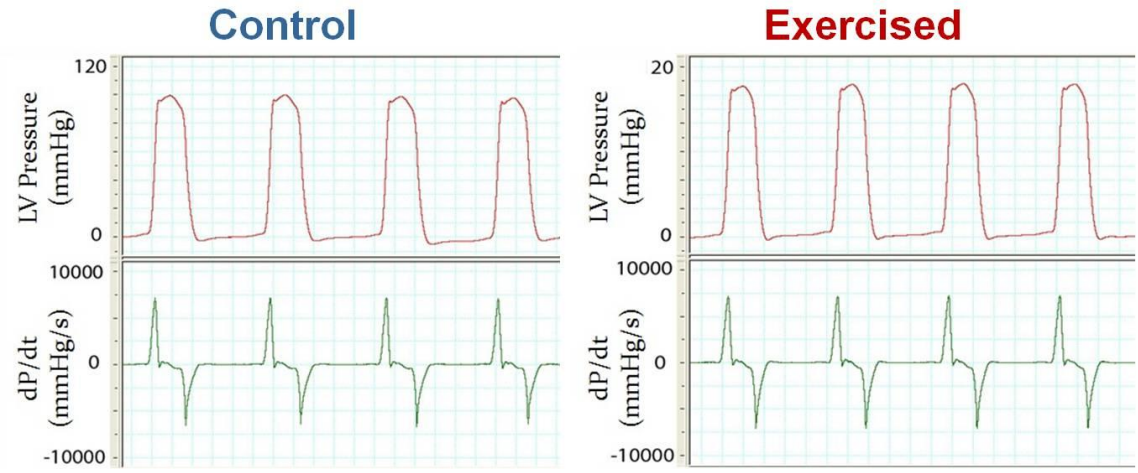

Figure 9. Representative left ventricular (LV) pressure relations and dP/dt curves
Representative LV pressure curves and dP/dt curves in one-one animal from control and exercised groups**.**

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Table 5. Hemodynamic parameters in untrained control and exercised rats

	Control (n=11)	Exercised (n=9)	p
HR, beats/min	235±7	236±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, µl	206.0±8.0	209.4±11.9	0.8099
LVESV, µl	99.5±6.7	75.4±5.0*	0.0126
SV, µl	145.4±7.7	175.4±8.5*	0.0172
CO, ml/min	32.3±1.9	44.7±3.7*	0.0053
CI, (ml/min)/kg BW	66.5±4.0	100.0±8.0*	0.0008
EF, %	64.1±1.5	73.7±0.8*	0.0001
SW, mmHg·ml	11.1±0.8	15.1±0.9*	0.0030
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
LV maximal power, mW	59.8±5.6	91.7±9.3*	0.0067

Values are means ± SEM. HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diatolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; SV, stroke volume; CO, cardiac output; CI, cardiac index; EF, ejection fraction; SW, stroke work; dP/dt_{max} and dP/dt_{min} maximal slope of the systolic pressure increment and the diastolic pressure decrement, respectively; τ, time constant of LV pressure decay; TPR, total peripheral resistance; BW, body weight. *$p<0.05$ vs. controls.

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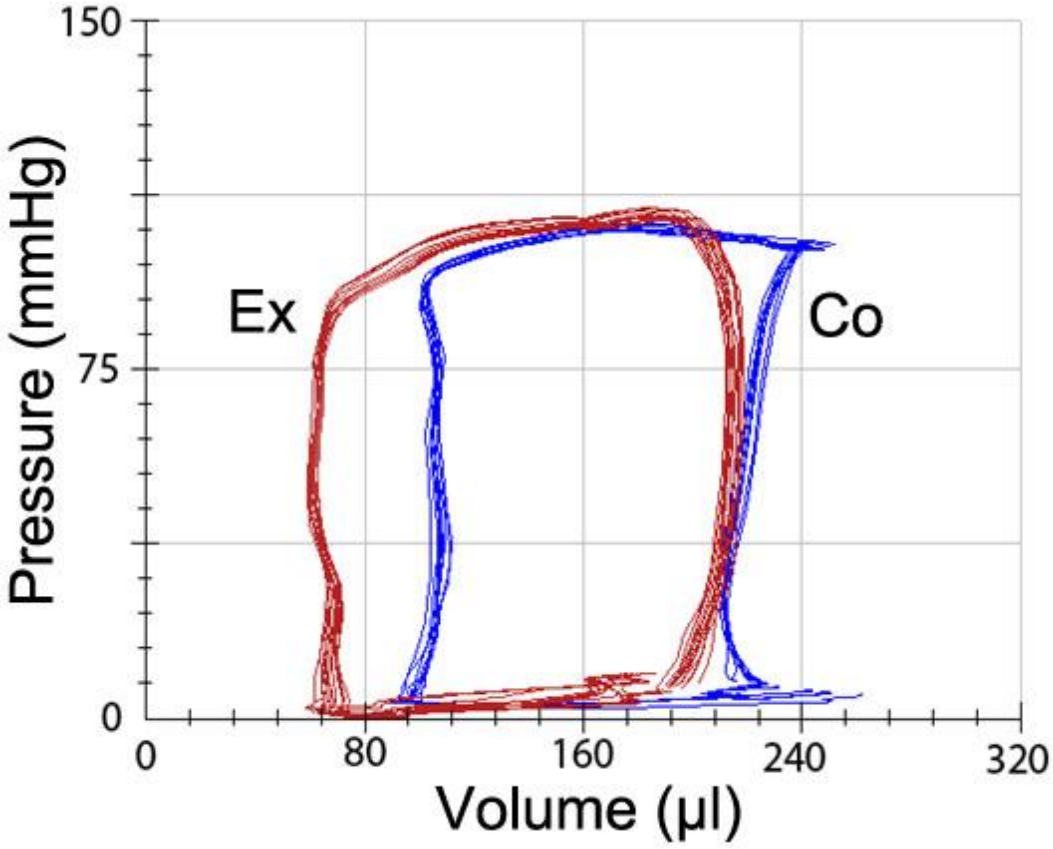

Figure 10. Steady-state left ventricular (LV) pressure-volume (P-V) loops

Original recordings of LV steady-state P-V loops obtained with the Millar P-V conductance catheter system 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.

Functional indexes derived from P-V analysis at different preloads. Figure 11. 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 in Figure 11., ESPVR was steeper in exercised than in control animals, suggesting increased contractility in trained heart. The EDPVR did not differ between the groups indicating unchanged LV stiffness in exercised rats.

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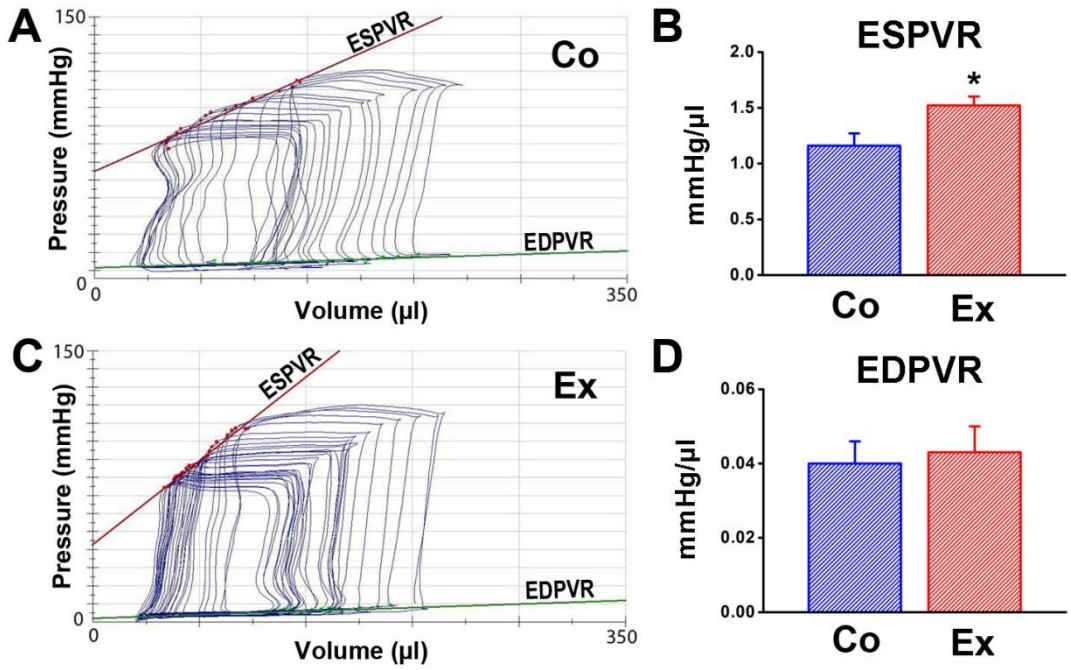

Figure 11. End-systolic pressure-volume (P-V) relationship (ESPVR) and end-diastolic P-V relationship (EDPVR)

End-systolic P-V relationship (ESPVR) and end-diastolic P-V relationship (EDPVR) in one representative animal from the exercised (A) and control (C) groups. Original recordings of representative P-V loops were obtained with a P-V conductance catheter system at different preloads during vena cava occlusion and showed increased slope of ESPVR (B) in exercised rats compared with sedentary controls. The steeper ESPVR indicated increased contractile function. The slope of EDPVR did not differ between the groups indicating unaltered diastolic stiffness (D). *$p<0.05$ vs. Co.

Figure 12.A shows PRSW (the slope of the linear relation between SW and EDV, a sensitive contractility parameter) in a representative exercised and control animal. The slope was steeper in trained rats than in control rats, indicating increased systolic performance. Compared with the corresponding control animals the overall PRSW values were significantly higher in exercised rats (Fig. 12.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. As Figure 12.C shows, the slope of this relation was steeper in trained animals than in untrained animals, indicating increased contractility in exercise-induced

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hypertrophy. The overall dP/dt_{max}-EDV slope values were significantly higher in exercised rats (Fig. 12.D).

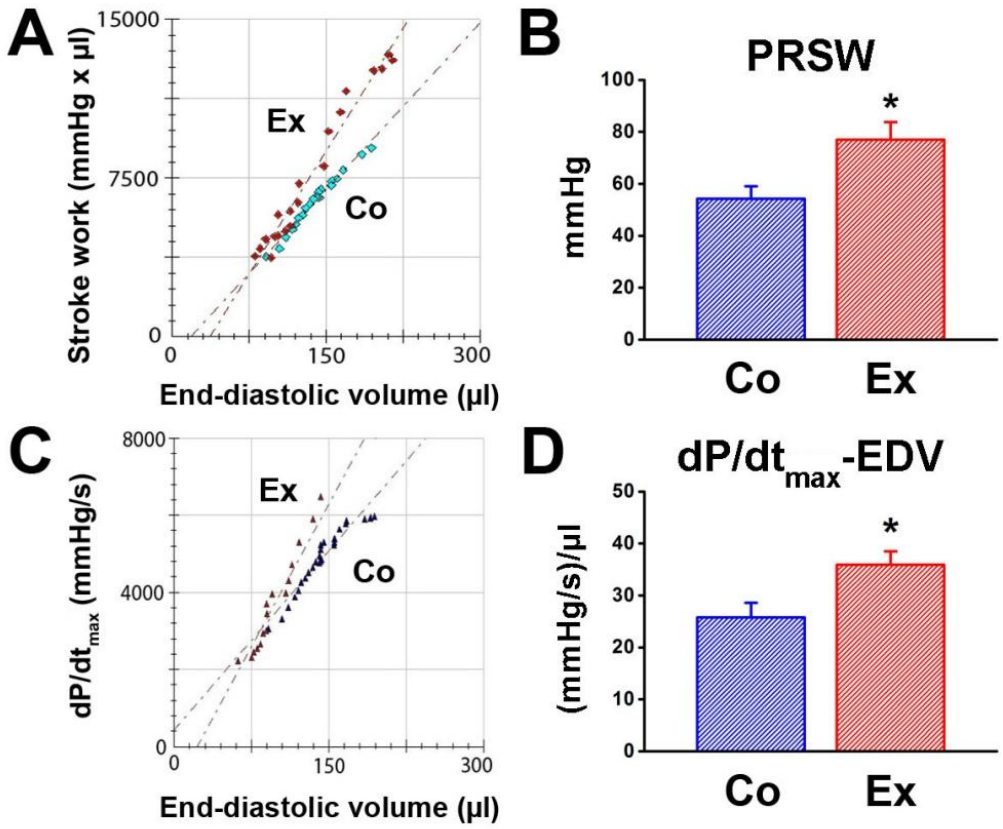

Figure 12. Pressure-volume loop derived left ventricular contractility parameters
Preload recruitable stroke work (PRSW), the slope of the relationship between stroke work (SW) and end-diastolic volume (EDV) (A); and maximal slope of the systolic pressure increment (dP/dt_{max}) - EDV relationship (C) 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 E_{es} and a decrease of E_a. Subsequently, VAC (E_a/E_{es}) was significantly decreased in trained rats suggesting improved mechanoenergetics (Fig. 13.). SW was increased in exercised rats and PVA did not differ between the groups. Eff and LV maximal power was increased in trained rats compared with control rats (Fig. 13., Table 5.).

