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ABSTARCT

Background: Heart failure with preserved ejection fraction (HFPEF) is the only cardiovascular disease with an increasing incidence and prevalence. Complex pathophysiology of the disease and multi-organ involvement confers with high mortality rates among patients. Despite rising prevalence and poor survival prognosis, there are no established treatments up to date to improve clinical outcomes. Exercise training is recommended to improve functional capacity and the quality of life in patients and could possibly outweigh any pharmacological intervention, since lifestyle dependent risk factors, physical inactivity and deconditioning contribute to the deterioration of HFPEF. However, the optimal dose of exercise, exerting beneficial effects on cardiac and renal function and structure is not yet established and require further evaluation in experimental studies. **Methods:** Female Dahl salt-sensitive (DS) rats were randomized into sedentary low-salt (LS), high-salt (HS) and three exercise training groups. The rats performed moderate intensity continuous treadmill running (MCT), 5-times 40 min/week at 60 % of maximal oxygen consumption (VO_{2max}); high intensity interval training high-volume (HIT-HV), 3-times/week, 4 intervals of 4 min at 90 % $VO_{2max,}$ separated by 3 min at 60 % VO_{2max} and high intensity interval training low-volume (HIT), 2 intervals of 4 min at 90 % $VO_{2max,}$ separated by 3 min at 60 % $VO_{2max.}$ Total distance run was given as a measure of running performance. Blood pressure (BP), echocardiographic evaluation of diastolic function and cardiac structure, hemodynamic measurements and blood analysis were performed. Body organs were harvested, tissue weights measured and subjected to histological analysis. Metabolic cages were used to collect urine samples and evaluate kidney function. Lastly, survival distribution was estimated using the Kaplan-Meier method. **Results:** All of the rats placed on high sodium diet (8.0 % NaCl), developed hypertensive BP levels, diastolic dysfunction and left-ventricular concentric hypertrophy, while ejection fraction remained normal. The levels of NT-proBNP were elevated and a mild kidney dysfunction developed. Exercise training did not lower BP and did not improve pathological changes in cardiac and renal function and structure. The mortality rates were not different between high sodium groups and the animals were prematurely dying from stroke at a high rate. **Conclusion:** Female DS rats placed on a high sodium diet, satisfied the clinical HFPEF diagnostic criteria and progressed into HF at week 35. Development of diastolic dysfunction was consistent with progressing decline in renal function. Although exercise did not alter the overall mortality observed in DS rats under high sodium intake, exercise trained groups had a modest delay in mortality compare to HS animals and HIT-HV and HIT-LV showed better estimated survival at the end of the study. The study clearly confirmed that stroke is the major cause of mortality in DS model and suggested that the high incidence of stroke might be related to the chronic high sodium intake.

FREQUENTLY USED ABBREVIATIONS

BNP	Brain natruiretic peptide
BP	Blood pressure
CKD	Chronic kidney disease
DR	Dahl-salt-resistant rat
DS	Dahl-salt-sensitive rat
EF	Ejection fraction
GFR	Glomural filtration rate
HF	Heart failure
HFPEF	Heart failure with preserved ejection fraction
HFREF	Heart failure with reduced ejection fraction
HIT-HV	High intensity interval training high-volume
HIT-LV	High intensity interval training low-volume
HR_{max}	Maximal heart rate
HS	High-salt
LA	Left atrium
LAVI	Left-atrial volume index
LS	Low-salt
LV	Left ventricle
LVEDP	Left-ventricular end-diastolic pressure
MCT	Moderate intensity continuous training
MET	Metabolic equivalent
NT-proBNP	N-terminal of the prohormone brain natriuretic peptide
NYHA	The New York Heart Association functional class
RAAS	Renin-angiotensin-aldosterone system
SD	Standard deviation
SV	Stroke volume
TD	Tissue Doppler
TL	Tibial length
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
vWF	Von Willebrand factor

INTRODUCTION

Overview

Cardiovascular diseases are the leading cause of death worldwide [1] and correlate with a number of risk factors present in modern societies, such as inactive lifestyle, poor diet and obesity [2]. Heart failure (HF) with a preserved ejection fraction (HFPEF) is one of the diseases with escalating prevalence and low survival rates, yet the underlying pathophysiology is still poorly understood and existing treatment strategies do not effectively reduce the high mortality rates among patients [3]. Unspecific etiology of the disease, varying extent of structural and functional changes, as well as multi-organ involvement, makes it challenging to target experimental studies and effectively address pathological mechanisms [4].

Current treatments of HFPEF focus on relieving symptoms of the disease and improving overall survival and exercise training has been proven to have a beneficial role in increasing exercise capacity and quality of life among HFPEF patients [3, 5]. Cardiorespiratory fitness is a well established predictor of overall mortality [6] and exercise training is a safe and promising HF treatment strategy [7]. However, the effects of exercise training on cardiac function, structure and periphery in HFPEF are still debated and different studies present varying results [8]. Moderate continuous exercise training has been recommended for HFPEF patients and is commonly applied in clinical and experimental trials [7]. At the same time, high-intensity interval training has been proven to be superior in improving exercise capacity [9] and induced number of beneficial changes in the structure and function of the myocardium in patients with systolic HF [10]. The optimal volume of exercise in HFPEF is not established and the dose-response relationship must be further determined. Experimental models should be applied to evaluate safety, efficiency and underlying mechanisms of different volumes of exercise in HFPEF.

Animal models are commonly used to study changes in myocardial structure and function and provide clear concepts for specific investigations in clinical trials [11]. Dahl-salt sensitive (DS) rat model is an established model of isolated diastolic HF and mimics disease characteristics observed in patients [12]. Combining novel exercise training protocols with varying volume of exercise in an experimental DS model of diastolic HF, will allow for studying the underlying pathology of the disease and will give a better insight into the physiological alterations induced by exercise training in cardiac, as well as kidney function

and structure. Most importantly, the study can be a contribution to understanding the exercise training effects on survival rates in DS rats and the predominant causes of mortality in this model.

Systolic and diastolic heart failure

HF is defined as an impaired ability of the heart to deliver oxygen and substrate to satisfy the working muscle, including the heart itself [3]. Typical clinical symptoms are fatigue, shortness of breath, signs of fluid retention and pathological changes in the structure or function of the heart at rest and mostly during physical exertion. Exercise intolerance is common and causes further progressive deterioration with worsening of the functional status and the quality of life in HF patients [13].

HF is typically subdivided into two groups: systolic or diastolic. The clinical distinction and the terminology used to describe the condition have been traditionally based on the measurements of left ventricular (LV) ejection fraction (EF) - defined as stroke volume (SV) divided by end-diastolic volume (EDV). According to this distinction, patients suffering from systolic HF have a reduced LV ejection fraction (HFREF) of less than ≤ 35 %, while diastolic HF is characterized as HFPEF of at least ≥ 50 % [3]. In systolic HF, when LV contraction and emptying are reduced, SV is maintained by an increase in end-diastolic volume due to the dilation and eccentric hypertrophy of the LV [3]. The main features of diastolic HF are impaired LV relaxation and increased LV stiffness, usually without present dilation of the LV but with traits of concentric hypertrophy, commonly induced by pressure overload [14].

HFREF is a better understood type of HF and has dominated the previous studies with more extensive exploration of underlying pathophysiology and treatment strategies, while the mechanisms behind and treatments for HFPEF are poorly understood and reflect on low survival among patients [3].

Prevalence and incidence of heart failure

The burden of the disease is escalating due to the new social and health challenges. Based on the epidemiological data [13, 15, 16], HF is evident in between 2 - 3 % of the population. About 15 million Europeans (of total 900) and 5.8 million Americans (of 300) suffer from HF [13, 17]. Since HF is mainly "a disease of the elderly" with prevalence rising after reaching 75 years of age, the overall incidence is increasing due to the aging of populations and improved survival rates for patients with cardiovascular diseases [18]. According to Lazzarini

et al. [19], HF is typically first diagnosed at the age of 80, because older patients are prone to longer exposure to cardiovascular and lifestyle related risk factors for development of HF. Additionally, physiological cardiac aging is associated with changes in cardiac structure and function, putting the elderly at greater risk of developing HF [19].

A number of studies point towards similar trends in distribution of systolic and diastolic HF [3, 20, 21], with at least 50 % of patients suffering from HFPEF. Additionally, since the new cases of HFPEF are mainly "age-specific", affecting older individuals, the proportions of HF distribution are progressing towards increasing HFPEF among HF patients. The changing profile of HF is also linked to the associated cardiovascular diseases underlying HFPEF, with higher prevalence of inactivity, hypertension, diabetes and atrial fibrillation in the general population [20, 21].

Prognosis

In general terms, survival rates are alarmingly poor in HF patients and about 50 % die within four years of the initial admission to the hospital [13]. The impact of age on mortality rates is shown to be particularly strong in HF patients; with an increase of 26 % for every 10 years increase in age following initial diagnosis [22, 23]. An analysis of 30 cohort studies, including data on 39.372 HFPEF and HFREF patients revealed 40 % mortality rates with a median follow-up of 2.5 years [22]. Cowie et al. [23] reported survival rates of 81 % at one month, 75 % at three months, 70 % at six months, 62 % at 12 months and 57 % at 18 months in a cohort of patients with both systolic and diastolic HF. Statistical data from US (latest available update: 2007) link HF to as many as one out of 9 deaths in total [17].

However, there is a lack of consensus about survival and mortality rates in systolic and diastolic HF. Some studies point towards similar prognosis [13, 24], while others argue for higher mortality rates among subjects diagnosed with systolic HF. Owan et al. [20] reported 29 % mortality rates for HFPEF versus 32 % for HFREF at one year of follow-up and respectively 68 % versus 65 % at five years. Survival rates in HFREF patients were improved over time, but not in the HFPEF, since there is a scarce evidence about effective treatment strategies for HFPEF. The mortality rates among HFPEF patients are expected to rise, based on the growing incidence and poor prognosis for the disease. Epidemiological evolution towards dominance of HFPEF in aging populations calls for better understanding of the disease and for development of novel treatment options.

Etiology of HFPEF

Etiological profile of diastolic HF differs from systolic HF. Typically HFPEF patients are older (mean age 71 vs. 66 years), diabetic, obese and of female gender. About 67 % of women compare to 42 % of men suffer from diastolic HF [17]. Hypertension and atrial fibrillation dominate the spectrum of main risk factors for developing HFPEF [25]. The primary and underlying risk for developing both types of HF relates to lifestyle factors such as body weight, smoking, alcohol intake, exercise habits and diet [2, 20, 26]. Obesity is especially prominent in patients with diastolic HF [27]. The lifetime risk of HF can be reduced by maintaining healthy lifestyle [2] and the primary prevention should focus on targeting individuals from the risk populations to decrease the incidence of HF [28]

Diagnostic criteria for HFPEF

Diagnostic criteria for establishing HFPEF are complex and require number of clinical assessments. The cut-off values for diagnosing HFPEF still vary between different studies, possibly contributing to the diverse study outcomes [29]. However, three obligatory conditions must be met to establish the diagnosis of HFPEF: presence of signs or symptoms of congestive HF, presence of normal or mildly abnormal LV systolic function and the evidence of LV diastolic dysfunction [3, 14].