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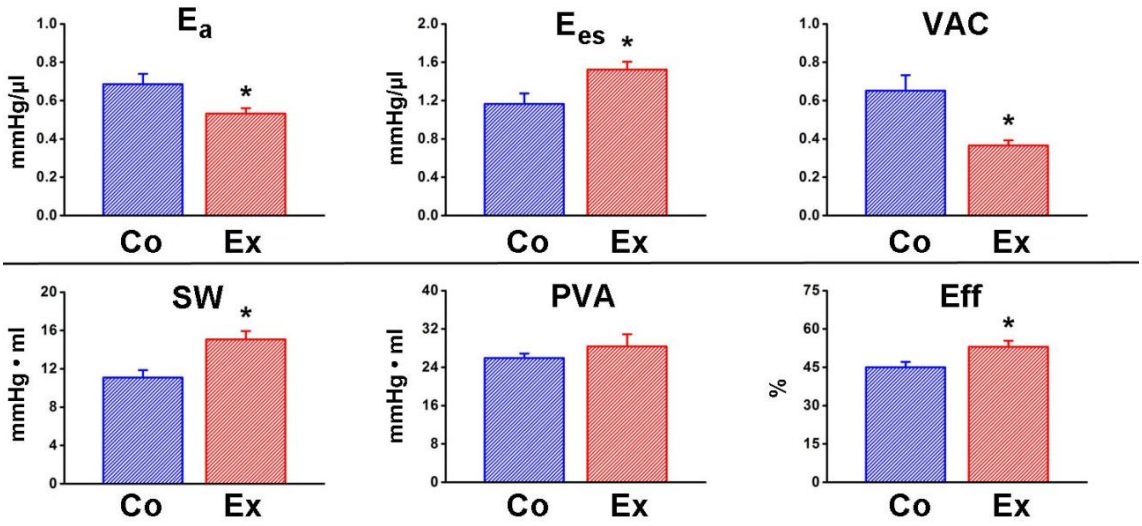

Figure 13. Cardiac mechanoenergetics after long-term exercise training

Upper panel: increased end-systolic elastance (E_{es}) and decreased arterial elastance (E_a) resulted in improved ventriculo-arterial coupling ($VAC=E_a/E_{es}$) in exercised rats compared with untrained controls. Lower panel: increased stroke work (SW) along with unaltered pressure-volume area (PVA) led to an amelioration of mechanical efficiency (Eff=SW/PVA) in trained rats. Note that all of these parameters suggest improved LV mechanoenergetics in exercise-induced cardiac hypertrophy. *$p<0.05$ vs. Co.

5.1.6. Reversibility of exercise-induced cardiac hypertrophy

As shown in Table 6., body weight and heart weight did not differ between the detrained exercised and detrained control groups. The mean cardiomyocyte width regressed to control levels after discontinuation of the training. No differences could be observed between the detrained exercised and detrained control groups regarding morphological and functional echocardiographic parameters, which suggests the complete reversibility of exercised-induced changes (Table 6.).

Table 6. Cardiac morphometric and echocardiography parameters in detrained control and detrained exercised rats

	Detrained control (n=5)	Detrained exercised (n=6)	p
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, μl/kg BW	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, μl/kg BW	546.1±16.5	543.2±39.4	0.9522
EFbp, %	56.0±0.5	59.1±1.6	0.1730

Values are means ± SEM. BW, body weight; HW, heart weight; LVAWTd and LVAWTs, left ventricular (LV) anterior wall thickness at diastole and systole, respectively; LVPWTd and LVPWTs, LV posterior wall thickness at diastole and systole, respectively; LVIVSTd and LVIVSTs, LV interventricular septal wall thickness at diastole and systole, respectively; LVEDD, LV end-diastolic dimension; LVESD LV end-systolic dimension; FS, fractional shortening; LVEDVsp and LVEDVbp, LV end-diastolic volume calculated using single-plane ellipsoid (sp) and biplane ellipsoid (bp) model, respectively; LVESV, LV end-systolic volume; SV, stroke volume; SVI, SV index; EF, ejection fraction.

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

5.2.1. 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 (Table 7.). The calculated LV mass and LV mass index values indicated LV hypertrophy after completion of exercise training protocol. Echocardiographic data were underpinned by post mortem measured heart weight data, which showed increased heart weight values. The difference was even more pronounced when heart weight to body weight ratio was calculated (Table 7.).

Table 7. Endurance exercise training induced left ventricular (LV) morphological changes and echocardiography data

	Control (n=12)	Exercised (n=10)	p
HW, g	1.34±0.03	1.48±0.03*	0.0071
HW/BW, g/kg	2.89±0.07	3.66±0.11*	<0.0001
LVAWTd, mm	2.15±0.02	2.36±0.03*	<0.0001
LVAWTs, mm	3.12±0.07	3.48±0.07*	0.0015
LVPWTd, mm	1.96±0.03	2.08±0.02*	0.0015
LVPWTs, mm	2.97±0.05	3.16±0.06*	0.0276
LVEDD, mm	6.69±0.09	6.61±0.07	0.4963
LVESD, mm	3.96±0.10	3.40±0.06*	<0.0001
FS, %	40.9±1.1	48.6±0.6*	<0.0001
LV mass, g	1.00±0.01	1.10±0.02*	<0.0001
LV mass index, g/kg BW	2.14±0.05	2.76±0.07*	<0.0001

Values are means ± SEM. HW, heart weight; BW, body weight; LVAWTd and LVAWTs, left ventricular (LV) anterior wall thickness at diastole and systole, respectively; LVPWTd and LVPWTs, LV posterior wall thickness at diastole and systole, respectively; LVEDD, LV end-diastolic dimension; LVESD LV end-systolic dimension; FS, LV fractional shortening; *p<0.05 vs. controls.

5.2.2. Baseline hemodynamics

There was no difference between the groups regarding pressure values (MAP, LVESP, LVEDP, dP/dt_{max} and dP/dt_{min}) and HR (Table 8.). LVESV was decreased in trained rats compared to control ones, along with unaltered LVEDV. Consequently our data of systolic parameters revealed an increase in SV, EF, CO and SW, while TPR has shown to be decreased in exercised animals (Table 8.).

Table 8. Baseline hemodynamic data of pressure-volume analysis

	Control (n=12)	Exercised (n=10)	p
HR, beats/min	410.2±8.2	401.8±8.4	0.4911
MAP, mmHg	144.4±2.7	141.9±5.8	0.5165
LVESP, mmHg	160.1±3.3	158.3±9.2	0.8476
LVEDP, mmHg	3.2±0.2	3.8±0.6	0.2257
dP/dt_{max}, mmHg/s	9228±360	9720±723	0.5270
dP/dt_{min}, mmHg/s	-12156±402	-12056±728	0.9012
LVEDV, µl	229.9±2.9	234.9±4.9	0.3669
LVESV, µl	111.3±1.8	100.1±1.9*	0.0005
SV, µl	118.7±3.5	135.6±4.3*	0.0073
EF, %	52.3±1.2	58.1±0.9*	0.0018
CO, ml/min	49.0±1.3	55.9±1.6*	0.0031
TPR, mmHg/(ml/min)	2.94±0.09	2.56±0.13*	0.0242
SW, mmHg·ml	14.1±0.6	17.8±0.9*	0.0027

Values are means ± SEM. HR, heart rate; MAP, mean arterial pressure; LVESP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diatolic pressure; dP/dt_{max} and dP/dt_{min} maximal slope of the systolic pressure increment and the diastolic pressure decrement, respectively; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; SV, stroke volume; EF, ejection fraction; CO, cardiac output; ; TPR, total peripheral resistance; SW, stroke work. *$p<0.05$ vs. controls.

5.2.3. Contractility indices derived from pressure-volume analysis at different preloads

Figure 14. shows representative original P-V loops recorded during reducing pre-load (transient occlusion of the inferior vena cava) in exercised and control animals. ESPVR, PRSW as well as dP/dt_{max}-EDV have been showed to be steeper in exercised animals compared to controls. The slope values of these relationships were significantly

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higher after endurance exercise training, indicating a markedly increased LV contractility (Fig. 14.).

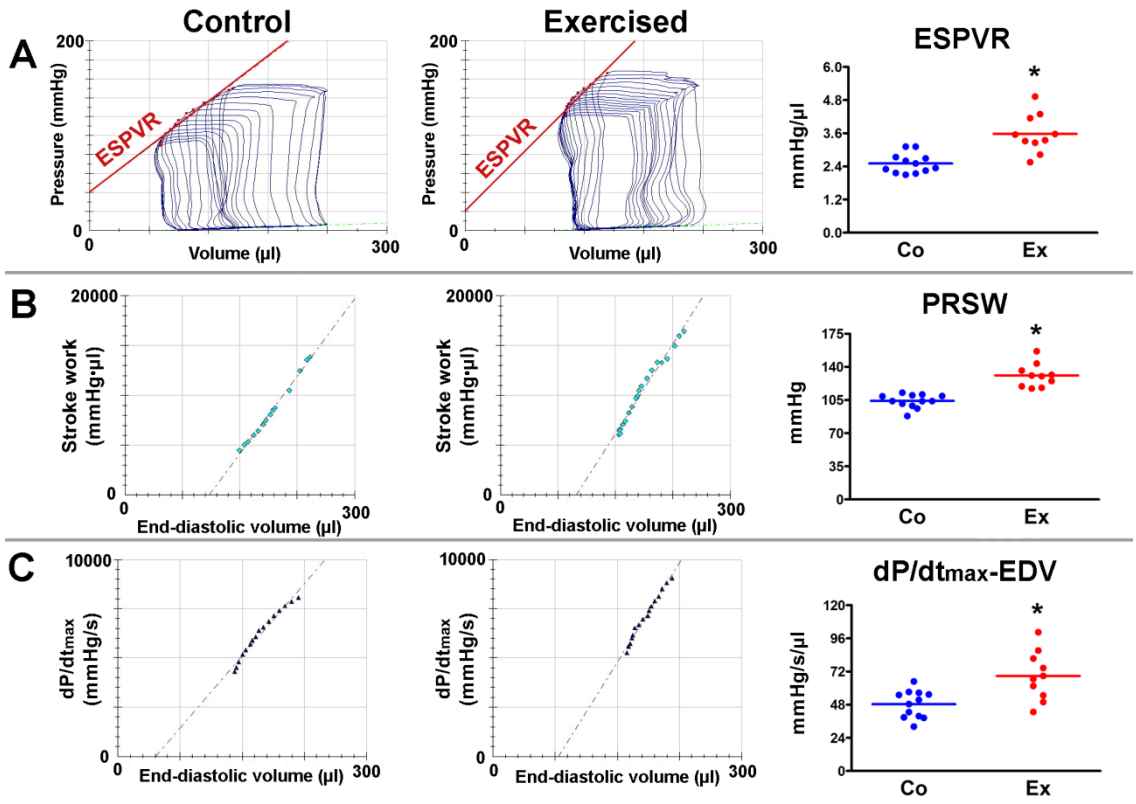

Figure 14. Contractility parameters measured by left ventricular pressure-volume (P-V) analysis.