Symptoms and signs

Many of the symptoms and signs of HF result from salt and water retention and are non-specific and cannot be discriminated between HF or other risk factors such as obesity or elderly age. At the same time, assessment of symptoms and signs is highly relevant in clinical practice to evaluate the patients response to treatments [3]. Typical signs and symptoms of congestive HF include pulmonary edema, ankle swelling, hepatomegaly, dyspnea on exertion and fatigue. Dyspnea is one of the earliest symptoms due to the pulmonary congestion, however is difficult to interpret in obese or elderly patients. Effort related dyspnea can be measured by exercise testing with the peak oxygen uptake (VO_{2peak}) values of $VO_{2peak} < 25$ $mL \cdot kg^{-1} \cdot min^{-1}$ (reduced) and $VO_{2peak} < 14$ $mL \cdot kg^{-1} \cdot min^{-1}$ (low) [14].

Normal or mildly abnormal left-ventricular systolic function

Objective evidence of structural and functional abnormalities is necessary to establish the diagnosis of HF and most common involves echocardiographic examination, also to determine EF. The cut-off values for defining EF differed in previous year, however LVEF $\geq$ 50 % is now commonly accepted and consistent with the presence of normal or mildly abnormal LV systolic function [30]. The definition of normal systolic function must be further extended by the measures of LV volumes. To exclude LV enlargement, LV end-diastolic volume index and LV end-systolic volume index cannot exceed 97 mL/m^2 and 49 mL/m^2, respectively, as proposed by Paulus et al. [14].

Evidence of abnormal left-ventricular relaxation, filling, diastolic distensibility and stiffness

In hypertensive patients, LV diastolic function abnormalities occur early in the course of the disease, often before detectable changes in LV structure [31]. The measurements of diastolic dysfunction can be obtained either invasively through cardiac catheterization, providing definite evidence of HFPEF or non-invasively through echocardiographic tissue Doppler (TD) evaluations.

The definite evidence for HFPEF in patients includes values of time constant LV relaxation > 48 ms, LV end-diastolic pressure > 16 mmHg, mean pulmonary capillary wedge pressure > 12 mmHg and diastolic stiffness modulus > 0.27. Elevated LV end-diastolic pressure (LVEDP) or pulmonary capillary wedge pressure in the presence of a normal LV index, point towards reduced LV end-diastolic distensibility (the position on a pressure-volume plot of the LV diastolic pressure-volume relation), [14].

Non-invasive diagnostic of diastolic LV dysfunction is preferably derived from myocardial TD. The E/E' ratio can be used as a marker of elevated filling pressures and refers to the ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E'). E wave depends on left-atrial (LA) driving pressure, LV relaxation and age, while E' depends mostly on LV relaxation kinetics and age. Therefore, in the E/E' ratio, the effects of LV relaxation and age can be eliminated and the ratio becomes a measure of LA driving pressure or LV filling pressure. The E/E' ratio exceeding 15, indicate highly elevated LV filling pressures and diagnostic evidence of LV diastolic dysfunction and the ratio lower than 8, marks low LV filling pressures, providing evidence of the absence of HFPEF. The ratio

ranging from 8 - 15 is suggestive, but non-diagnostic and calls for additional non-invasive investigation to confirm the diagnosis [14].

Inconclusive E/E' ratio can be supplemented with a marker of LA volume index (LAVI), proposed as a robust biomarker of diastolic LV dysfunction and cardiovascular risk. LAVI is strongly associated with the severity and duration of diastolic dysfunction and increases progressively with worsening of the disease. LAVI > 40 mL/m^2 provides sufficient evidence of diastolic LV dysfunction. Furthermore, an electrocardiogram stating atrial fibrillation can be considered as an evidence for diagnosis [14]. Alternative supplementary non-invasive measurement can be the E/A ratio, describing peak flow velocity during early filling (E) to atrial systole (A) or Ard-Ad index referring to the difference in duration of pulmonary venous and mitral flow at atrial contraction [31].

Hormonal responses to HF are also typically measured when the disease is suspected. Atrial and brain natruiretic peptides (ANP, BNP), the products of atrial and ventricular myocardium, are produced in a response to increased atrial or ventricular stretch and their compensatory response results in vasodilation and enhanced LV relaxation. Cardiac myocytes produce pro-BNP, which is cleaved and detectable in blood as NT-proBNP (N-terminal of the prohormone brain natriuretic peptide) and BNP and serve as HF biomarkers. NT-proBNP levels correlate with early diastolic LV relaxation and LV stiffness modulus. To consider HFPEF diagnosis in patients, a high predictive value was set at 220 pg/ml of NT-pro BNP and 200 pg/ml of BNP [14].

Structural changes

Patients with HFPEF present concentric LV hypertrophy, characterized by a high LV wall mass-volume ratio and large cardiomocyte diameter [27]. Cardiac hypertrophy is one of the most severe changes following HFPEF and correlates with increased mortality; however the patterns of remodeling in HFPEF have been inconsistent between the studies. About 40 % of HFPEF patients do not meet the criteria for LV hypertrophy and can even present with traits of eccentric remodeling [32, 33]. At the same time, the extent of concentric LV remodeling is an important implication for the diagnosis and can be considered as a solid evidence of diastolic LV dysfunction. When TD yields non-conclusive results (15 > E/E' > 8), the LV mass index > 122g /m^2 (females) or > 149g/m^2 (males) claim sufficient evidence for establishing HFPEF diagnosis [14].

LA dimensions can be also enlarged in HFPEF, with the extent of atrial remodeling serving as an objective index of severity of HF. Changes in the LA are of special significance, because they indicate the scope of pathological changes over prolonged period of time with the increased LA size reflecting the severity and chronically increased LV filling pressures [32]. Summarized diagnostic criteria, as proposed by Paulus et al. [14] are presented in Figure 1.

Current treatment recommendations

According to the guidelines of European Society of Cardiology [3], the main objectives for the management of HF focus on relieving symptoms of the disease, preventing hospital admissions and improving overall survival. Systolic HF is commonly managed by pharmacological treatments, such as neurohumoral antagonists (ACE inhibitors, ARB's, MRA) or by device treatments, proven to be effective in improving clinical outcomes. On the contrary, there are no established treatments up to date, proving to reduce morbidity and mortality in HFPEF patients. Current treatment of HFPEF is limited to the management of comorbidities and prescription of diuretic medications. Specific drugs can improve diastolic function or structure, however without correlation to clinical outcomes. Different drug trials performed (ACE-inhibitors, ARB's, ß-blockers), showed no reduction in hospitalizations or cardiovascular deaths among HFPEF patients, as summarized by Butler et al. [4, Table 2] and McMurray et al. [3].

Unsuccessful trials in HFPEF can relate to unspecific etiology of the disease and large heterogeneity and variation between patients. Foremost, there is an urge to identify phenotypic characterization and categorization of the treatment groups, to improve targeting of experimental studies. As described earlier, there is a lack of consensus about exact definition and classification of HFPEF patients and the grades of diastolic dysfunction and structural remodeling can vary in-between patients. The pathophysiology of HFPEF is complex and relates not solely to cardiac function and structure, but also to peripheral factors, systemic and pulmonary vascular abnormalities, end-organ involvement, kidney disease and comorbidities [4].

Exercise training

The current treatment guidelines recommend exercise training for HF patients, listing a number of benefits such as improvement of functional capacity, health-related quality of life and reduced HF hospitalizations [3]. Exercise training is now recognized to be effective in

improving HF specific exercise intolerance and applicable for stable HF patients from NYHA class I-III [7]. Recommended training intensity is the moderate continuous aerobic training at 40-50 % VO_{2peak} at the beginning and increasing up to 70-80 % of VO_{2peak}. However, the further statement about exercise in HF underlies the importance of individualized approach in choosing optimal training protocol. Benefits of different modalities such as intensive interval training or resistance training are also pointed out and should be taken into consideration [7].

Exercise training and mortality

Cardiorespiratory fitness, commonly expressed as exercise capacity is a well established predictor of overall mortality, both in healthy subjects and in patients with cardiovascular diseases [6]. Exercise can be used as an effective primary and secondary prevention, attenuating the risks of premature death in subjects already affected by the disease [34]. The evidence of protective effects of exercise on cardiovascular diseases and all-cause mortality is solid and extensive, including large cohort studies with long follow-up periods [5].

VO_{2peak} has been shown to have a stronger survival predictive power than other established risk factors or clinical variables. Applying the measure of metabolic equivalent (MET, oxygen uptake of ~3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) to assess the exercise capacity; an increase in 1 MET only, corresponds to 12 % improved survival rates [35]. Furthermore, individuals with the lowest VO_{2peak} benefit the most from improvements in overall fitness. The protective effect of exercise training is therefore graded and subjects with the highest fitness level have the lowest risk of premature death, indicating a dose-response relationship. Among patients with cardiovascular diseases, there is a nearly linear correlation between the reduction in risks of mortality with improved VO_{2peak} [35].

Physical fitness is a strong predictor of overall and cardiovascular mortality even when the different risk factors such as smoking, resting heart rate, systolic blood pressure (BP) and bodyweight are taken into account [36]. In other words, subject with high levels of fitness and other risk factors for cardiovascular diseases may be at lower risk of premature mortality than sedentary subjects with no risk factors for cardiovascular diseases [34].

Despite well-established knowledge about beneficial effects of exercise, patients suffering from cardiovascular diseases are often categorized as generally inactive. Dontje et al. [37] examined the levels of physical activity among 68 HF patients; showing low levels of daily physical activity and sedentary lifestyle in over half of the study participants.

Furthermore, VO_{2peak} is not only the predictor of mortality but also of healthcare expenses, including patients with and without cardiovascular diseases. The costs of healthcare were substantially reduced 5.4 % by average with an increase in 1 MET, proving the fitness levels not to be only the expression of health but also the indicator of economic resource utilization; as an example the combined costs of inactivity and associated obesity approximate 9.4 % of all the health care expenses in the US annually [38].

Up to date, the studies examining effects of exercise training on reducing mortality and improving survival exclusively involved HFREF patients. Most studies reported statistical association between improved exercise capacity and better clinical outcomes, such as reduced mortality and hospitalization [39-42]. A small proportion of studies examined the mortality rates during the time-limited follow-up periods (2.5 - 10 years) and similarly, reported improved survival rates and a reduced number of adverse cardiac events [43, 44]. The subject of HFPEF and the effects of exercise interventions on mortality among patients, is even less explored and call for a more comprehensive evaluation.