The slope of end-systolic P-V relationship (ESPVR) (A); preload recruitable stroke work (PRSW), the slope of the relationship between stroke work and end-diastolic volume (B); and maximal slope of the systolic pressure increment - end-diastolic volume relationship (dP/dt_{max}-EDV) (C) in representative rats from control (Co) and exercised (Ex) groups. As seen on the dot plots, all of these contractility parameters are markedly increased in the Ex group, suggesting improved inotropic state in exercise induced cardiac hypertrophy. Horizontal lines represent mean values. *$p<0.05$ vs. Co.

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5.2.4. 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. 15. and 16.).

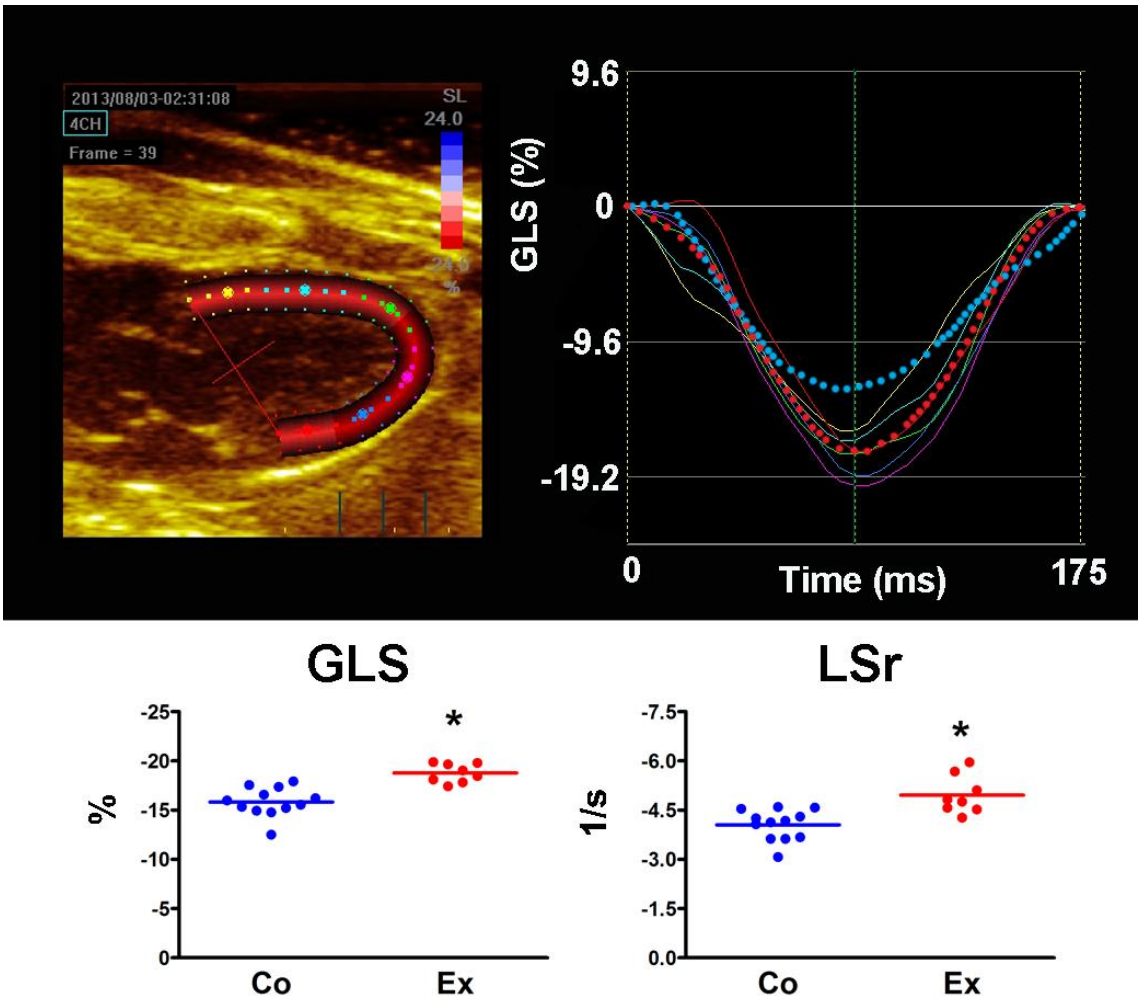

Figure 15. Speckle-tracking analysis of long-axis recordings

Representative original long-axis recordings of an exercised (Ex) 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 (Co) rat (blue dotted line). This figure illustrates the determination of the global longitudinal strain (GLS). The negative peak of the averaged curve represents the global longitudinal strain. As depicted by the dot plots, both GLS and longitudinal starin rate (LSr) were increased in exercised rats. Horizontal lines represent mean values. *p<0.05 vs. Co.

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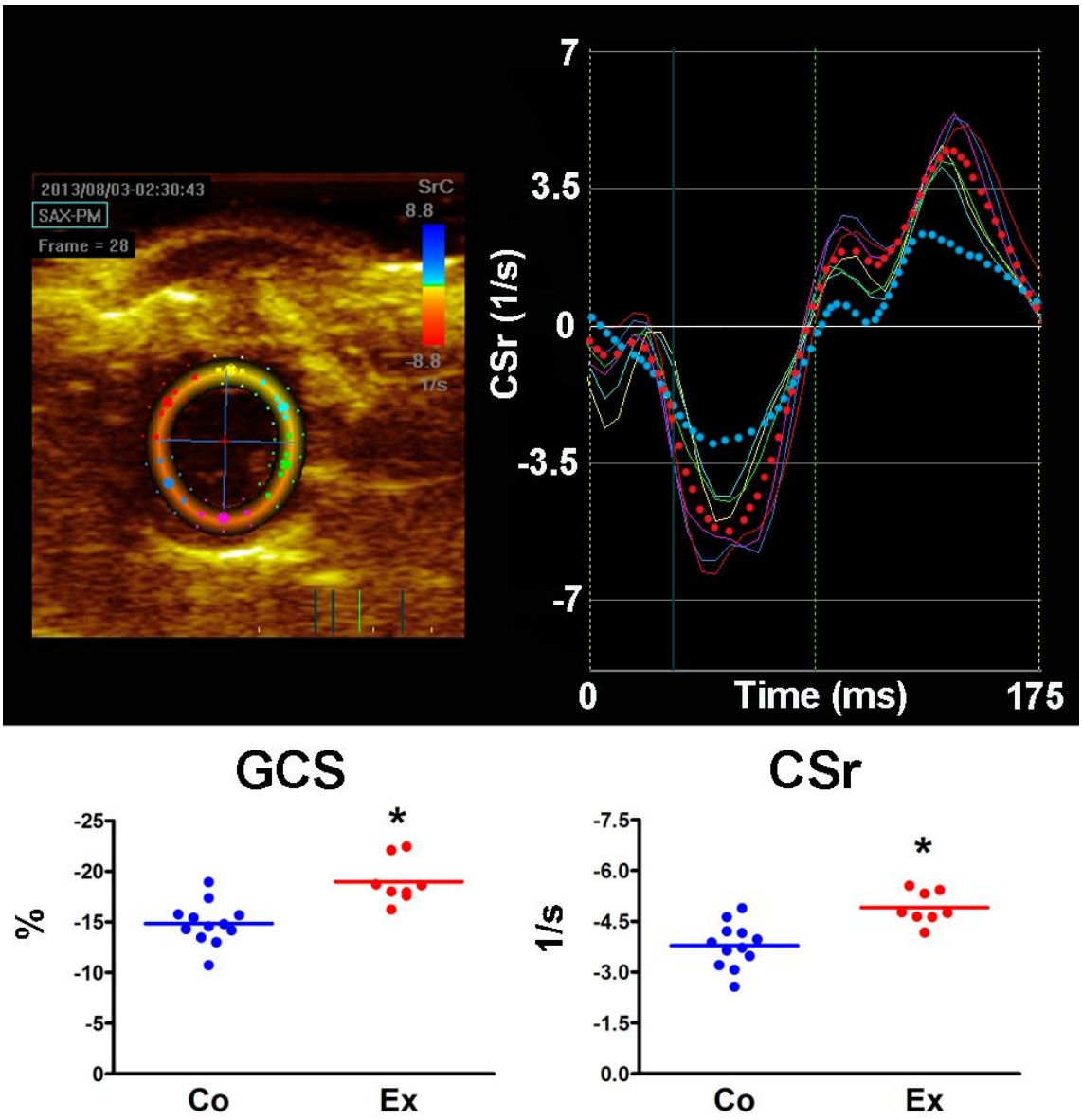

Figure 16. Speckle-tracking analysis of short-axis recordings

Representative original short-axis (at mid-papillary level) recordings of an exercised (Ex) 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 (Co) rat (blue dotted line). This figure illustrates the determination of circumferential strain rate (CSr). The peak negative value of the averaged curve is the systolic strain rate. As depicted by the dot plots, both global circumferential strain (GCS) and CSr was increased in exercised rats. Horizontal lines represent mean values. *p<0.05 vs. Co.

5.2.5. 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; Fig. 17.). PRSW was strongly related to GLS (r=-0.64, p=0.002), LSr (r=-0.71, p=0.001), less to CSr (r=-0.51, p=0.023), while tended to be correlated with GCS (r=-0.34, p=0.082). We also found moderate correlations between dP/dt_{max}-EDV and strain parameters (GLS: r=-0.59, p=0.005; LSr: r=-0.57, p=0.009; GCS: r=-0.46, p=0.042; CSr: r=-0.51, p=0.021).