Exercise training and HFPEF

Abnormal diastolic function considerably decreases cardiac output; the heart is unable to satisfy metabolic demands of the body and, as a result, exercise capacity is largely impaired. In addition, increase in diastolic pressures elevates lung blood volume and triggers pulmonary congestion and dyspnea, reducing exercise tolerance in HFPEF patients [45]. HFPEF is a complex disease and the multiple components of cardiovascular system and bodily organs are affected. The rationale behind exercise intolerance is still inadequately understood and the peripheral, endothelial, vascular, chronotropic and systolic factors are believed to affect diminished exercise capacity as much as diastolic dysfunction [46].

One of the first studies [47] on diastolic HF and physical activity examined the effects of 12-week home-based, low-to-moderate intensity (40 % and 60 % of maximal heart rate (HR_{max})) walking intervention in elderly women. The results showed improved 6-minute walk test and enhanced quality of life. Walking distance was also improved and correlated with better exercise self-efficacy [48].

Similarly, studies investigating effects of exercise training among older HFPEF patients (70 ± 6 years, 83 % females, BMI over 30 and functional class > II) reported improved $VO_{2\ peak}$, self-reported quality of life, power outputs and exercise time [49]. The intervention consisted of combined treadmill walking and cycling for 16 weeks/ three times a week, one hour

continuous training, starting at 40 % of HR reserve the first two weeks, followed by 60 % - 70 % over the next 14 weeks. Abnormal diastolic relaxation was evident in 71 % of the patients. Baseline Doppler echocardiography measurements reported increased LV mass and mass/volume ratio indicative of concentric LV remodeling and impaired relaxation. The exercise intervention did not affect the diastolic parameters. BNP levels also did not change, although the authors mentioned that the patients were stable, well compensated and the baseline BNP levels were generally low, concluding that the results may not apply to individuals with more severe HF [49].

Smart et al. [50] aimed the exercise intervention to examine changes in cardiac function more closely, in addition to exercise capacity. Patients were generally younger (67 ± 5.8 years) and presented with a moderate diastolic dysfunction, defined by either delayed relaxation or pseudonormal filling. The training group underwent ergometer cycling intervention for a standard period of 16 weeks, three sessions/ week at an intensity of 60 - 70 % $VO_{2peak.}$ Similar to other studies, the patients increased their VO_{2peak} by 24 %. Results from echocardiographic examination did not show significant difference in neither E/A or E/E' ratios. The study concluded that the improvements facilitated by exercise training were limited to gains in exercise capacity, but the mechanisms behind are uncertain and could be related to the peripheral rather than cardiac factors.

The study performed by Edelmann et al. [51] is apparently the only trial directly observing improved diastolic function, as a result of exercise training in HFPEF patients. The study population consisted of 56 % females, 65 ± 7 years of age. 72 % of patients had a grade-I diastolic dysfunction, 89 % had overweight and 86 % had hypertension. Exercise intervention was a combination of aerobic endurance training (cycling at 50 - 60 % $VO_{2\ peak}$) and resistance training from week 5 (60 - 65 % of one repetition maximum). The increase in $VO_{2\ peak}$ (38 %) correlated with a reduction in E/E' ratio (-3.2). The atrial volume was decreased, possibly demonstrating a lower need for LV compensation and better relaxation during diastole. LV volume index was not changed, however it might be a misleading result, due to the possible athletic hypertrophy effects. Correspondingly, the levels of BNP were not elevated, indicating reduced severity of the pathological hypertrophy. Additionally, the level of type-I collagen was reduced by exercise training, indicating lower degree of myocardial stiffness. Improved diastolic function may be associated with reduction in collagen turnover, affecting stiffness of the muscle, the overall pump function and exercise capacity [27].

Alves et al. [52] analyzed how the exercise training intervention influence LV systolic function and structure in both HFREF and HEPEF patients. HFPEF group included 31 subjects, at younger age (62.9 ± 10.2 years), mostly males (22/ total 31), obese and of NYHA class II (39 %) and III (55 %). Training consisted of intervals of treadmill running or cycling, at 70 - 75 % HR_{max} and 1-min active recovery at 45 - 55 % HR_{max}. Interval aerobic training resulted in better LVEF in all patient groups, with HFPEF patients showing improvement from 56 to 58 %. Exercise tolerance improved by 45 %. Also, diastolic function expressed by E/A ratio increased and the E wave deceleration time decreased. An interesting finding of this study related to enhanced systolic function, also in patients with HFPEF. Myocardial contractile dysfunction might be explained by concentric remodeling and LV stiffness. Although the systolic dysfunction is not as much expressed as in HFREF, the small alteration can also contribute to exercise intolerance in HFPEF patients. Alves et al. [52] hypothesized further, that since the concentric remodeling and LV dimensions in HFPEF were not affected by exercise training (in contrast to HFREF), the improved contractile function could have occurred through reduced ventricular stiffening and lower collagen deposition.

There is a lack of consensus whether the main attributor to improved exercise capacity in HFPEF undergoing exercise training relates to cardiac factors or to the adaptations in the periphery. HFPEF is nearly exclusively affecting older individuals, and it is known that aging results in deterioration of skeletal muscles, such as decrease in relative number of type-II fibers and capillary density, associated with reduced exercise performance [53]. Additionally, the alterations in skeletal muscle of HF patients relate to the reduction of type-I, slow-twitch oxidative fibers, further contributing to severe exercise intolerance [54].

Kitzman et al. [55] conducted a study to determine whether exercise intolerance in HFPEF is caused by skeletal muscle abnormalities. A number of 22 older HFPEF patients (70 ± 7 years) were included, with a control group of 43 age-matched healthy subjects. 82 % of patients were females, NYHA class-II (77 %) to III (23 %) and overweight. In HFPEF patients, VO_{2peak} was reduced compared to control group, as well as 6-minute walk-distance. HFPEF patients had a reduced percentage of type-I fibers, type-I/ II fiber ratio, capillary to fiber ratio, with the changes related to lower VO_{2peak} values. Overall, the percent of type-II fibers was greater in HFPEF than in control group of the same age. Taken together, the results from this study confirmed that abnormalities in skeletal muscles in HFPEF exceed changes that would be expected from aging alone and help to explain severely impaired exercise capacity.

Additionally, Haykowsky performed a number of studies [56-58] examining skeletal muscle abnormalities in relation to impaired VO_{2peak} in older HFPEF patients and pointed out a number of alterations, that together with reduced cardiac output contribute to exercise intolerance. HFPEF patients showed reduced percent total and lean body mass compared to healthy, age-matched control group and increased thigh intermuscular fat and intermuscular fat/skeletal muscle ratio, that both related to lower VO_{2peak} [58]. Moreover, HFPEF patients showed reduced peak exercise arteriovenous-oxygen difference, suggesting inefficiency of the muscles to extract the substrate [56].

In summary, the numbers of studies examining the effects of exercise training in HFPEF are limited, but existing findings from small-scale studies are promising and indicate possible new treatment strategies for this patient population. The common consensus relates to the beneficial role of exercise in increasing exercise capacity and related quality of life, but the impact of exercise on diastolic function and peripheral adaptations is still debated and different studies presents varying results [8].

Different training intensities

All of the described trials operated with moderate training intensities, ranging from 40 % - 75 % of HR_{max}, as recommended by current guidelines [3]. Over the past years, a number of studies have demonstrated the superiority of high-intensity interval training over moderate intensities in improving maximal oxygen uptake (VO_{2max}), [9]. The underlying principle behind the high-intensity interval training is to combine bouts of intensive exercise with lower intensities that allow for recovery and as a result, prolong the exercise time performed at higher intensities [59].

Interval training has been shown to exert greater adaptations also in HF patients. Smart and Steele [60] examined the effects of continuous and intermittent training in 23 HFREF patients. Both groups performed stationary cycling for 16 weeks at the intensity of 70 % $VO_{2peak,}$ three times per week, either 30 minutes continuously or 60 minutes intermittently (60 seconds work - 60 seconds rest). The results showed 13 % VO_{2peak} increase in continuous exercise group versus 21 % increase in the intermittent group. Functional capacity was significantly more improved in the group performing intermittent training sessions.

Wisloff et al. [10] compared moderate continuous training (70 % HR_{peak}) with aerobic interval training (95 % HR_{peak}) in the group of 27 post-infarction HFREF patients (mean age 75 years). Exercise capacity increased in both groups following 12 weeks of training. Changes in

the interval group were especially pronounced with $VO_{2\,peak}$ improving by as much as 46 % compared to 14 % in the moderate intensity group. Importantly, intensive interval training initiated reverse LV remodeling with diastolic and systolic diameters decreasing by 12 % and 15 %, respectively. Bio-markers of hypertrophy and severity of HF (pro BNP) decreased by 40 %. Systolic function was improved by increasing LVEF by 10 percentage points and the SV by 17 %. Diastolic function and ventricular relaxation improved by 49 %, as well as the endothelial function measured by flow-mediated dilation. Rognmo et al. [61] demonstrated in a large multicenter study, that both high-intensity and moderate-intensity training were safe in coronary heart disease patients, with a low risk of cardiovascular events compared to number of exercise hours.

Exercise at moderate intensity improved $VO_{2\,peak}$ in patients but was not sufficient to induce intrinsic changes in the heart itself, to reverse the pathologies following HF, while high-intensity exercise was shown to benefit the heart and produced significant biological and clinical improvements [10]. Thus, the extent of structural and functional changes of the cardiomyocytes depends predominantly on the training intensities, both in patients and healthy humans, and the adaptations are integral to the improvements in VO_{2max} [59].

The data on HFPEF and the optimal volume (frequency x intensity x duration) of exercise are still limited and further research is needed to determine the dose-response relationship. The experimental study by Emter et al. [62] reported sudden death of several younger rats, when the animals were running at higher speeds (17.5 m/min) and no incidences of death when the running speed was reduced to 14 m/min. Similarly, several experimental studies designed to measure the independent effects of exercise training on mortality in rat-models, have shown varied results. Studies utilizing low-intensity exercise intervention in spontaneously hypertensive rats reported increased survival and attenuated development of HF [63]; including a number of improved cellular and systemic physiological alterations, such as delaying the onset of LV dilation, restoration of coronary capillary growth and suppression of pathological cardiomyocyte enlargement and fibrosis [62, 64]. However, other studies reported spontaneous deaths in a substantial number of rats (67 %), performing free-running wheel-exercise without additional medical treatment [65].

Therefore, the optimal intensity of training is still questioned and the diverse results from previous protocols on exercise and HFPEF emphasize the importance of establishing a threshold exercise intensity to prevent disease development and improve diastolic function.

Heart failure and kidney dysfunction

As discussed earlier, pathophysiology of HFPEF is complex and relates not solely to cardiac function and structure, but also to peripheral factors and end-organ involvement [4]. Chronic kidney disease (CKD) is a common co-morbidity in HF, and similarly, patients with kidney dysfunction are at higher risk of developing cardiovascular diseases. Briefly, CKD is characterized by the pathological structural remodeling or markers of kidney damage; including abnormalities in the composition of urine, such as elevated proteinuria excretion or elevated levels of creatinine in blood serum. A typical feature of CKD is a reduced glomural filtration rate (GFR), often estimated by the measurements of creatinine clearance. In patients, $GFR < 60$ mL/min/1.73 m^2 for ≥ 3 months is indicative of CKD [66].