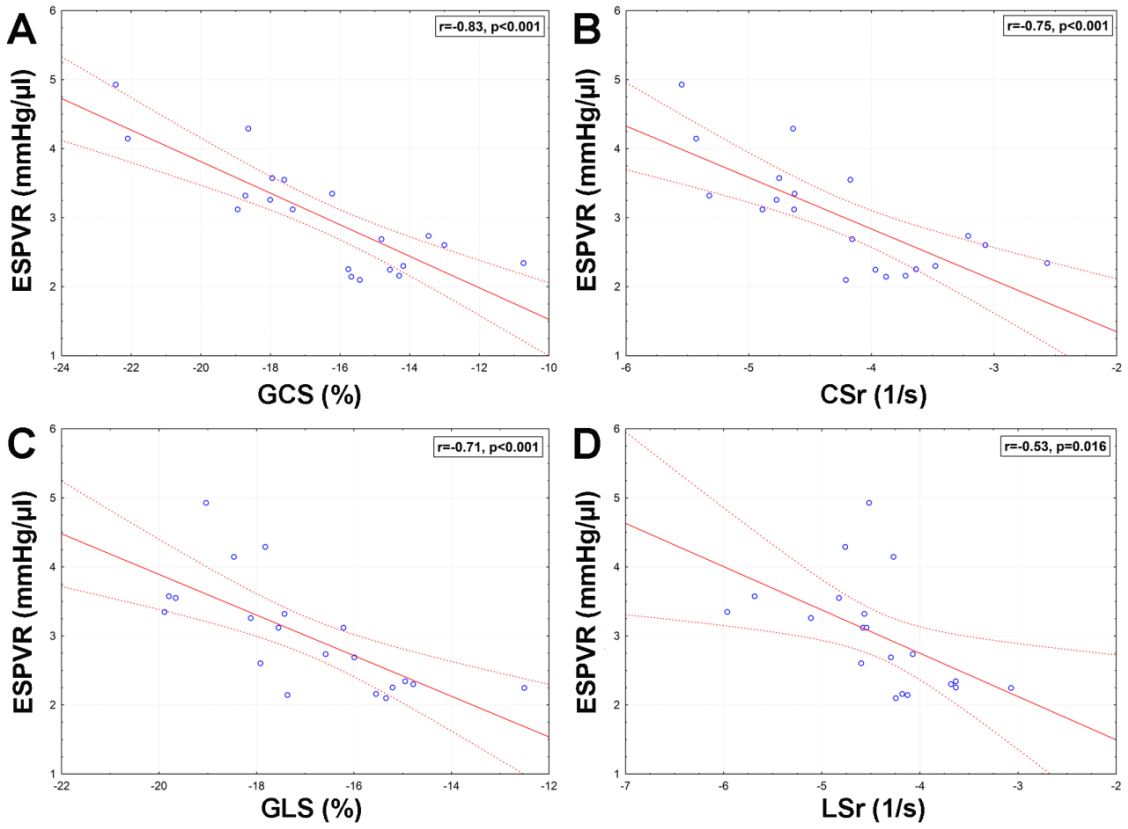

Figure 17. Correlations of the slope of end-systolic pressure-volume relationship (ESPVR) and strain parameters

Correlations between ESPVR and global circumferential strain (GCS) (A), systolic strain rate (CSr) (B) and global longitudinal strain (GLS) (C), systolic strain rate (LSr) (D) in trained and control rats (n=20).

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5.3. Cardiac effects of acute, exhaustive exercise

5.3.1. Body weight and heart weight

The body weight did not differ before and after the acute exhaustive exercise. Body weight loss to baseline body weight ratio during the exercise protocol was significantly increased in exercised rats. Heart weight measured immediately after the hemodynamic measurements was similar in exercised and control animals (Table 9.).

Table 9. Body and heart weight data of control and acute exercised rats

	Control (n=22)	Acute exercised (n=22)	p
Body weight before AEE, g	356±8	368±9	0.3050
Body weight after AEE, g	347±7	350±8	0.7417
Body weight loss, %	2.4±0.4	4.7±0.3*	<0.0001
Heart weight, g	1.13±0.04	1.14±0.04	0.8777

Values are means ± SEM. AEE: acute exhaustive exercise. *p<0.05 vs. controls.

5.3.2. 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 (Table 10.).

Table 10. Biochemical parameters measured from blood plasma in control and acute exercised rats

	Control (n=12)	Acute exercised (n=12)	p
Cardiac troponin T, ng/ml	0.025±0.006	0.131±0.022*	0.0001
CK, U/l	284.5±37.5	2029.3±461.4*	0.0011
LDH, U/l	541.5±58.6	836.7±130.1*	0.0011
AST, U/l	79.3±2.7	247.6±32.2*	<0.0001
Creatinine, µmol/l	34.6±3.0	33.6±1.9	0.7837

Values are means ± SEM. CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase. *:p<0.05 vs. controls.

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5.3.3. 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. 18.).

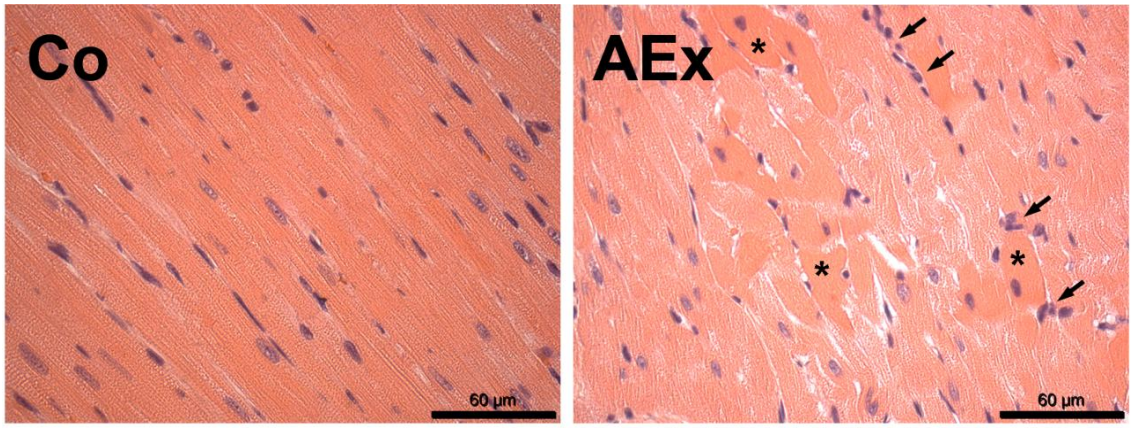

Figure 18. Histological investigation of hematoxylin-eosin (HE) stained left ventricular (LV) myocardium of control (Co) and acute exercised (AEx) rats

Hematoxylin-eosin 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 (Fig. 19.A).

Nitrotyrosine-staining showed increased tyrosine nitration in the myocardium of rats underwent excessive exercise (Fig. 19.B).

The number of TUNEL-positive cardiomyocyte nuclei were significantly increased in the exercised group (Fig. 19.C).

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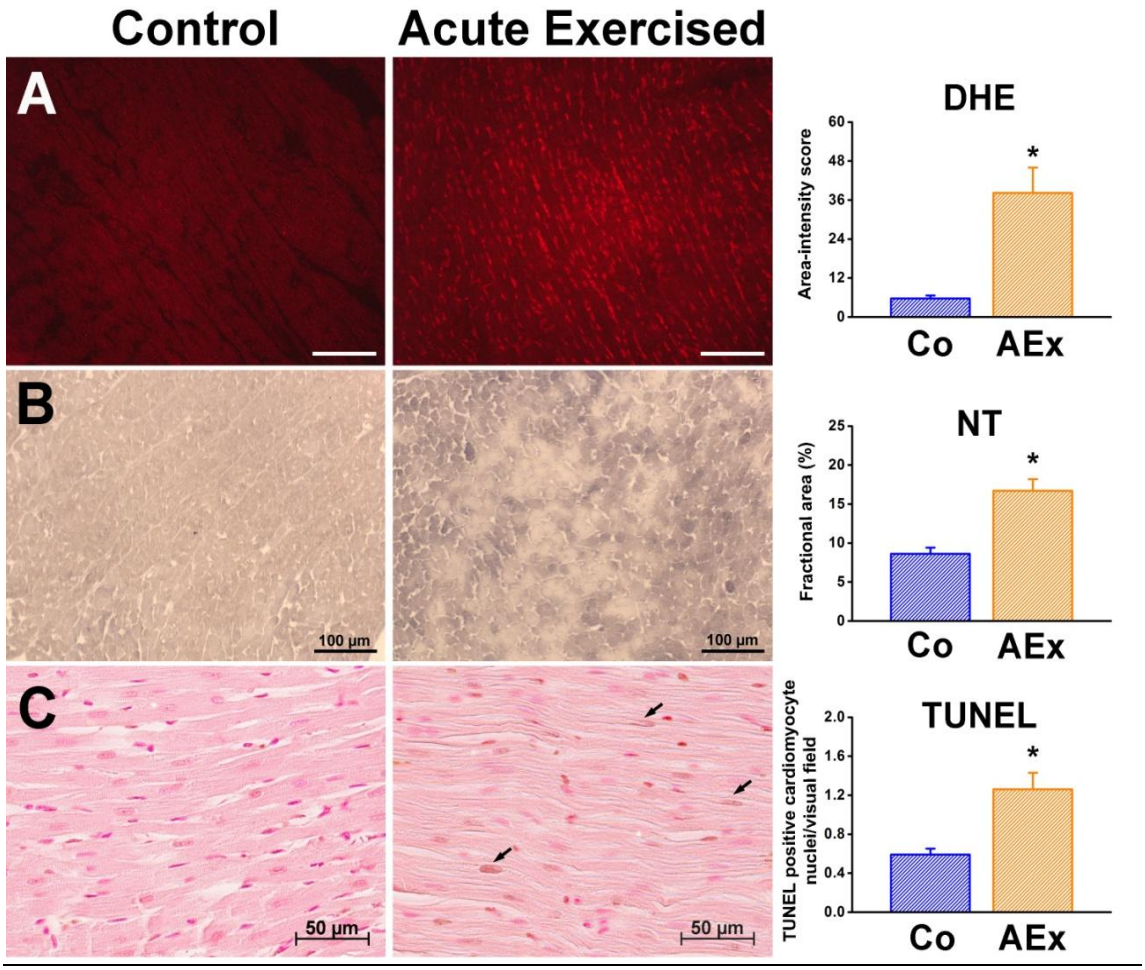

Figure 19. Histological analysis of left ventricular (LV) myocardium of control (Co) and acute exercised (AEx) rats

Panel A: Myocardial dihydroethidium (DHE) staining showed more intense red fluorescent signal (typically nuclear position) in LV myocardium after exhaustive exercise indicating increased superoxide formation (magnification 200x, scale bar 100 µm). Panel B: Nitrotyrosine (NT) immunostaining revealed increased tyrosine nitration (dark grey color indicates nitrotyrosine positive area) in the myocardium of exercised group (magnification 200x, scale bar 100 µm). Panel C: Acute exercised rats showed increased number of TUNEL-positive cardiomyocyte nuclei (see arrows) compared to control, suggesting enhanced myocardial apoptotic activation (magnification 400x, scale bar 60 µm). *$p<0.05$ vs. Co.

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5.3.4. 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 (Fig. 20.).

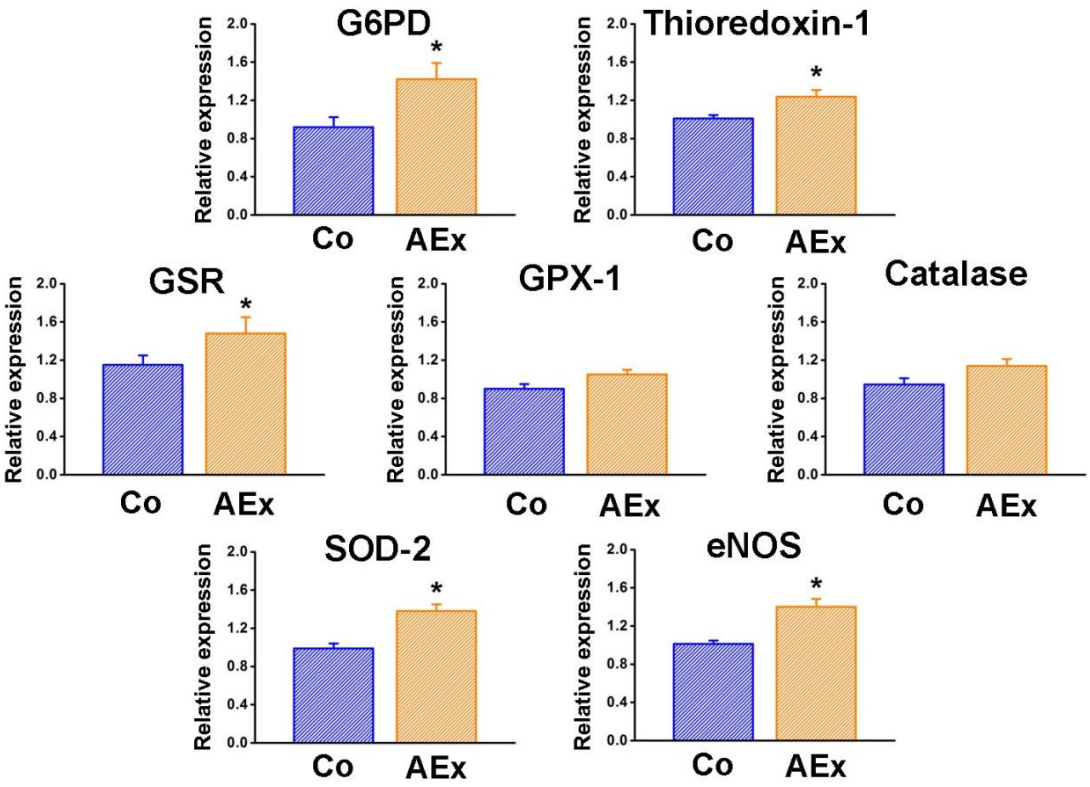

Figure 20. Oxidative stress related myocardial gene expression analysis after exhaustive exercise

Relative myocardial expression of genes related to oxidative stress: glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase 1 (GPX-1), glutathione reductase (GSR), thioredoxin-1, catalase, superoxide dismutase-2 (SOD-2) and endothelial nitric oxide synthase (eNOS) in control (Co) and acute exercised (AEx) rats. *$p<0.05$ vs Co.

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The 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 (Fig. 21).

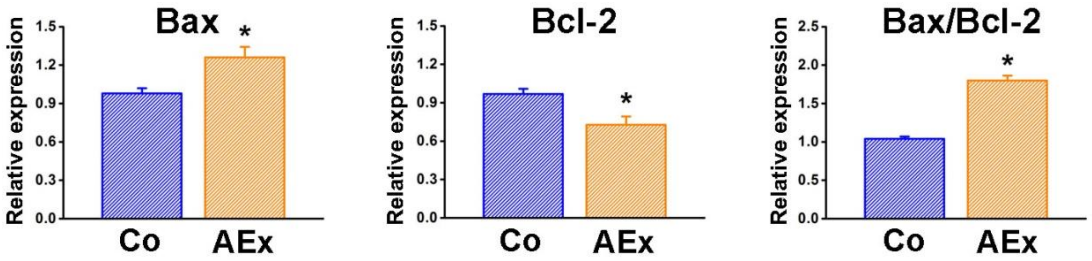

Figure 21. Myocardial gene expression related to apoptosis after exhaustive exercise

Relative myocardial expression of genes related to apoptotic signaling (Bax, Bcl-2 and Bax/Bcl-2 ratio) in control (Co) and acute exercised (AEx) rats. *$p<0.05$ vs Co.

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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-β1 showed a strong tendency toward higher values after exercise, however without reaching the level of statistical significance (Fig. 22.).

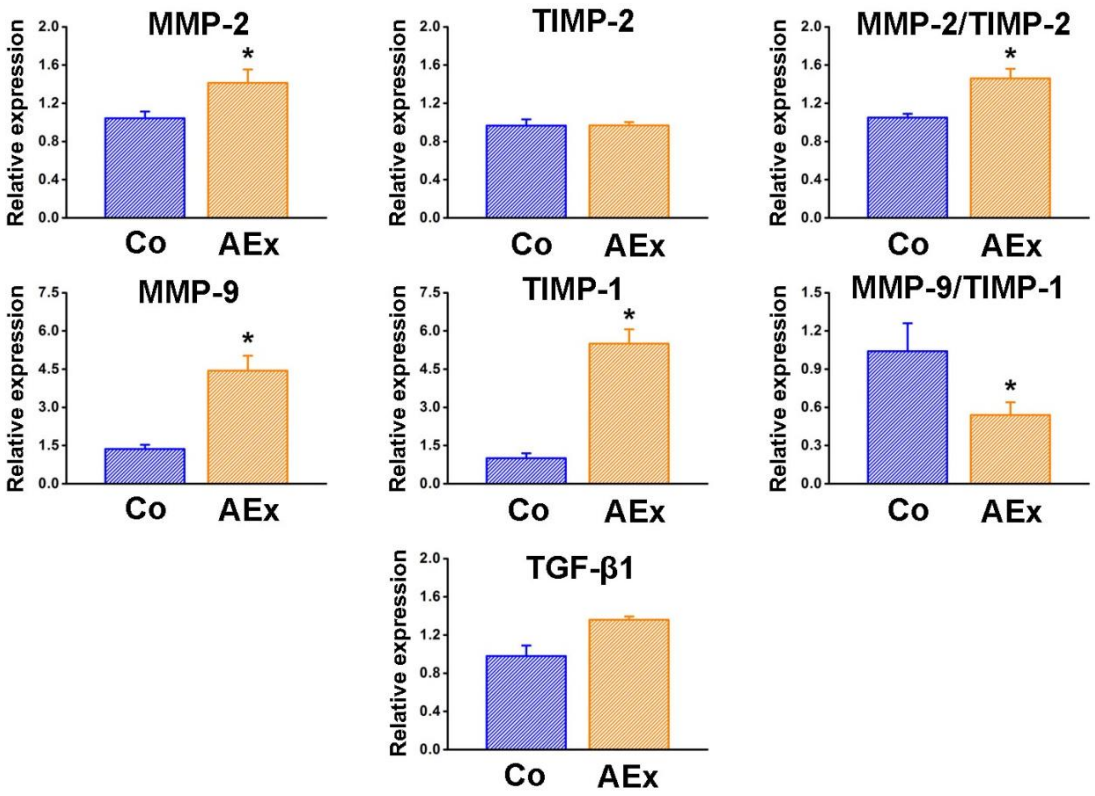

Figure 22. Myocardial gene expression of related to extracellular matrix (ECM) turnover after exhaustive exercise

Relative myocardial expression of genes related to extracellular matrix (ECM) turnover: matrix metalloproteinase (MMP)-2, tissue inhibitor of metalloproteinase (TIMP)-2, MMP-2/TIMP-2 ratio, MMP-9, TIMP-1, MMP-9/TIMP-1 ratio) and transforming growth factor β1 (TGF-1) in control (Co) and acute exercised (AEx) rats. *$p<0.05$ vs Co.

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5.3.5. Hemodynamic parameters

Baseline hemodynamic data. Figure 23. 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 (Table 11.). The decreased width of baseline P-V loops after exhaustive swimming reflects reduced SV along with unaltered LVEDV and increased LVESV. EF, CO and CI decreased significantly suggesting deteriorated systolic performance in rats after exhaustive exercise. TPR and E_a were increased in rats after intense exercise (Table 11.).

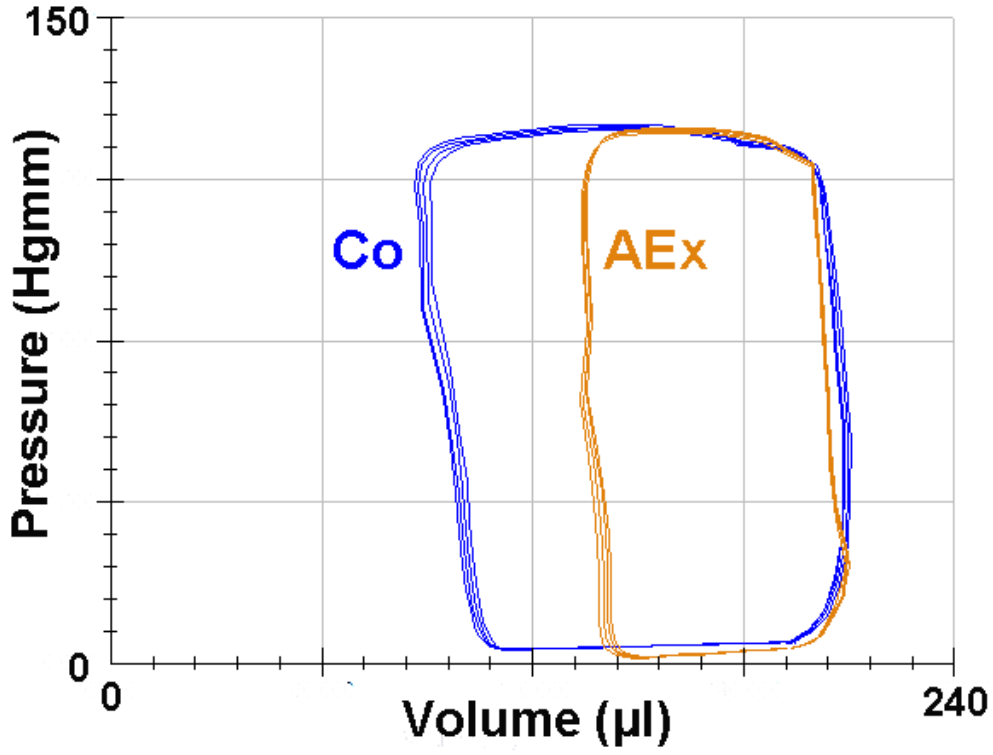

Figure 23. Steady-state left ventricular pressure-volume (P-V) loops after exhaustive exercise

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.

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Table 11. Hemodynamic parameters in control and acute exhaustive exercised rats

	Control (n=10)	Acute exercised (n=10)	p
HR, beats/min	419±13	418±8	0.9436
MAP, mmHg	112.5±4.4	109.8±3.5	0.6400
LVESP, mmHg	125.7±4.4	125.1±3.7	0.9272
LVEDP, mmHg	3.9±0.6	4.4±0.3	0.4208
LVEDV, µl	214.7±15.5	211.9±13.7	0.8943
LVESV, µl	100.1±6.7	140.4±8.5*	0.0015
SV, µl	139.1±9.4	102.3±11.3*	0.0226
CO, ml/min	58.5±4.7	42.4±4.5*	0.0235
CI, (ml/min)/kg BW	173.1±16.8	123.2±12.2*	0.0271
EF, %	59.5±1.8	44.1±3.4*	<0.0001
SW, mmHg·ml	13.4±0.6	9.1±0.1*	0.0030
dP/dt_{max}, mmHg/s	8350±465	7700±458	0.3329
dP/dt_{min}, mmHg/s	11095±741	10183±693	0.3805
τ(Weiss), ms	7.8±0.3	7.9±0.3	0.6876
τ (Glanz), ms	10.9±0.6	11.2±0.6	0.7297
TPR, mmHg/(ml/min)	2.02±0.16	2.84±0.29*	0.0228
Slope of EDPVR, mmHg/µl	0.036±0.004	0.041±0.004	0.3133
Maximal power, mW	110.4±16.5	58.7±6.1*	0.0009

Values are means ± SEM. HR, heart rate; MAP, mean arterial pressure; LVESP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diastolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; SV, stroke volume; CO, cardiac output; CI, cardiac index; BW, body weight; EF, ejection fraction; SW, stroke work; dP/dt_{max} and dP/dt_{min} maximal slope of the systolic pressure increment and the diastolic pressure decrement, respectively; τ, time constant of LV pressure decay; TPR, total peripheral resistance; EDPVR, end-diastolic pressure-volume relationship. *$p<0.05$ vs. controls.

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Systolic and diastolic functional indexes derived from P-V analysis at different preloads. Figure 24.A 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 excessive exercise. PRSW were significantly decreased after intense exercise indicating impaired contractility (Fig. 24.B). As Figure 24.C shows, 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.