HF adversely affects renal function and renal insufficiency further deteriorates HF, producing a vicious circle. CKD and HF share the same underlying risk factor contributing to worsening of the condition, namely, hypertension. The co-occurrence of CKD and HF poorly affect the clinical outcomes and confer with higher mortality rates among patients. The physiological interplay between CKD and HF is mostly related to increased salt retention and following volume expansion through increased renin production, upregulation of neurohormonal pathways and inflammatory mechanisms. Correspondingly, HF worsens CKD by decreasing renal perfusion, caused by lower cardiac output, and by activation of the renin-angiotensin aldosterone system (RAAS), [67]. Ang II, the primary biologically active product of RAAS, stimulates peripheral vascular resistance, in order to elevate BP and volume to restore renal perfusion, and in result increases cardiac afterload. LV is forced to work against higher pressures, what in response stimulates structural pathological remodeling [68, 69].

Patients with CKD have a high incidence of diastolic dysfunction and structural cardiac changes, such as increased LV mass and raised troponin-T levels, associated with myocyte damage [70]. In a study of disadvantageous effects of kidney dysfunction on LV diastolic function in patients with normal EF, a number of structural and diastolic abnormalities increased in parallel with the severity of kidney dysfunction. Changes were observed in LA diameter, interventricular septum thickness and posterior wall thickness. Assessment of diastolic function by TD showed increasing E/A ratio with progressing kidney dysfunction, observed even in patients with early stages of CKD [71]. Declining diastolic function and pathological structural changes were associated with adverse cardiac events and higher mortality rates compared to CKD patients without a specter of such risk factors [70].

Additionally, the majority of patients with CKD do not progress into advanced stages of renal disease because a mortal event is usually preceding the progression to end-stage renal disease [72]. A large study examining the rates of end-stage renal disease, cardiovascular and non-cardiovascular deaths and risk factors for end-stage renal disease, revealed that older adults (mean age 75 years) with CKD are more likely to die from any cause than to progress into the final stages of renal disease. During a follow-up of 14 years, 44 % of deaths were due to the cardiovascular causes, predominantly HF [73].

The current guidelines on CKD advocate lifestyle modifications such as changes in the diet, with an emphasis on low sodium intake and exercise training, as a primary disease prevention [66]. The statement on exercise and CKD recommends aerobic exercise at an intensity of > 60 % of maximum capacity to improve cardiorespiratory fitness, however the optimal dose of exercise that exerts most beneficial changes on renal parameters is still unclear [74].

Animal models of heart failure

Animal models are commonly used in translational science to study the changes in myocardial structure and function, vasculature and muscles, that otherwise would be challenging to study in human subjects. Animal models are specially valuable for studying underlying mechanism of a disease. Human diseases can be reproduced in animal species and experimental models provide good evidence for disease causation. The main goal of animal studies is to provide a clear concept for selected and specific investigation in humans [11].

Rodent models, particularly rat, are most commonly used in the study of cardiovascular and renal disease [75]. As discussed earlier, HF and kidney dysfunction are closely linked together, with hypertension as a primary and underlying risk factor. This physiological interplay was used, to generate experimental models that reflects the disease in patients. The first animal models utilized mechanical constriction of blood supply to the kidney to induce elevated BP [76, 77]. The pressure overload models are still commonly used to study the adverse effects of hypertension on cardiac and renal physiology.

Aortic Banding Model, with surgical narrowing of the aorta by about 50 %, produces LV hypertrophy, increases LV stiffness and reduces relaxation, what makes it interesting for studying diastolic dysfunction [78]. Around 18 - 20 weeks of compensated hypertrophy, LV systolic pressure decreases, accompanied by increasing LV volumes, reduced EF and progression into HF [79].

Spontaneously Hypertensive Rat is another model of primary hypertension, with reported increase in wall thickness, impaired relaxation and chamber stiffness. In this model, hypertension begins when the animals are approximately two months of age and is followed by a long period of compensated hypertrophy. However, the indications of LV pump dysfunction appear at 18 months of age with a prevalent impaired contractile function and LV dilation [79, 80].

Moreover, the alterations in cardiac structure and function can be reduced directly by inducing renal damage. In 5/6 Nephrectomy Model, one kidney is removed and after interval of 1 - 2 weeks, the contralateral kidney is either subjected to ligation of two or three branches of its renal artery [75] or 5/6 of the total renal mass is removed [81]. Nephrectomized rats, already four weeks after surgery, show reduced cardiac output, hypertensive BP levels and LV hypertrophy characterized by increased cross-sectional area of cardiomyocytes [82, 83]. At the same time, contraction force declines and both LV contraction and relaxation time are prolonged [81].

Dahl-salt-sensitive rat model

Overall, the animal models of HFPEF are scarce and, as presented, often associate with gradual transition into systolic HF [11, 80]. One of the broadly used and recognized HFPEF models is Dahl-salt-sensitive (DS) rat model, since it mirrors the isolated diastolic HF observed in clinical practice [12], what makes it the model of choice for the current study. Originally, DS rats are a strain of Sprague-Dawley rats, that show hypersensitivity to intake of sodium and placed on a high-salt (HS) diet are prone to develop concentric LV hypertrophy without dilation of the chamber and decreased LV fractional shortening associated with systolic HF [12, 79].

The time-frame of initiating the diet and the amount of sodium supplied in the chow is essential for the course of cardiac remodeling and the life expectancy of the animals [84]. Placing the 7-week old DS rats on a HS diet (8 % NaCl) is shown to cause a steep elevation in BP, compensated LV hypertrophy at 13-weeks and signs of HF at 19-weeks, including increase in E/A, LVEDP, lung edema and congestive liver [12]. Moreover, salt and water handling impairments in DS rats affect number of extra-cardiac factors, such as kidney function, leading to the progressive worsening of HF [85]. The abnormal DS kidney tends to retain NaCl at a higher rate due to decrease in GFR, showing impaired reserve capacity for increasing GF [86]. The combination of cardiac and renal dysfunction in this model allows for

studying of HFPEF closely related to disease etiology observed in clinical settings, possibly explaining the complex underlying mechanisms.

Study aims and hypothesis

Taking into account the growing dominance of HFPEF in aging populations, rising healthcare expanses and high mortality rates among HFPEF patients, there is a need to study the mechanisms of the disease and to develop novel treatment strategies. Considering beneficial effects of exercise training on improving exercise capacity, quality of life and clinical outcomes in HF patients, it is important to establish an optimal training protocol and to determine its impact on HFPEF pathophysiology. The animal model of DS rat gives a better chance to evaluate development of HFPEF, changes in function and structure of the myocardium, multi-organ involvement, mortality and its underlying causes.

Therefore, the main aim of the study is to examine the development of HFPEF in DS rats and to evaluate the effects of different training volumes, as dose-response relationship, between exercise training, cardiac function, structure and mortality. Moreover, considering the connection between kidney function and HFPEF, renal parameters and biomarkers of severity of HF will be measured and evaluated.

The study hypothesis is that exercise training will alter cardiac function and structure in DS rats, possibly preventing the development of HFPEF and will prolong the time until mortal event happens. In addition, we hypothesize that training intensity is of a greater significance than training duration on improving HFPEF pathophysiology and related mortality rates.

METHODS

Animal model

A total number of 120 female DS rats were included in the study and the animals were obtained from Charles River Laboratories International (Connecticut, USA) The female gender was chosen purposefully, since HFPEF is more common among females [17] and most of the studies up to date enroll male rats. The rats were divided into five groups: low-salt (LS) sedentary group (n = 20), high-salt (HS) sedentary group (n = 40) and three exercise groups. Low-salt laboratory chow (Special Diet Services, Witham, Essex, UK) containing 0.3 % NaCl was fed to 120 weaning rats until the diet was switched to HS (8 % NaCl) in 100 of the rats at 7-weeks of age. All of the rats were housed under identical conditions in a 12-hours light/dark cycle and given food and water *ad libitum*. All animal procedures were approved by The Norwegian Animal Research Authority, in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) [87].

Exercise training

Three different exercise protocols on a treadmill (Linton Instrumentation, Diss, UK) were completed and all the three groups exercised over a period of 28 weeks. In a well-established protocol on a treadmill [88], the rats performed moderate intensity continuous training (MCT), 5-times 40 min per week at 60 % VO_{2max} (25° elevation). The high-intensity interval training, high-volume (HIT-HV) animals ran 3-times per week, alternating between 4 min running at 90 % VO_{2max} and 3 min active recovery at 60 % VO_{2max}. The high-intensity interval training, low-volume (HIT-LV) group performed only two intervals per day (14 min), while the HIT-HV group ran four intervals per day (28 min), as presented by Figure 2.

Oxygen uptake / total distance run

VO_2 was measured at rest and during the exercise protocol test in a metabolic modular treadmill (Columbus instruments North Hauge Avenue, Columbus Ohio) and a "maximally achieved" O_2 uptake was detected at the limit of animal tolerance. Briefly, the animals were submitted to 15 min of warming up at 25° inclination at 40 - 50 % of VO_{2max}, and treadmill band velocity was increased by 1.8 m min^{-1} every 2 min until they were unable to run further. The criterion for reaching VO_{2max} was a leveling-off of VO_2, despite increased running speed.

The protocol used has previously been validated and described in detail [88]. In this study, the VO_{2max} was measured at a baseline and 4 times during the period of exercise training to adjust for exercise intensity. Total distance run was given as a final summary of running performance and calculated from the total duration and speed protocol (same as described above).

Non-invasive blood pressure measurements

Non-invasive BP measurements were performed by tail cuff Plethysmography for rats (CODA system, Kent Scientific Co., Torrington, CT, USA), every two weeks, after a 7-day acclimatization period. Non-anaesthetized rats were restrained in a thermic plastic chamber and the temperature was maintained between 32 and 34° C. Systolic BP measurements of individual animals represent the average of ten high-quality acquisitions. These measurements were recorded 18h or more after the last exercise training session in the trained rats.

Echocardiography

Transthoracic echocardiography was performed using Vevo 2100 system (Visual Sonics, Ontario, Canada) with a 24-MHz transducer. DS rats were lightly anesthetized but spontaneously breathing (1.5 - 2 % of isoflurane inhalant mixed with 1 L/min 100 % O_2) in the supine position on a heating platform to maintain the body temperature at about 37°C. HR and respiratory physiology were continuously monitored by ECG electrodes. The transducer was placed gently in the left parasternal position by an observer blinded to study group. Blinding was also maintained during all of the analyses. LA dimensions were measured in long axis view at the end-systole, with an M-mode cursor line placed through the LA walls at the level of the aortic valves, with two-dimensional guidance. The LV structural parameters measured from short axis view in M-mode were used in the calculation of LV ejection fraction. In order to estimate LV mass, the area-length method was used [89, 90]. Doppler recordings were obtained from the apical 4-chamber view by positioning the Doppler sample volume parallel to the flow direction. The mitral inflow measurements performed were mitral valve (MV) early wave peak and MV atrial wave peak, with MVE/A ratio calculated. Myocardial velocities were recorded at the level of basal septal segment from the apical view using pulsed Doppler tissue imaging. Early (E') and late (A') diastolic waves were measured, and E/E' ratio was calculated. All measurements were performed excluding the respiration

peaks and obtained in triplicate. All calculated parameters were automatically computed by the Vevo 2100 standard measurement package.

The animals were followed until the terminal event occurred or until the study ended when the remaining animals were 34 or 35 weeks of age. The end of the protocol was determined by echocardiographic measurements and LV filling velocities. When the E/A, E/E' and LA dimensions achieved marked difference from LS, the indices for diagnosing diastolic HF were met and the animals were subjected to sacrifice.

Metabolic cages

Urine samples were collected from rats placed in individual metabolic cages (Nalgene, Rochester, NY, USA) for a period of 24h, three times during the study protocol, at weeks: 13, 19, 28 and included 50 rats. Animals had a free access to drinking water (250 mL bottles) and laboratory chow, according to the allocated diet. The animal's body weight and amount of chow and water consumed were measured and the sodium intake calculated. Urine samples were used to determine: urine output, microalbuminuria, serum creatinine, albumin/creatinine ratio and creatinine clearance. After measuring urine volumes, urine samples were centrifuged at 3000 rpm for 10 min at room temperature and then stored at -20°C. Samples were further subjected to the analysis of serum creatinine by enzymatic method (Roche Modular, Mannheim, Germany) and albumin by immunological method and nephelometry (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring), [91].

Briefly, albuminuria (mg) describes increased urinary excretion of albumin and microalbuminuria (mg/24h^{-1}) refers to albumin excretion above the normal physiological range, but below the level of detection by tests measuring total protein. Creatinine, a waste product of muscle metabolism, is commonly used as a marker of efficiency of kidney filtration. Albumin/creatinine ratio (mg/μmol/L) is an anternative, highly accurate method for quantitative evaluation of proteinuria, that corrects for variations in urinary concentration due to different status of hydration. Creatinine clearance (ml/min^{-1}) estimates the levels of creatinine in a 24h sample of urine to the creatinine level in blood plasma, indicating the filtration capacity of the kidneys [66]

Hemodynamic measurements

Hemodynamic measurements were performed as terminal procedures in all the groups. Shortly, after induction of anesthesia (2 % Isoflurane) and intubation of the animals, the right carotid artery was cannulated with a conductance catheter (1.4 Fr.; Transonic Scisense Inc., London, CA) with a pressure transducer that was advanced across the aortic valve into the LV. LV end-systolic and end-diastolic pressures were measured. After withdrawal of the catheter into the aorta, phasic and mean arterial pressures were recorded. All of the data were recorded on LabChart 7 software (AD Instruments, UK).

Blood analysis

Following hemodynamic measurements, the animals were terminated under deep isoflurane anesthesia (5 %), and the blood was obtained from the LV. NT-proBNP level was measured in plasma (NT-proBNP ELISA kit from CUSABIO, China) according to the manufacturer's specification to determine the levels of a cardiac hypertrophy marker. Plasma was collected using EDTA as an anticoagulant and centrifuged for 5 minutes at 3000 x g, 4°C within 30 minutes of collection. Serum was collected using separator tube allowing samples to clot for 30 min at room temperature before centrifugation for 5 minutes at 3000 x g. Aliquots were stored at -80 °C.

Organ weights

Heart and lung weights were measured immediately after their excision. The hearts were divided into four chambers. Left and right atria and left and right ventricles were weighted. LV was stored at -80°C for protein analysis. The lung wet-to-dry weight ratio was used as an index of lung water accumulation after chronic cardiac pressure overload. The lungs were placed in an oven at 37°C after the first weighting. Every day for 4 days, the lungs were re-weighed as dry weight. The wet-to-dry weight ratio was calculated by dividing the wet by the dry weight.

Histology

Hearts of 3 rats from each study group were perfusion-fixed with a 4 % paraformaldehyde and arrested in diastole, embedded in paraffin and cut into 4-μm sections. Paraffin sections were stained with Haematoxylin and Eosin (H&E) staining for routine histopathological analysis, and Wheat germ agglutinin (WGA)-immunofluorescence staining for cardiomyocyte

cross-sectional area measurement. The cross-sectional area of cardiomyocytes was determined in sagital sections (4-chamber view) of the LV after staining of plasma membrane with rhodamine-labeled wheat germ agglutinin (WGA), (Vector Laboratories, Inc.). The cross-sectional area was determined from 4 different areas of the free-wall of the LV (40x magnification), in 15 individual myocytes per frame, by digital planimetry using the ImageJ software. Summarized experimental design is presented in Figure 3.

Analysis of morbidity and mortality

All rats were under regular veterinary inspections and were controlled for signs of morbidity or moribund state. HS diet and hypertension are associated with stroke mortality in DS model [92] and the animals were observed for clinical signs of stroke throughout the study. Clinical signs will vary depending on the type of stroke, underlying cause and the extent of damage exerted on brain cells. However, typical signs of stroke in rats usually involve abnormal breathing, decreased spontaneous activity, incoordination, falling to one side, hunched posture or paralysis [93].

Statistical analysis

The Kaplan-Meier method (GraphPad Prism, version 5.0) was used to estimate survival distribution and to generate survival curves and log-rank test (IBM SPSS , version 21) was applied to determine survival rates and check the difference in survival distribution between the groups. Data are presented as mean ± standard deviation (SD). Differences between HS and LS groups were calculated using Student's T-test. Differences between all experimental groups were analyzed using 1-way ANOVA test. When the comparisons between the groups indicated statistical significance Tukey or Games-Howell post hoc, all-pairwaise multiple comparisons tests were performed. The significant levels were set to the *p-value < 0.05*.

RESULTS

Results are presented as two separate parts. First, with the focus on DS rat model and the effects of HS diet on development on HF, with comparison between HS and LS groups. Next, the effects of exercise training are described, including all of the exercise groups.

Characterization of a diastolic heart failure rat model

Systolic blood pressure and hemodynamic measurements

HS diet introduced at 7 weeks of age resulted in steep elevation of systolic BP from week 9 in HS group, while LS animals maintained normal (146 ± 8.7 mmHg) levels of BP throughout the protocol (Figure 4.) The HS rats started to reach significantly elevated BP at week 13 (174.6 ± 15.6 mmHg), with an evident increase from the baseline measurements ($p < 0.05$). The difference between HS and LS was apparent from the week 15 (193.8 ± 10.6 vs. 147.9 ± 12.5 mmHg, respectively, $p < 0.05$). Invasive measurements with a catheter inside the LV confirmed high levels of systolic BP and the end-point values were 208.6 ± 23.9 mmHg for HS and 147.4 ± 15.9 mmHg for LS.

Structural remodeling

High BP levels in HS animals were accompanied by a 22 % increase in LV mass (calculated by echocardiographic exam) compare to LS, with an average values of 1114.7 ± 341.9 vs. 883.8 ± 212.8 mg for HS and LS, respectively *(p < 0.05)*. The increase in LV mass of the HS animals was noted at week 14 *(p < 0.05),* and a significant difference between HS and LS rats was apparent from week 12 *(p < 0.05)*. Magnitude of differences in LA dimensions was less evident between the groups when analyzed by echocardiography, with significant difference apparent only at week 21. However, *ex vivo* measurements of tissue weights revealed higher LA weights in HS animals, especially when normalized to tibial length (TL), as presented in Table 1. In addition, cardiomyocyte cross-sectional area was enlarged in HS group *(p < 0.05)* and the analysis of LV hypertrophy index, defined as LV weight/ TL ratio, showed significant difference between the groups (Figure 4.).

Diastolic function and NT-pro BNP

Echocardiographic data showed EF over 50 % throughout the protocol, both in HS and LS animals. Nonetheless, EF decreased progressively in both groups towards the end of the study, with a significant decline firstly apparent at week 23 in HS and week 25 in LS animals. Statistical difference between the groups was reported at week 27 with an average EF of 68.4 $\pm$ 6.2 % for HS and 82.1 $\pm$ 2.8 % for LS $(p < 0.05)$, (Figure 6 A.). EF decreased from 85.9 $\pm$ 5.0 (Pre) to 67.7 $\pm$ 6.6 % (week 35) for HS and from 88.3 $\pm$ 3.4 to 80.2 $\pm$ 5.4 % for LS, corresponding to 21 % vs. 9 %, respectively.

Echocardiographic examination throughout the study, determined a shift in myocardial flow velocities and diastolic E/A and E/E' ratios. Marked decline in E/A ratio was recorded at week 21 in HS group, from 1.6 $\pm$ 0.3 (Pre) to 0.8 $\pm$ 0.2. Thereafter, the E/A increased again towards 1.8 $\pm$ 0.4 at the end of the protocol. Overall, there was no difference between the groups, except from week 24 (Table 2.). Similar changes were observed in E/E' ratio, however the E/E' was significantly elevated at the end of protocol in HS group, reaching the values of 17.8 $\pm$ 4.8 vs. 11.2 $\pm$ 3.7 in LS $(p < 0.05)$, (Figure 6.). Invasive hemodynamic measurements at the end of the study confirmed elevated LVEDP levels in HS animals, with an average of 14.0 $\pm$ 8.3 mmHg vs. 5.9 $\pm$ 2.5 mmHg for LS $(p < 0.05)$, (Figure 6 B.). In addition, the plasma level of NT-pro BNP, the marker of severity of HF, was measured at the study end-point, indicating elevated NT-proBNP in HS group (64.4 $\pm$ 45.1 pg/mL) vs. LS (17.7 $\pm$ 13.5 pg/mL), $(p < 0.05)$, (Table 2.).

Renal function

Table 3. summarizes data on renal function from a sample of 50 rats. Urine output, microalbuminuria and albumin/creatinine ratio were significantly increased in HS vs. LS animals $(p < 0.05)$. Urine output was markedly higher in HS (285 $\pm$ 96 μl.min^{-1}.kg^{-1}) vs. LS (23 $\pm$ 2 μl.min^{-1}.kg^{-1}), corresponding to 82 % increase. Microalbuminuria, from a 24h urine collection were also elevated in HS rats (95 $\pm$ 42 mg.24h^{-1}) vs. LS (0.65 $\pm$ 0.34 mg.24h^{-1}), a 99% increase. Similarly, albumin/creatinine ratio was largely increased in HS (1137 $\pm$ 460) vs. LS (7.9 $\pm$ 3.9), corresponding to 99 % increase. Creatinine and creatinine clearance were not different between the groups.

Signs or symptoms of heart failure

Tissue weights harvested at the study end-point, indicated development of pulmonary edema in HS animals, defined as increased ratio of wet/dry lung weight (HS 5.49 ± 1.2 vs. LS 4.71 ± 0.5, $p < 0.05$), (Figure 7. and Table 1.). The total distance run (m), a measurement of exercise performance and tolerance, was no different between HS and LS animals at the end of the study (Figure. 7), with the average values of 282.7 ± 116.1 m for HS and 238.6 ± 59.6 m for LS.