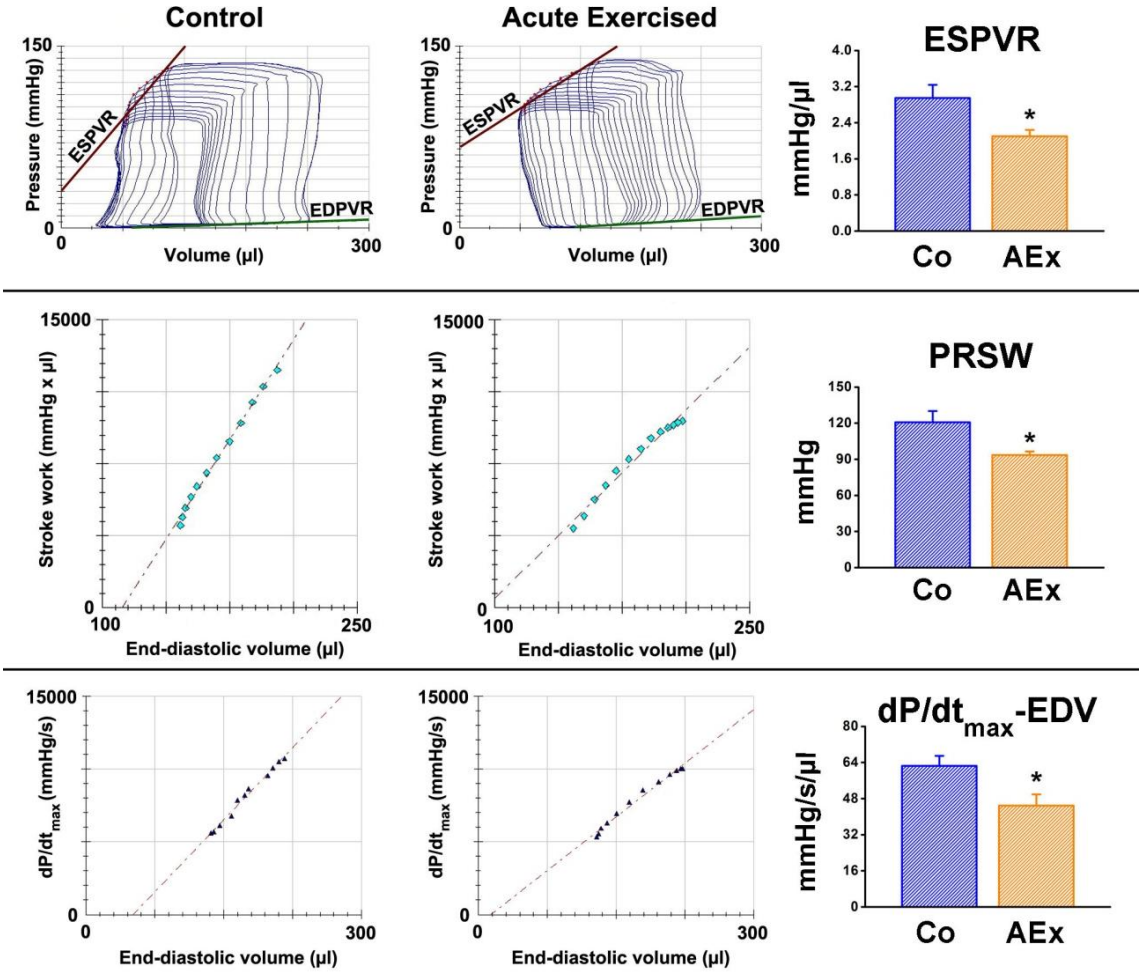

Figure 24. Pressure-volume (P-V) loop derived load independent left ventricular contractility parameters after acute exhaustive exercise

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. SW was decreased in acute exercised animals. P-V analysis revealed a significant decrease in E_{es} and an increase of E_a. Subsequently, VAC was significantly increased in exhaustively exercised rats suggesting contractility-afterload mismatch (Fig. 25.). Despite the decreased SW in exercised rats, pressure-volume area did not differ between the groups. Mechanical efficiency and maximum power of LV work were also impaired in exercised rats compared to the controls (Fig. 25., Table 11.).

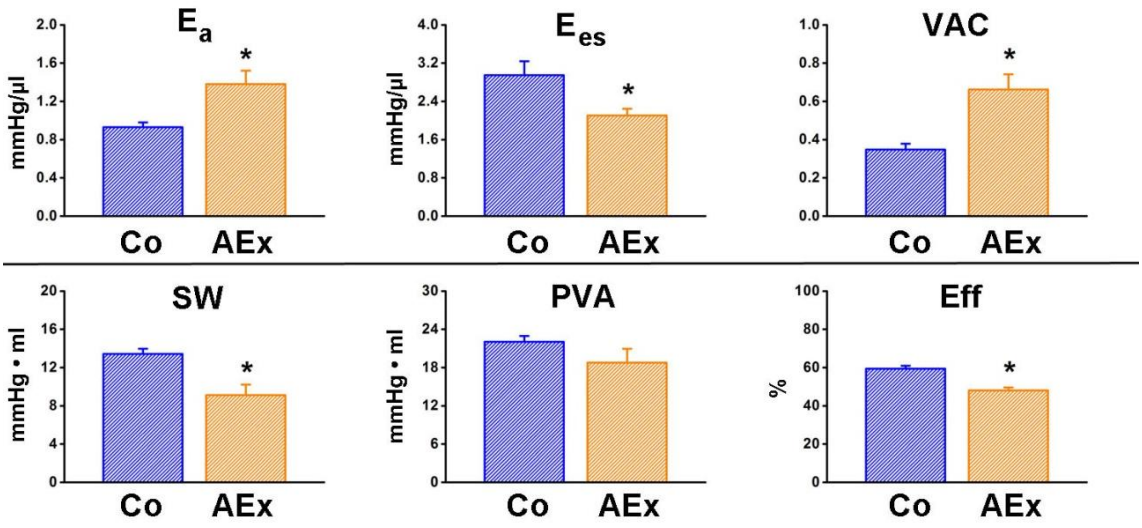

Figure 25. Cardiac mechanoenergetics after acute exhaustive exercise

Upper panel: Decreased end-systolic elastance (E_{es}) and increased arterial elastance (E_a) resulted in deteriorated ventriculo-arterial coupling ($VAC=E_a/E_{es}$) in acute exercised (AEx) rats compared with controls (Co). Lower panel: Decreased stroke work (SW) along with unaltered pressure-volume area (PVA) led to reduced mechanical efficiency (Eff=SW/PVA) in the swimming group. All of these parameters suggest impaired LV mechanoenergetics after exhaustive exercise. *p<0.05 vs. Co.

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6.Discussion

6.1. Long-term exercise-induced cardiac changes

In the present study we provide a reliable characterization of cardiac function in a rodent model of endurance exercise training using the method of LV P-V analysis and measuring load-independent functional indexes.

6.1.1. Exercise-induced left ventricular hypertrophy

The adaptation of myocardial tissue that accompany exercise training in experimental animal models depends on factors, such as modality, intensity, duration and frequency of the physical activity (Wang et al., 2010). According to literature data and results of our own previous pilot studies we established a rat model of robust cardiac hypertrophy induced by swim training. Although the duration of swim exercise varies considerably in previously published works, it generally induces hypertrophy of the myocardium by as much as 15 % (Kaplan et al., 1994; Wisløff et al., 2002; Medeiros et al., 2004). Our present data on rats that underwent a 12-week intensive (200 min/day) swim exercise training program showed a more robust cardiac hypertrophy as indicated by heart weight and calculated LV mass index values (Table 2. and 4.). According to the results of echocardiography measurements we observed significantly increased LV wall thickness and LV mass index values in exercised animals compared with controls, which is in line with previous studies (Wang et al., 2008; Bocalini et al., 2010). The histomorphometry of LV myocardium showed increased cardiomyocyte width (Fig. 6.C) in exercise-induced cardiac hypertrophy. This finding is consistent with the features described in isolated cardiomyocytes (Wang et al., 2008; Wisløff et al., 2001) as well as in LV myocardium (Medeiros et al., 2004) derived from animals underwent endurance training.

To confirm the physiological nature of the observed myocardial hypertrophy and exclude the possible role of the swimming-associated stress response we examined conventional markers of pathological hypertrophy (TGF-beta, beta-MHC and Masson's trichrome staining (Bernardo et al., 2010)) as well as biomarkers of stress (ACTH and cortisol (Contarteze et al., 2008)). The observed unaltered amount of myocardial collagen, unchanged TGF-β and β-MHC expression and stress biomarker values serve

as evidence to rule out stress induced remodeling and prove the physiological background of massive LV hypertrophy observed in our swimming rats (Fig. 7., Table 3.).

Previous studies showed increased LV end-diastolic dimensions in human athletes (Fagard, 2003) as well as in trained experimental animals (Benito et al., 2011) suggesting an exercised-induced dilation of the LV. In contrast, other researchers reported unaltered LV end-diastolic dimensions after long-term endurance exercise training in rats (Wang et al., 2008; Kemi et al., 2013). In the present study, both in vivo echocardiography and invasive hemodynamic measurements showed unaltered LVEDV and decreased LVESV in our exercised rats (Tables 4. and 5.). Recent data suggesting the importance of the training program characteristics (type, duration, intensity) might provide a reasonable explanation to these differences (Pluim et al., 2000; Naylor et al., 2008).

6.1.2. Systolic function and cardiac contractility

Previous studies showed improved contractility on isolated cardiomyocytes (Kemi et al., 2004; Wisløff et al., 2001) as well as on retrogradely perfused Langendorff hearts (Libonati, 2012) of endurance trained animals, however there is limited available data on sensitive contractility parameters assessed in vivo.

We found in our rat model that exercise training was associated with markedly increased dP/dt_{max} and significantly increased EF (Table 5.). Although dP/dt_{max} has been widely used as a cardiac contractility parameter, it is well recognized that it is dependent on loading conditions, especially on changes in preload (Kass, 1995). Classical echocardiographic parameters of cardiac contractility, FS and EF are also known to be influenced by both preload and afterload and therefore can not reliably be used to assess contractile function in models where loading is altered. The method of simultaneous LV pressure and volume analysis by the miniaturized pressure-conductance catheters allowed us to calculate highly sensitive and reliable load-independent indexes of LV contractility.

Historically, ESPVR (E_{es}) has been proposed as a fairly load-insensitive index of ventricular contractility. According to present results, ESPVR was significantly increased in exercised animals, indicating an improved contractile state of the LV

myocardium (Fig. 11.). However, because this relation can be altered not only by changes in the inotropic state but also by changes in chamber geometry and other diastolic factors, we also calculated other parameters. PRSW represents the slope of the linear relation between SW and EDV. It has been described as a parameter independent of chamber size and mass, and it is sensitive to the cardiac contractile function of the ventricle. Although it integrates data from the entire cardiac cycle, it is influenced most of all by the systole (Kass, 1995). PRSW was also significantly increased in exercised rats compared with control rats (Fig. 12.). A previous investigation has demonstrated that the slope of the relation between dP/dt_{max} and EDV, another P-V loop-derived index, has been referred as a sensitive but less load-dependent parameter of LV contractility (Little, 1985). This sensitive contractility index was also augmented in exercised animals compared with untrained controls (Fig. 12.).

6.1.3. Diastolic function

Diastolic LV stiffness is predominantly affected by alterations in myocardial structural changes (advanced glycation end-products (AGE) deposition, interstital fibrosis and remodelling) (van Heerebeek et al., 2008). Indexes of LV stiffness and compliance (LVEDP and slope of EDPVR, Table 5. and Fig.11.) were not significantly different between exercised and control animals despite the significant increase of ventricular wall thickness and marked myocardial hypertrophy. This is in accordance with Libonati, who described unaltered LV stiffness in isolated perfused hearts of trained rats (Libonati, 2012).