VO_{2max}, could not be considered as objective in our study, due to the trained animals getting accustomed to electrical grid at the bottom of the treadmill. Sedentary animals were not in contact with electrical grid on a regular basis, and generally performed better under VO_{2max} testing than trained animals, possibly biasing results. To avoid the bias, the method of exercise testing was changed to open chamber, without electrical stimulation, but with a direct supervision and encouragement of each animal by a blinded to the study group observer and total distance run was given as an evaluation of running performance.

Mortality and stroke

The Kaplan-Meier survival curve (Figure 8.) illustrates how the survival distribution compared between the groups during a period of 258 days. The mean number of survived days was highest for LS group (209 days), compared to HS group (173 days). The Log-rank pairwise comparison test showed significant differences in survival distribution between HS and LS animals ($p < 0.05$). Only 1 animal from LS group died during the study (5 %), while mortality in HS group approached 57.5 % (Table 4.). None of the animals showed signs of HF preceding mortal event, defined as labored respiration, loss of activity, peripheral edema or sudden variation in body weight. The main established cause of death was stroke, with a total mortality by stroke of 40 % in HS group and 5 % in LS.

Effects of exercise training

Systolic blood pressure

The progressive increase in BP levels over time among the training groups was the same as for HS animals (Figure 9.) There was no difference in systolic BP between HS and exercise groups. LV catheterization confirmed high systolic BP and the end-point values were 213.8 ± 28.3 mmHg for MCT, 211 ± 26.1 mmHg for HIT-LV and 205.4 ± 26.3 mmHg for HIT-HV.

Structural remodeling

Generally, the increase in LV mass, as well as the changes in LA dimensions among all the training groups followed similar patterns as for HS. Figure 10. represents 3 time-points for echocardiographic examination of LV structural remodeling. LV mass was significantly different from LS from week 21 *(p < 0.05)*, but there was no difference between the animals performing exercise training and HS group. There was no statistical difference in atrial remodeling between training groups in comparison to LS and HS, but the end-point measurements of tissue weights were higher among all the groups following HS diet. Cardiomyocyte cross-sectional area was larger among HS groups *(p < 0.05)*, except from HIT-LV that showed no difference vs. LS. Analysis of LV hypertrophy index showed significant differences between all HS groups and LS *(p < 0.05)*, (Figure 10).

Diastolic function and NT-pro BNP

The echocardiographic examination showed EF above 50 % in all of the training groups throughout the study. Gradual decline in EF was observed towards the end of the protocol and was consisted with changes reported in HS group (Figure 11 A.). There was no difference in EF between training groups or vs. HS *(p < 0.05)*. Final measurements reported EF of 65.7 ± 7.9 % MCT, 63.4 ± 9.0 % HIT-LV and 59.7 ± 10.9 % for HIT-HV.

Echocardiography evidenced no difference between groups in E/A ratio. Similarly to HS rats, training groups showed a decline in E/A around week 21, with the values returning back to baseline measurements towards the last week of the study. The E/E' ratio was increased in exercising animals vs. LS, but the differences were not statistically significant for MCT and HIT-HV groups. Only HIT-LV group showed statistical differences vs. LS *(p < 0.05)*. Exercising animals were not different from HS *(p < 0.05)*, (Figure 11 B and C.).

LVEDP was elevated in all of the training groups; however there was no difference vs. HS or in-between the groups. MCT had the highest LVEDP levels from among all of the training groups (13.9 ± 6.4 mmHg), closely approximating HS animals (14.0 ± 8.3), followed by HIT-LV (11.3 ± 5.3 mmHg) and HIT-HV (9.8 ± 6.3 mmHg). In addition, NT-proBNP levels were significantly elevated in all of the training groups vs. LS *(p < 0.05)*. The highest levels of NT-proBNP were evident in HIT-LV, HIT-HV and MCT, respectively. Data are presented in Table 2.

Renal function

In general terms, all of the trained animals resembled changes in renal function corresponding to those described in HS. Urine output was lower in trained animals compare to HS, but still exceeded the numbers reported in LS *(p < 0.05)*. Microalbuminuria levels were also insignificantly lower than in HS, but significantly elevated vs. LS *(p < 0.05)*. Albumin/creatinine ratio was significantly increased in trained groups vs. LS animals *(p < 0.05)*, but not different from HS. There was no difference in-between the training groups. Data are presented in Table 3.

Signs or symptoms of HF

The ratio of wet/dry lung weight was higher in MCT (5.14 ± 0.3) and HIT-HV (5.0 ± 0.3) vs. LS but not in HIT-LV (4.56 ± 1.02), however, statistical test did not show significant difference between the groups (Table 1.) VO_{2max} was not different between the groups at the baseline measurements. Total distance run, evaluated at the end of the study, was higher among the training groups and the differences were significant from sedentary LS and HS animals *(p < 0.05)*. There was no significant difference in between the training groups (MCT, 481 ± 82.6 m; HIT-LV, 381.4 ± 74.1 m and HIT-HV, 455.3 ± 154 m), (Figure 12. and 13).

Mortality and stroke

The training protocol was well-tolerated by all the animals and no deaths occurred during the training sessions. The log-rank pairwise comparison showed statistically significant distribution between LS animals and all the training groups *(p < 0.05)*, also after Bonferroni correction (Figure 14.) There was no significant difference in survival distribution between HS and all the training groups, also when the training groups were collapsed together. Table 3 shows the number of total adverse events for each group, including death by stroke and

animals found dead or sick but without established cause of death. Stroke was the main cause of mortality in all of the groups. Next, the number and percentage of death by stroke in each group was specified. Death by stroke was highest in MCT (60 %), followed by HIT-HV (40 %) and HIT-LV (35 %). Similar to the general survival distribution, the log rank test detected differences in stroke survival between the groups ($p < 0.05$), with the main difference allocated between LS and the remaining groups. The pairwise comparisons revealed no differences in stroke survival between HS and all the training groups.

DISCUSSION

The present study was performed to establish clear criteria for HFPEF in DS rats and to examine progression of the disease. Following, the effects of different exercise volumes were evaluated, as a dose-response relationship, between exercise training, cardiac function and structure, as well as renal function. In addition, the study aimed to examine mortality and its underlying causes in DS rat model.

Characterization of an experimental model of HFPEF - Dahl Salt-Sensitive rats.

Blood pressure

The DS rats submitted to prolonged HS diet showed a gradual and accentuated elevation of systolic BP, reaching severe hypertensive values. The characteristics of the development of hypertension in DS rats were consistent with other studies [12, 84, 85]. Steep rise in BP was evident already from the week 13, early in the course of the diet, and similar time-course was reported in other studies [12, 84].

Structural changes

Echocardiographic examinations showed an increase in the estimated LV mass from week 14, leading to progressive remodeling of LV due to the high sodium intake and associated pressure overload. At the cellular level, histopathological image analysis at the end of the study revealed an enlargement in the cardiomyocyte cross-sectional area in HS rats and higher LV hypertrophy index compared to LS group. Clinically, cardiac hypertrophy, commonly relates to the history of hypertension and is considered to be a major cause of diastolic HF [12, 94]. In concentric hypertrophy, cardiomyocytes grow in a transverse direction, while the cell length remains constant. Thus, the increase in cardiomyocyte diameter is often accompanied by collagen deposition, fibrosis and subsequently, myocardial stiffness [27]. Moreover, the changes observed in the LA dimensions, estimated by echocardiography, were less conclusive and as presented in the results, the difference between the groups was only apparent during the week 21. However, the *post mortem* examination of the LA weights showed higher values in HS rats compared to LS animals.

Therefore, DS rat model seems to reflect the changes observed in HFPEF patients, with typical structural characteristics, such as LV hypertrophy, concentric remodeling and LA enlargement [32]. Interestingly, echocardiographic indices of diastolic dysfunction did not have prognostic value in patients, but the scope of structural changes, especially LA enlargement was associated with an increase in cardiovascular events. The rationale behind can be explained by changes in LA size resulting from the severity and duration of increased LV diastolic pressures, while E or E/E' ratio represent LV diastolic pressures at one point in time [32].

Diastolic and systolic function

One of the main criteria for diagnosing diastolic HF relates to EF and LV systolic properties. All of the animals in HS group showed EF above 50 %, considered to be within normal range. Although EF presented clinically normal values, a progressive decline in EF was evident from week 23 and reached statistically significant difference from LS at week 27.

Echocardiographic assessment of diastolic function in the HS animal showed E/A ratio decreasing towards week 21 and gradually increasing thereafter, however no statistical significance was detected. Generally, lower E/A ratio indicates reversed filling velocity and impaired relaxation corresponding to mild diastolic dysfunction and higher E/A reflects severe diastolic dysfunction and restrictive filling patterns [30]. Similar changes were evident for E/E' in HS rats, with a marked drop at week 25 and then gradual increase towards significantly elevated ratio, indicative of high filling pressures, at week 35. In the study by Klotz et al. [85], the increase in E/A and E/E' ratios was also not significant and the conclusion about the extent of diastolic dysfunction over time could not be established. Doi et al. [12] reported increased E and decreased A wave at week 19 and significantly higher E/A ratio among HS groups versus control. Additionally, direct hemodynamic measurements of LV cavity pressures were performed at the end of the present protocol and showed elevated LVEDP in HS rats.

One of the studies evaluating changes in myocardial structure and function in DS rats over time, was performed by Klotz et al. [85] and the animals were consecutively sacrificed at weeks: 12, 16, 20 to gain an insight into underlying pathologies. The diet protocol was the same as in the present study but the animals were of a male gender. Similarly, the study reported increased LV mass (> 30 %) and high LVEDP, reaching the values of ~ 25 mmHg at week 20. EF increased at weeks 8 and 12, but declined progressively thereafter. Early in the

time-course of the study (week 12), there was an evidence of passive diastolic dysfunction characterized by shift towards lower volumes, but with an enhanced systolic performance. At the same time, other indices of HF such as elevated LVEDP and increased lung weight were not present. During the weeks 16 and 20, the LVEDP was elevated, accompanied by a reduction in EF and renal dysfunction. Pulmonary edema also developed at weeks 16 and 20, correlating with elevated LVEDP, and was consistent with a transition to HF in male rats. Similar time-course of HF development was observed in the study by Doi et al. [12] performed on male DS rats, with the animals showing overall signs and symptoms of HF at week 19, such as concentric LV hypertrophy, elevated LVEDP, lung edema and larger percentage area of fibrosis.

Furthermore, Klotz et al. [85] have suggested that emerging diastolic dysfunction is initially compensated by enhanced systolic performance and there is no physiological evidence of a reduced pump function. Over time, corresponding to 20 weeks, end-systolic and end-diastolic pressure-volume relation did not vary from the control, but HF characterized by high LVEDP and pulmonary edema developed, despite of overall pump function remaining normal. Results suggest that in a setting of chronic hypertension, HFPEF might not be always a consequence of diastolic dysfunction. Underlying HFPEF pathology can occur as a result of salt and water handling impairments, such as observed in renal dysfunction .

Kidney function

In HS animals urine output, microalbuminuria and albumin/creatinine ratio were significantly increased compare to LS, but the levels of plasma creatinine and creatinine clearance remained at a similar level. CKD usually progresses in stages, with kidney damage induced by hypertension, as a initial step towards decreased GFR, kidney failure and death. GFR can still be preserved in the early stages of kidney damage and among patients, there is a higher prevalence of earlier stages of CKD [66]. In DS rats, creatinine clearence, the marker of GFR was still maintained, while elevated excretion of microalbuminuria and high albumin/creatinine ratio were suggestive of ongoing pathological changes. Microalbuminuria, defined as a prolonged excretion of albumin at a mild elevation, is typically observed in persistent hypertension and is the first marker of kidney dysfunction. However, the presence of microalbuminuria in urine is not exclusively associated with renal pathology [95]. Elevated levels of microalbuminuria correlate with HF, LV dysfunction and hypertrophy. The link between cardiovascular disease and leakage of microalbuminuria into urine is unclear, but

possibly relates to endothelial damage, chronic inflammation and the RAAS activation [96]. Additionally, the accumulation of proteinuria in the proximal tubular cells further deteriorates kidney function, by triggering proinflammatory and profibrogenic pathways, that contributes to renal scarring [97].

Salt and water handling impairments are the main characteristics of DS model and kidney dysfunction is commonly observed, however, the descriptive parameters differ between the trials. Histological examination of the kidney in a DS model, placed on 8 % NaCl diet, showed initiated sclerosis and collapse of the glomeruli, associated with cellular infiltration, and the arterioles were affected by extensive perivascular fibrosis [12]. Similar findings were reported by Inoko et al. [84], with a collapse of capillary lumens and tubular atrophy. Moreover, the levels of creatinine were higher than in control group and most of the DS rats reached high creatinine levels appoximating 1.0 mg/dL at the terminal stage. In the study by Klotz et al. [85] plasma creatinine gradually increased in aging animals and reached the values of 0.52 ± 0.20 mg/dL (week 8), 0.43 ± 0.19 (week 12), 0.51 ± 0.26 (week 16) and 0.77 ± 0.21 (week 20), illustrating a progressing dysfunction of the kidney.

In HFPEF patients, lower estimated glomerular filtration rate (GFR) and urine dipstick proteinuria were found be a consistent independent predictor of clinical outcomes, such as all-cause hospitalization and death [67]. Additionally, higher level of proteinuria was a risk factor for rapid progression of CKD, worsening of cardiac function and death from cardiovascular causes [98]. High stroke mortality among HS animals in our study relates to the findings from the clinical studies [72, 73], where most of the patients died in the early stages of CKD due to cardiovascular events, possibly explaining why we did not observe more pronounced changes in the kidney function.

Exercise capacity

Interestingly, exercise capacity was not reduced in HS compare to LS, contradictory to exercise intolerance reported in patients [45]. Lack of exercise intolerance is especially intriguing, considering the development of pulmonary edema in HS rats, associated with effort related dyspnea in patients [14]. The underlying causes can be related to discrepancies between human and animals studies. HF patients are generally characterized as inactive [37] and present with a number of risk factors such as obesity, elderly age and associated co-morbidities, likely contributing to exercise intolerance [3, 53], while animals in the current

study were observed as relatively active and most importantly, lack the specter of risk factors the patients present with.

Effects of exercise training on HFPEF in Dahl Salt-Sensitive rat model.

One of the main objectives of the current study was to evaluate the structural and functional alteration, observed in the heart and the kidneys, induced by different training volumes in the DS model of HFPEF. The study hypothesized that exercise training will prevent the development of HFPEF and prolong the time until adverse event happens and that intensity of training is of a greater importance than the duration. From a pathophysiological point of view, exercise could by far outweigh any pharmacological intervention in this heterogeneous syndrome, since lifestyle dependent risk factors, physical inactivity, and physical deconditioning contribute to the deterioration of HFPEF.

Blood pressure

Participation in regular aerobic exercise is commonly associated with lower BP values in hypertensive patients [99] and animals [100] and improves overall cardiovascular risk profile. However, in DS rat model, the patterns of hypertension development were similar between HS sedentary and training animals and none of the exercise training regiments reduced BP levels. The same findings were reported in previous studies on BP levels in DS rats [101, 102]. Overton et al. [102] hypothesized that the combination of chronic exercise and high sodium consumption produces elevated blood volumes. Higher blood volumes can offset the influences of decreased sympathetic nervous system activity, normally associated with aerobic training, on lowering resting BP [103]. Similar explanation was proposed by Miyachi et al. [64], indicating that increased venous return with exercise, leads to increase in cardiac output and in the setting of high-salt intake, contributes to preserved hypertension.

Structural changes

Structural remodeling of the heart followed the same patterns as described in HS rats and the differences between exercising groups were not significant, with all the animals developing LV hypertrophy. Cardiac hypertrophy associated with hypertension can be seen as initially adaptive response to increased external load, resulting in lower ventricular stress by increasing LV wall thickness [64]. However, prolonged cardiac overload eventually results in

HF. In fact, increased LV mass is of a high prognostic relevance in hypertensive patients, predicting higher incidence of clinical events and death [104].

Miyachi et al. [64] also reported an increase in LV mass of DS rats, but the changes in LV posterior wall thickness and intraventricular septum thickness were attenuated by exercise training, indicative of reduced LV concentric remodeling. The authors showed that LVEDP was also reduced in trained animals compare to control, in contrast to elevated values observed in our female rats, with MCT reaching the values closely approximating HS. In the study by Miyachi et al. [64], exercise lowered the extent of LV interstitial fibrosis and restored myocardial capillary density, preventing myocardial hypoxia than can potentially develop as the pathological hypertrophy progresses. The findings of the study were very promising, however the study ended when the male rats were 18 weeks old, in contrast to 35 weeks in our female rats. Sedentary HS male rats achieved LVEDP of 8 mmHg compare to 14.0 mmHg in sedentary females, possibly pointing towards more severe levels of diastolic dysfunction in the current study.

Diastolic and systolic function, pulmonary edema and NT-proBNP levels

The extent of changes in diastolic function of DS rats performing exercise training, was similar to the findings observed in HS animals. EF declined in a similar pattern and was significantly reduced compare to LS, however remained > 50 % throughout the study. Differences in E/A ratio were insignificant between all the groups. E/E' ratio reached high values and was significantly elevated in HIT-LV group versus LS, but the changes were insignificant for MCT and HIT-HV. The ratio of wet/dry lung weight was insignificantly higher versus LS, but the extent of pulmonary edema was lower than in HS animals. Similar finding was reported in the study by Miyachi et al. [64] where exercise training prevented increase in lung/TL ratio.

NT-proBNP levels were significantly elevated versus LS and were comparable to values in HS animals. BNP and NT-proBNP are released by cardiac ventricles in a response to volume expansion or pressure overload. The physiological function of the hormones counteracts pathways such as RAAS, involved in progressive worsening of HF. The plasma concentration of BNP and NT-proBNP correlates well with systolic and diastolic LV dysfunction [105]. Consistently, higher levels of BNP and NT-proBNP are evident in renal disease, independently of the stage of CKD. NT-proBNP is influenced in a greater extent by the

degree of renal function, presenting as a strong prognostic indicator of hospitalizations and mortality [106].

Exercise and kidney function

The changes induced by exercise training on renal function, observed in DS female rats, were limited and there was no significant benefit of exercise on the kidney and its functional paramenters, such as albuminuria or creatinine clearance, compare to HS sedentary animals.

On the contrary, studies utalizing low-intensity training in different rat models, observed beneficial effect of exercise on renal function. Emter et al. [62] reported suppressed levels of proteinuria in a study of spontaneously hypertensive HF rats undergoing low-intensity treadmill running for 6 months. Another study in spontaneously hypertensive rats, performing 20 weeks of low-intensity (55 % VO_{2max}) treadmill running showed beneficial modifications in ultrastructural renal morphology with exercise training [107]. However, it is important to note that in this case, exercise training reduced the BP levels compare to sedentary animals by 26 % (from 186 ± 5.1 to 138 ± 3.7 mmHg), in contrast to our study, where the severe BP levels were not modified by exercise training. The kidney exposed chronically to elevated BP levels loses its ability to regulate internal pressures, resulting in progressing nephrosclerosis. Thus, hypertension promotes the progression of renal disease by worsening glomerular injury and increasing proteinuria; proteinuria further aggravates renal damage [97] and the extent of renal damage corresponds with deterioration of cardiac function [98].

The current guidelines and statement on exercise training in CKD recommended aerobic exercise training at an intensity > 60 % of maximum capacity to improve cardiorespiratory fitness among CKD patients [66, 74], however the effect of high-intensity training on renal function remain unclear. As discussed by Aparicio et al. [108], strenuous exercise can result in muscle damage, evidenced by high blood levels of muscle proteins such as creatinine kinase, lactate dehydrogenase and myoglobin and increasing plasma concentration of these proteins can impair renal function. To investigate the effects of high-intensity training on plasma, urinary and morphological renal markers, the authors performed a strength training study in rats. Wistar rats performed a strength training protocol on a treadmill, with weights tied to the cord of the tail, and run 3 - 4 sessions per week at a speed of 35cm/s. The results displayed higher plasma urea, albumin and creatine kinase concentrations in the training group versus control. The levels of plasma creatinine and testosterone level were lower among exercising animals. Also, renal interstitial connective tissue was 30 % higher. The

study concluded that high-intensity resistance training promoted a worse morphological renal profile that could possibly correlate with a higher risk of kidney disease.

Exercise capacity

Exercise capacity, expressed as total distance run, was better following exercise training, with an evident difference from sedentary controls, both HS and LS. Improvement in exercise capacity was in agreement with gains observed in patients and the findings were common for all the studies, independently of the scope of changes in diastolic function and structure [49, 50].

Most of the studies observing improvement in cardiac parameters with exercise training, included patients with mild to moderate diastolic dysfunction and younger age (62.9 ± 10.2 - 65 ± 7 years of age), [51, 52]. In contrast, *ex vivo* examinations of the animals in the current study point towards severe pathological changes in cardiac structure and function. Most importantly, hypertension, the major risk factor underlying pathological pathways of HFPEF was controlled in patients, but not in animals. Control of hypertension is considered as a primary treatment strategy in HFPEF and patients are commonly placed on diuretic medications, to control sodium and water retention [3]. This risk-factor distinction between patients and animals, may explain different outcomes of exercise interventions and limited extend of improvements on cardiac structure and function, independently of exercise dose applied.