Improved ventricular relaxation has been observed in exercised animals (as indicated by decreased τ, a load-independent index of active relaxation; Table 5.). Ventricular relaxation is an ATP-dependent active process, depends mostly on Ca^{2+} uptake by the sarcoplasmatic reticulum, during the first third of diastole. The detected functional differences might reflect improved Ca^{2+}-handling as suggested by Kemi et al. and improved energetic state of the myocardium (Kemi et al., 2014).

6.1.4. Mechanoenergetics

We used P-V analysis to characterize mechanoenergetic changes in exercise-induced cardiac hypertrophy. We determined E_{es} (end-systolic elastance, can be

identified as the slope of ESPVR) and E_a (arterial elastance), which are relative load-independent indices of LV contractility and vascular loading, respectively. E_a is an integrative index that incorporates the principal elements of arterial load, including peripherial vascular resistance, total arterial compliance, characteristic impedance and systolic and diastolic time intervals (Sunagawa et al., 1983). The parallel increase of myocardial contractility (E_{es}) and the decrease of E_a led to a highly significant optimization of the ventriculo-arterial coupling ratio in exercised animals (Fig. 13.). Ventriculo-arterial coupling (E_a/E_{es}) is an important determinant of net cardiac performance and energetics (Kass and Kelly, 1992). Improved ventriculo-arterial coupling in exercise-trained animals reflect a more appropriate matching between the LV and the arterial system, which results in an optimal transfer of blood from the LV to the periphery without excessive changes in pressure (Chantler et al., 2008). This phenomen is in line with a human echocardiographic study, where an improved vetnriculo-arterial coupling was observed in athlete's heart (Florescu et al., 2010).

Stroke work (SW) is determined as the area within the P-V loop and represents the external mechanical work performed by the left ventricle during a single heart cycle. P-V area (PVA) has been described as an index of LV total mechanical energy and is linearly related to total myocardial oxygen consumption (Suga, 2003). Increased SW along with unaltered PVA leads to increased mechanical efficiency (SW/PVA) in exercised animals compared with controls (Fig. 13.), suggesting an optimization of metabolic efficiency in exercise-induced cardiac hypertrophy: increased mechanical work along with similar myocardial oxygen consumption. This is in accordance with the observation that physiological hypertrophy is associated with a more effective metabolism (Laaksonen et al., 2007).

6.1.5. Reversibility of exercise-induced cardiac hypertrophy

Our cardiac morphometric results after discontinuation of intense exercise training and 8 weeks of detraining (Table 6.) confirm the complete reversibility of the observed cardiac phenotype (and reflect the age-related continuous increase in rat body dimensions). Functional indices measured by echocardiography (EF, FS) suggest the complete regression of exercise-induced alterations, however further investigations are warranted to confirm the functional reversibility of athlete's heart. These findings

further support the physiological nature of exercise-induced myocardial hypertrophy in our rat model, and are in accordance with previous observations (Bocalini et al., 2010; Benito et al., 2011).

6.2. Correlation of contractility indices of pressure-volume analysis and speckle-tracking echocardiography

In this investigation we examined whether non-invasive speckle-tracking echocardiography could be feasible to detect LV contractility alterations in animal models of athlete's heart.

6.2.1. Left ventricular hypertrophy

In agreement with results of other research groups, an increase in post mortem assessed heart weight has been observed in our rat model of exercise-induced cardiac hypertrophy. The observed increase in cardiac mass was underpinned by increased wall thickness values and calculated LV mass data using echocardiography (Table 7.). The degree of cardiac hypertrophy was comparable to other small animal models of exercise-induced cardiac hypertrophy (Wang et al., 2010).

6.2.2. Baseline hemodynamic data

Regular exercise training induced physiological hypertrophy is associated with normal or enhanced function of the heart (McMullen and Jennings, 2007). Echocardiographic data indicated an increased fractional shortening in trained animals which was the consequence of decreased end-systolic dimensions along with unchanged end-diastolic dimensions. Accordingly, our baseline hemodynamic data obtained with the pressure-volume system showed an increase in systolic parameters (SV, EF, CO and SW) in trained animals along with unaltered pressure values and heart rate as well as with decreased TPR (Table 8.). These results are in good agreement with our previously described hemodynamic data of exercise induced hypertrophy using another anesthesia protocol (ketamine-xylazine).

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6.2.3. Sensitive left ventricular contractility indices derived from pressure-volume analysis

Contractility is the capacity of the myocardium to contract independently of alterations in preload or afterload. The slope of the end-systolic P-V relationship (ESPVR) is the most commonly used and perhaps the most reliable index of LV contractility in the intact circulation and is almost insensitive to alterations in preload or afterload (Cingolani and Kass, 2011). As shown in Figure 14., ESPVR was steeper in trained rats indicating an improved inotropic state of the LV myocardium. During the transient occlusion of vena cava inferior two additional sensitive contractility indices could be acquired: the slope of the linear relation between SW and EDV, the so-called preload recruitable stroke work (PRSW) and the slope of the linear relation between dP/dt_{max} and EDV (dP/dt_{max}-EDV) (Pacher et al., 2008). Both of these indices were increased in trained hearts compared with control ones, confirming the improved contractile state in exercise induced cardiac hypertrophy (Fig. 14.).

6.2.4. Strain and strain rate measured by speckle-tracking echocardiography

The search for powerful systolic parameters is an ongoing quest for echocardiographic research, but precise evaluation of supernormal function is even a major issue. Speckle tracking echocardiography gained particular interest as it allows quantitative evaluation of myocardial motion both at global and regional levels (Popovic et al., 2007). Superiority of speckle tracking derived parameters in detecting subtle myocardial injury was suggested by numerous works not just in humans but also in animal models (Kim et al., 2012; Kramann et al., 2014). Strain indices were showed to be able to sensitively and continually reflect the progression of heart failure as well (Koshizuka et al., 2013). Nevertheless, available data encompasses the value of strain indices in reduced myocardial function exclusively, but less is known about its added value in supernormal states, especially in the trained heart. In our experiments both longitudinal and circumferential strain and strain rate successfully reflected increased contractile function (Fig. 15. and 16.).

6.2.5. Correlation between strain parameters and sensitive contractility indices

We found robust correlations between invasive contractility indices, and longitudinal or circumferential strain and strain rate (Fig. 17.). In a recent publication, Ferferieva and coworkers demonstrated similar results regarding circumferential strain and PRSW (Ferferieva et al., 2013). Nevertheless, their experiments were conducted in mice models of transaortic constriction and myocardial infarction, whilst our correlations originated from an athlete's heart model of supernormal contractility - a scenario where conventional echocardiography usually lacks the power to precisely measure myocardial function. They also compared tissue Doppler imaging (TDI) and speckle tracking measurements of strain parameters and demonstrated the superiority of TDI in case of higher heart rates. Temporal resolution is an obvious advantage of the Doppler technique, however, its angle-dependency is certainly an issue in terms of reproducibility (Fontana et al., 2012). Because of that reason and also the possibility of measuring longitudinal strain and strain rate, we propose STE as the method of choice during resting conditions. Longitudinal strain gained huge value in human echocardiographic examinations. Despite the fact that tracking algorithm of our software is developed for a use on apical images in humans, the unusual delineation of the region of interest in our experimental settings was feasible. Furthermore, longitudinal strain and strain rate were also found to be robust parameters (Fig. 15.). Longitudinal and circumferential deformation can represent the function of different layers of myofiber architecture and therefore, valuable regional alterations (i.e. subendocardial ischemia) could be assessed as well (Ishizu et al., 2014). However, limitations of the speckle tracking technique known from human investigations may also apply: acquisition of proper images with optimal spatial and temporal resolution is of high importance (Blessberger and Binder, 2010). Out-of-plane speckle motion to reduce tracking quality is implied in the 2D approach.

In a rat model of athlete's heart speckle tracking derived indices were in close relationship with invasive load-independent measures of cardiac contractility. The observed correlations between P-V analysis and strain parameters are promising in terms of widespread use of speckle tracking echocardiography during consecutive evaluation of physiological myocardial hypertrophy in small animal models.

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6.3. Acute exhaustive exercise-induced cardiac changes

In the current study we have validated our rat model of acute exhaustive exercise induced myocardial injury by confirming key mechanisms of cardiac damage. We provide the first detailed hemodynamic characterization and described several aspects of LV dysfunction after acute exhaustive exercise.

6.3.1. Biomarkers of myocardial injury

In the present study an obvious myocardial injury was observed after exhaustive swimming exercise. Release of cTnT as well as non-specific cardiac enzymes CK, LDH and AST were markedly increased after exhaustive exercise (Table 10.), which is in line with previous observations on animal models (Chen et al., 2000; Nie et al., 2010; Li et al., 2012) and numerous human exercise studies (Scharhag et al., 2008; Shave et al., 2010). In accordance with recent literature data our HE staining of LV myocardium showed signs of sporadic cardiomyocyte damage after exhaustive exercise (Li et al., 2012) (Fig. 18.).

6.3.2. Cardiac dimensions and baseline hemodynamics

Although numerous human studies investigated cardiac function after a prolonged exercise both in nonelite subjects and in elite athletes by using echocardiography, there is controversial literature data about systolic and diastolic functional changes of LV (Oxborough et al., 2010). Li et al. showed a significant impairment of cardiac function of experimental animals subjected to exhaustive physical activity both in vivo (echocardiography) and in vitro (Langendorff model) (Li et al., 2012). To the best of our knowledge, the present work is the first one that describes LV pressure and volume relations in detail and provides characterization of LV dysfunction in vivo after exhaustive exercise. P-V analysis clearly demonstrated significantly increased LVESV along with unchanged LVEDV, thus decreased SV and EF in rats underwent our exhaustive exercise protocol (Fig. 23.). These cardiac dimensions are consistent with previous experimental results (Li et al., 2012) and correspond with human echocardiography data suggesting systolic impairment after exhaustive exercise (Neilan et al., 2006c; Middleton et al., 2006).

In our rat model exhaustive exercise was associated with unchanged dP/dt_{max} and LVESP, but markedly decreased EF (Table 11.). Although dP/dt_{max} and EF have been widely used as cardiac contractility parameters, it is well recognized that they are dependent on loading conditions, and therefore cannot reliably be used to assess LV contractile function in models where loading is altered (Kass, 1995). The method of simultaneous LV pressure and volume analysis performed by the miniaturized pressure-conductance catheter allowed us to calculate highly sensitive load-independent indexes of LV contractility.

Indices of LV stiffness (LVEDP and slope of EDPVR) were not significantly different between exercised and control animals, as well as unchanged ventricular relaxation has been observed after intense exercise (as indicated by τ and dP/dt_{min}; Table 11.). The observed unchanged diastolic function is in line with recent findings on isolated rat hearts (Reger et al., 2012). Nevertheless there are several human studies describing a transient diastolic dysfunction (decreased E/A ratio assessed by echocardiography) after exhaustive exercise (Oxborough et al., 2010). It is possible that the impairment of active relaxation after prolonged exercise shows a normalization after 2 hours as this process is ATP dependent and can reflect on promopt metabolic changes in human studies, measured immediately following acute exertion. Further experimental reports are required for appropriate comparison of human and animal diastolic values.

6.3.3. Left ventricular contractility

The slope of ESPVR has been described as a fairly load-insensitive index of LV contractility, which was significantly decreased after intense exercise, indicating a deteriorated inotropic state of the LV myocardium (Fig. 24.). PRSW and dP/dt_{max}-EDV also represent sensitively myocardial contraction and indicated a marked impairment of LV contractility (Fig. 24.). To our best knowledge, this is the first evidence showing impaired in vivo contractility after prolonged, exhaustive exercise.

6.3.4. Mechanoenergetics

P-V analysis was used to characterize mechanoenergetic changes after exhaustive exercise. Above we described principal elements of LV mechanoenergetics. Increased E_a showed an increased afterload which suggests detrimental changes in the vascular system of acute exercised rats. Moreover, TPR was increased after exhaustive exercise. In contrast to those observations in athlete's heart, in our model of exhaustive exercise the parallel decrease of myocardial contractility (E_{es}) and the increase of E_a led to a significant impairment of the VAC in acute exercised animals (Fig. 25.). Impaired ventriculo-arterial coupling in the exercised group reflects a mismatch between LV contractility and afterload after exhaustive exercise, which resulted in a suboptimal transfer of blood from the LV to the periphery with more excessive changes in pressure.

We also described other mechanoenergetic aspects of LV performance. Decreased SW along with unaltered PVA leads to decreased mechanical efficiency in exhaustive exercised animals compared with controls, suggesting a deterioration of metabolic efficiency after such exercise: decreased mechanical work along with similar myocardial oxygen consumption (Fig. 25.). According to our knowledge the present work is the first to demonstrate impaired mechanical efficiency and vetriculo-arterial coupling after exhaustive exercise.

6.3.5. Exhaustive exercise-induced oxidative stress

Even though the exact mechanisms responsible for exercise-induced myocardial injury are still not well understood, there have been accumulated evidence indicating that exhaustive exercise causes imbalance between ROS and antioxidant defense, resulting in oxidative stress in the myocardium (Muthusamy et al., 2012). Increased formation of ROS (by the mitochondrial electron transport chain, NAPDH and xanthine oxidases (Pacher et al., 2007) activate a broad variety of hypertrophy signaling kinases and transcription factors and induce apoptosis by DNA and mitochondrial damage and activation of proapoptotic signaling kinases (Sabri et al., 2003). A robust generation of ROS and thus increased oxidative stress was observed by DHE-staining in the myocardium of exhaustive exercised rats compared to controls (Fig. 19.A). A recent experimental study of exhaustive exercise showed the key role of Nrf2, the primary transcriptional regulator of antioxidants, including G6PD, GPX-1, GSR and catalase,

which are upregulated as a compensatory reaction to ROS overproduction. Correspondingly we found significantly increased myocardial gene expressions of endogenous antioxidants, such as G6PD, GSR as well as thioredoxin-1 after exhaustive exercise. In accordance with Muthusamy et al. (Muthusamy et al., 2012), we observed an upregulation of mitochondrial SOD-2 in the myocardium, suggesting mitochondrial superoxide generation as a result of exhaustive exercise (Fig. 20.). Increased superoxide concentration reduces the bioavailability of NO by chemical inactivation to form toxic peroxynitrite. A markedly increased protein nitration was observed by nitrotyrosine staining (Fig. 19.B), suggesting increased nitrative stress, which could be the consequence of peroxynitrite formation (Pacher et al., 2007). Peroxynitrite can also "uncouple" eNOS to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress (Forstermann, 2010). In our study the myocardial expression of eNOS increased in response to oxidative stress, which might reflect upregulation as a reaction to decreased NO bioavailability and eNOS uncoupling. Moreover, nitration of several myocardial proteins can have a potential deleterious effect on myocardial contractility and increased peroxynitrite formation can induce apoptosis as well as matrix metalloproteinase activation (Pacher et al., 2007).

6.3.6. Oxidative stress-induced apoptosis and dysregulation of matrix metalloproteinases

The ratio of proapoptotic and antiapoptotic proteins (e.g. Bax/Bcl-2) regulates myonuclei integrity and cell survival by controlling mitochondrial membrane permeability (Hengartner, 2000). According to our results exhaustive exercise resulted in a markedly increased Bax/Bcl-2 ratio, thus enhanced apoptotic signaling in the myocardium (Fig. 21.). This finding has further been supported by increased number of TUNEL positive cardiomyocyte nuclei, suggesting increased DNA fragmentation after acute exercise (Fig. 19.C). This is in accordance with an investigation showing exhaustive exercise-induced increased proapoptotic activity in a small number of samples (Huang et al., 2009).

ROS can stimulate cardiac fibroblast proliferation and activate matrix metalloproteinases, which play a key role in the homeostasis of extracellular matrix in the myocardium. Sustained MMP activation (increased expression of MMPs or

downregulation of their endogenous inhibitors, TIMPs) might influence the structural properties of the myocardium by providing an abnormal extracellular environment, which the cardiomyocytes interact with (Kandasamy et al., 2010; Tsutsui et al., 2011). As recent investigations showed, expression and activity of MMP-2 and MMP-9 are increased in skeletal muscle after a single bout of exercise (Koskinen et al., 2001). The observed significant changes of MMP-2/TIMP-2 as well as MMP-9/TIMP-1 ratio suggest MMP dysregulation in myocardium of exercised rats (Fig. 22.). These findings raise the possibility that enhanced oxidative stress can be a stimulus for myocardial MMP activation, which might play an important role in the development of exhaustive exercise-induced cardiac dysfunction. TGF-β1 is a pleiotropic cytokine, which is involved in cardiac injury, repair and fibrotic remodeling (Dobaczewski et al., 2011). A strong tendency toward upregulation of TGF-β1 expression after exhaustive exercise suggests the activation of reparative and profibrotic mechanisms after myocardial injury induced by prolonged exercise.

6.4. Limitations

The interpretation of results from the present work is limited to young male rats. The possible influence of gender, age or species should be assessed in future studies. Swimming has been suggested to be effective to induce cardiac hypertrophy in animals even though it is associated with an important stress response (Contarteze et al., 2008). In order to minimize the influence of stress on the development of exercise-induced cardiac hypertrophy, rats of the exercised group were gradually acclimated with swimming training and also untrained control rats were placed into the water for five minutes at every training session. According to the plasma concentrations of stress biomarkers this regular swim training protocol was not associated with increased stress response in our rats. Swimming with a workload attached to the tail has been suggested to evoke exhaustive exercise-induced cardiac injury and oxidative stress in animals (Nie et al., 2010). In order to minimize the influence of stress in the model of acute exhaustive exercise-induced myocardial damage, all rats were familiarized with swimming 48h before the formal swimming protocol.

P-V loop analysis, which is widely used in large animals as well as in rodents, has become a requirement for the assessment of LV function because it is the only technique

that allows the measurement of LV performance independent from loading conditions. The proper measurements of absolute volumes is the most vulnerable part of the P-V technique. Nevertheless, as our results demonstrate, the baseline P-V data of control rats were very similar to those reported in a recent methodological study (Pacher et al., 2007). Moreover absolute volume data from the P-V analysis were in correspondence with those assessed by echocardiography.

We should also comment on the unchanged heart rate values in our model of exercise-induced LV hypertrophy. Measuring resting heart rate in rats requires special equipments, which was not avilable in our laboratory. Resting bradycardia in athletes is a consequence of shifted autonomous balance: particularly an increase in parasympathetic activity. We hypothesized that this increased activity can be altered by anesthesia resulting in no difference regarding heart rate. However this similarity in heart rate provide a more comparable way to characterize myocardial mechanics.

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7. 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 LV performance, in the present study we demonstrated systolic (improved contractility) and diastolic (improved active relaxation and unchanged LV stiffness) functional amelioration in exercised animals. 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 P-V 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 LV 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.

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8. Summary

The role of physical exercise in the prevention and treatment of cardiovascular diseases has been well described. Long-term exercise training-induced cardiac alterations have been investigated intensively in the past decades, however limited data is available of fuctional consequences. Although the beneficial effects of exercise training, recent studies have documented elevations in biomarkers consistent with cardiac damage after bouts of prolonged exercise in apparently healthy individuals, which has raised concerns about cardiovascular health consequences of such exercise. Our purpose was to describe the detailed hemodynamic alterations in a rat model of athlete's heart. We also aimed at understanding the biochemical, molecular biological, structural and functional alterations in the heart after exhaustive exercise in a rat model.

Our research group provided the first characterization of in vivo hemodynamic left ventricular (LV) alterations in athlete's heart by pressure-volume analysis after confirming swimming training-induced reversible physiological cardiac hypertrophy in our rat model. Based on reliable, load-independent indices, markedly improved contractility and ameliorated active relaxation was found in athlete's heart along with unchanged LV stiffness. Ameliorated mechanoenergetics were indicated by improved mechanical efficiency and optimized ventriculo-arterial coupling. In our additional experiment, speckle-tracking analysis derived strain parameters correlated strongly with invasively measured sensitive contractility indices. These correlations are promising in terms of widespread use of speckle-tracking echocardiography for non-invasive evaluation of physiological myocardial hypertrophy in small animal models.

Cardiac effects of prolonged, strenuous exercise were also investigated. Acute exercise induced cardiac injury and nitro-oxidative stress in our rat model and for the first time we described in vivo LV dysfunction in detail by using pressure-volume analysis. Our data demonstrated systolic functional deterioration (reduced contractility) in exhaustive exercised animals along with unchanged diastolic function and impaired mechanoenergetics (decreased efficiency, ventriculo-arterial mismatch) 2 hours after finishing a prolonged, exhaustive exercise. 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.

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9. Összefoglalás

A rendszeres fizikai edzés kardiovaszkuláris betegségek prevenciójában és terápiájában betöltött szerepe jól ismert. A hosszú távú fizikai edzés okozta kardiális változásokat számos kutatócsoport vizsgálta az utóbbi évtizedekben, azonban a funkcionális következményekről korlátozott ismeretanyag áll rendelkezésünkre. A testedzés kedvező hatásai ellenére az utóbbi években hosszútávú kimerítő fizikai terhelés hatására miokardiális károsodást jelző biomarkerek felszabadulását észlelték, amely kétségeket vetett fel az ilyen típusú testmozgás kardiovaszkuláris következményeivel kapcsolatban. Célunk volt részletes hemodinamikai jellemzést adni a sportszív patkánymodelljéről bal kamrai (BK) nyomás-térfogat analízis segítségével, valamint megvizsgálni a kimerítő fizikai terhelés okozta biokémiai, molekuláris biológiai, strukturális és funkcionális kardiális változásokat.

Az úszóedzés által előidézett BK hipertrófia fiziológiás természetének és reverzibilitásának igazolása után annak első in vivo hemodinamikai jellemzését adtuk. A nyomás-térfogat analízis során nyert elő- és utóterheléstől független indexek jelentősen növekedett BK-i kontraktilitást, javult aktív relaxációt és a jelentős hipertrófia ellenére változatlan falmerevséget mutattak. A javult mechanoenergetikát a BK működésének fokozott hatásfoka és a kedvezőbb ventrikulo-arteriális kapcsolás mutatta. Kiegészítő kísérletünkben a speckle-tracking analízis során nyert strain paraméterek szoros korrelációt mutattak az invazívan mért, érzékeny kontraktilitás-indexekkel, mely ígéretessé teszi a noninvazív speckle-tracking echokardiográfiát a sportszív kifejlődésének nyomon követésére kisállatmodellben.

Az egyszeri, hosszan tartó, kimerítő fizikai terhelés kardiális hatásait is vizsgáltuk. Patkánymodellünkben igazoltuk az akut fizikai terhelés okozta szívizomsérülést és nitro-oxidatív stresszt és részletesen leírtuk a kimerítő fizikai terhelés okozta BK diszfunkciót. Adataink a BK-i szisztolés funkció (csökkent kontraktilitás) és a mechanoenergetika (csökkent hatásfok, ventrikulo-arteriális kapcsolás zavara) romlását mutatták változatlan diasztolés funkció mellett 2 órával a kimerítő terhelés után. A kardiális nekroenzimek emlkedése mellett detektált csökkent szívfunkció, valamint a proapoptotikus és profibrotikus aktiválódás felveti a kérdést, vajon a hosszan tartó, megerőltető fizikai terhelés képes-e tartós miokardiális károsodást és diszfunkciót előidézni.

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11. List of publications

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