Mortality in Dahl Salt-Sensitive rat model

Kaplan-Meier survival analysis confirmed higher mortality rates among animals placed on a HS diet, compare to LS control, as commonly presented in literature [12, 84, 92]. Additionally, exercise training did not modify the total mortality distribution among the groups. However, the animals from HIT-HV and MCT, respectively, started to die at a later time-points than HS group and the delay in adverse events was especially noticeable in HIT-LV group, possibly suggesting a modest delay in mortality rates among HIT-LV rats. Examining the curves end-cumulative survival proportion, the proportion of rats dying towards the end of protocol did not appear very different between the groups, with animals dying at similar rate, with the exception of LS group. MCT and HS group had the lowest survival expectancy towards the end of protocol, while HIT-HV and HIT-LV had a higher survival at the same time-points.

The extended time-course of the present study can possibly explain lack of significant differences in mortality rates between sedentary and trained groups, compare to previous trials. Miyachi et al. [64] found that survival rates improved in DS rats following swim training, independently of hypertensive status of the animals. As mentioned earlier, the study protocol ended when the animals were 18 weeks compare to 35 weeks in our female rats. Another small-scale study [101] reported improved survival in DS rats, following low-intensity exercise training by approximately 30 % compare to non-exercise HS control group. However, the study was exclusively descriptive and did not examine underlying cardiac physiology. In addition, the protocol ended when the animals were about 12 weeks of age. In the current study, HS animals also started to die at an earlier rate than the animals from training groups, with 2 HS animals dead by the week 12, compare to no mortality in the training groups. This possibly indicates that varied duration of study protocols can shape survival distribution. Additionally, the studies describing development of diastolic HF in DS rats over time were performed on males [12, 85], alike the small trials demonstrating better survival in trained animals [64]. There is a scarce evidence on development of HF and mortality in female DS rats and the current results suggest delayed onset of the disease in females, questioning the extent of gender differences in this model.

Stroke mortality

The leading cause of death in DS rats placed on a HS diet was stroke. Exercise training seemed to modestly prolong time until adverse event happened, but did not affect overall stroke incidence reported at the end of the study. The cases of death by stroke in DS rat model were previously reported and showed that the animals were spontaneously dying from intracranial hemorrhage [12, 84]. On the other hand, Werber et al. [92] showed higher proportion of animals presenting with cerebral infarction versus cerebral hemorrhage.

Altogether, stroke is a third major cause of morbidity and mortality around the world. The prevalence is increasing with age, with 43 % of people $\geq$ 85 years old experiencing stroke and women are at a higher risk than men [17]. Elevated BP level is a powerful determinant of stroke, both of ischemic and hemorrhagic origin. High BP damages arterial walls, providing a lodging place for plaque build-up and increases the risk of thrombi and ischemic stroke. Chronic high BP can also directly generate blood vessels ruptures and cerebral bleedings, causing hemorrhagic stroke [109]. Increased prevalence of cardiovascular disease, stroke and stroke mortality correlates with dietary patterns associated with higher levels of BP, such as

high sodium diet [110]. However, different animal studies indicate that high BP alone, is not solely responsible for the incidence of stroke. Werber et al. [92] conducted one of the first studies examining factors influencing stroke incidence in DS rats. Male DS rats, developed high BP levels, but the amount of sodium in the diet had a greater significance for predicting stroke than high BP levels.

To get an insight into possible cause of high mortality in DS rats, despite exercise training, it is important to take a brief look at the origin of the model. Shortly, the DS rat model emerged when the effects of HS diet on BP of rats were studied and an observation was made, that there is a varied hypertensive response to different amounts of salt in the diet. Moreover, a marked degree of individual variation in the response of BP to salt was noted. Taking advantage of this observation, the rats were selectively inbreed from one strain of Sprague-Dawley rats for sensitivity (DS) or resistance (DR) to the hypertensive effects of HS intake. After three generations of selective breeding, the two lines were separated, with DS rats responding to HS feeding with a steep elevation in BP and DR maintaining normal BP levels despite the high sodium intake [111, 112].

Tobian and Hanlon [113] performed a study to determine whether DR rats consuming HS diet (0.8 % NaCl) will develop arterial lesions, despite normal BP levels. At the end of 8 weeks of the feeding protocol, 53 % of the rats consuming HS diet died, compare to no mortality in the LS group (0.3 % NaCl). After additional 7 weeks, all of the HS rats were dead, while after 15 weeks 88 % of LS rats were still alive. BP between the two groups was similar throughout the study and averaged 158.8 mmHg, corresponding to mild hypertension in this model. Autopsy of the brain of HS rats revealed many small brain infarcts, concluding cerebral vascular disease with brain infarction or brain edema as a leading cause of death. This study suggests, that even in the case of salt-resistant strain of rats, which develops only mild hypertension no different from the control group, high sodium intake is sufficient to induce stroke and greatly decreases survival. Consequently, the effects of HS diet seem to go beyond pathophysiological changes induced by high BP levels.

A recent study by Dmitrieva and Burg [114] linked serum sodium levels to the increased risk of stroke. The study confirmed the hypothesis that in the presence of high sodium blood concentrations, the secretion of von Willebrand factor (vWF) by endothelial cells is stimulated and leads to hypercoagulability and thrombosis.

The vWF is one of the key components of blood initiating clots. vWF is a large blood glycoprotein involved in hemostasis and is produced by endothelial cells. Originally, vWF circulates in the blood in a globular latent form, with the binding sites not exposed. A clot occurs at first, when a specific signaling pathway is activated, for example in stenosed arteries under high shear stress and when the platelets bind to the binding sites on vWF [115]. Elevated levels of vWF are therefore a major risk of thrombogenesis, especially in subjects with pre-existing cardiovascular conditions [116]. A mice model of dehydration was chosen for the described study, to examine whether high concentration of NaCl will result in vWF production in endothelial cells *in vivo*. Mice were fed gel food containing 30 % water as the only water source in the diet, and responded with a small increase in plasma sodium (5mmol/L), considered to be within physiological range. However, modest elevation of plasma sodium was sufficient to induce the expression of transcription factor (NFAT5) that contributes to vWF production in endothelial cells. Clinical indicators of increased ongoing coagulation (D-dimer) were present in mice blood and confirmed that increased secretion of vWF from endothelial cells elevates vWF in blood and promotes thrombosis.

The study indicates that increased sodium intake affects blood coagulability, thrombogenesis and consecutively, the risk of stroke. At the same time, the model illustrates that damage to endothelial cells is not only caused by hemodynamic factors, such as high systolic BP, but also by high concentration of sodium in the blood. DR rats, considered as one of the most salt resistant rodent models [113] had a high mortality by stroke, regardless of only mildly elevated BP levels. From this perspective, high incidence of stroke in DS rats could be linked to prolonged HS intake and possibly explains lack of major protective mechanisms induced by exercise training

STUDY LIMITATIONS

Animal models are broadly used in the studies of cardiovascular and renal disease, however there is a number of discrepancies between experimental and clinical studies. The disadvantages are predominantly related to the anatomical, physiological and pathological differences between animals and humans. With a regard to cardiac physiology, cardiovascular diseases are slowly developing in humans, compared to rapid progression into disease in laboratory rats. Consequently, cardiovascular disease is more common among older humans, while rats develop the disease at younger age [11].

Maintained exercise tolerance in HS rats is one of the examples of such discrepancies. HFPEF patients are generally inactive and present with a number of co-morbidities that limit exercise tolerance [37]. HS rats in this study, maintained normal body weight and activity levels corresponding to LS animals. It is difficult to predict in what extent maintained activity level contributes to better physiological outcomes in sedentary animals, than what would be expected in sedentary patients.

At the same time, the animals subjected to HS diet showed a steep elevation in BP levels that was not affected by exercise training. Hypertension is one of the first treatment targets for cardiovascular disease and patients are commonly prescribed antihypertensive medications [3]. Severe levels of BP, as reported in our rats, could never be observed in clinical settings. Thus, a direct comparison between experimental and clinical studies is challenging and it is difficult to recreate the scope of risk factors and progression of HFPEF in an identical manner.

One of the major methodological limitations of the study was related to invalid VO_{2max} measurements, caused by variations in running performance of the rats, due to the different responses to electrical grid stimulation. VO_{2max} is a "golden standard" in measuring exercise capacity and regular testing could give a better insight into how aerobic capacity correlates with a number of physiological parameters observed in the study.

Another unanswered question relates to the extent of physiological versus pathological LV remodeling early in the course of the study. Measuring NT-proBNP throughout the study, not solely at the end of the protocol, would give a better indication about severity of HF at different time-points and the extent of protective effects of different exercise volumes.

With a regard to the discussed effects of sodium on increased thrombogenesis, the measurements of vWF production in DS rats would provide a better insight into the mechanism behind frequent occurrence of stroke. Lastly, considering the high incidence of stroke in DS rats, possibly caused by high and prolonged salt feeding, questions the choice of the optimal animal model for studying HFPEF, especially in a longitudinal manner.

CONCLUSION

Taken together, the data obtained in the present study provide an insight into development of HFPEF in DS rat model and evaluate the effects of three different exercise protocols on structural and functional cardiac and renal parameters.

The DS model illustrates the effects of high sodium intake on severely elevated BP levels, leading to progressive remodeling of LV, confirmed by enlargement of cardiomyocytes diameter and indicative of concentric hypertrophy. At the same time, the study demonstrates the development of diastolic dysfunction in DS model, confirmed by highly increased LVEDP, presence of pulmonary edema and elevated NT-proBNP levels. Moreover, the ongoing kidney dysfunction was consistent with the concepts of HFPEF being a cardio-renal syndrome.

Additionally, the study shows that, although exercise training is not altering the changes in cardiac function and structure and not changing the overall mortality observed in DS rats under high sodium intake, the exercise trained groups had a modest delay in mortality compare to HS animals and the interval groups had a higher survival expectancy than HS and MCT at the end of the study.

Furthermore, the cause of mortality was reported, providing the first clear evidence that stroke is the leading cause of death in DS rats exposed to prolonged HS diet. This finding is possibly indicating that the adverse effects of excessive salt consumption, go beyond the pathological effects of hypertension and might be directly contributing to high stroke mortality in this model.

PERSPECTIVES

The current study evaluated the primary prevention of HFPEF by exercise training, and provided precise diagnostic criteria for female DS rats. The next step, considering the findings from this study, should focus on secondary prevention in rats with already established HFPEF. Analyzing the impact of different exercise protocols in HFPEF rats, could provide an insight into the effects of exercise on delaying or reversing the pathological changes in the heart and on the progression of kidney disease.

Moreover, utilizing provided exact diagnostic criteria and the time-frame for HFPEF development, an objective comparison between male and female rats could be possible. Including both male and female animals in the study, would help to understand the extent of gender discrepancies in this heterogeneous syndrome.

Most importantly, secondary prevention would give a picture of mortality rates beyond the time-frame of the current study. Considering the observations that high-intensity interval training groups had higher survival rates at the end of the protocol, a survival study could better verify these observations and answer the question about optimal exercise dose to improve survival in HFPEF. If confirmed, such findings would be of a great clinical importance, since none of the established treatments to date reduces the high mortality rates among HFPEF patients.

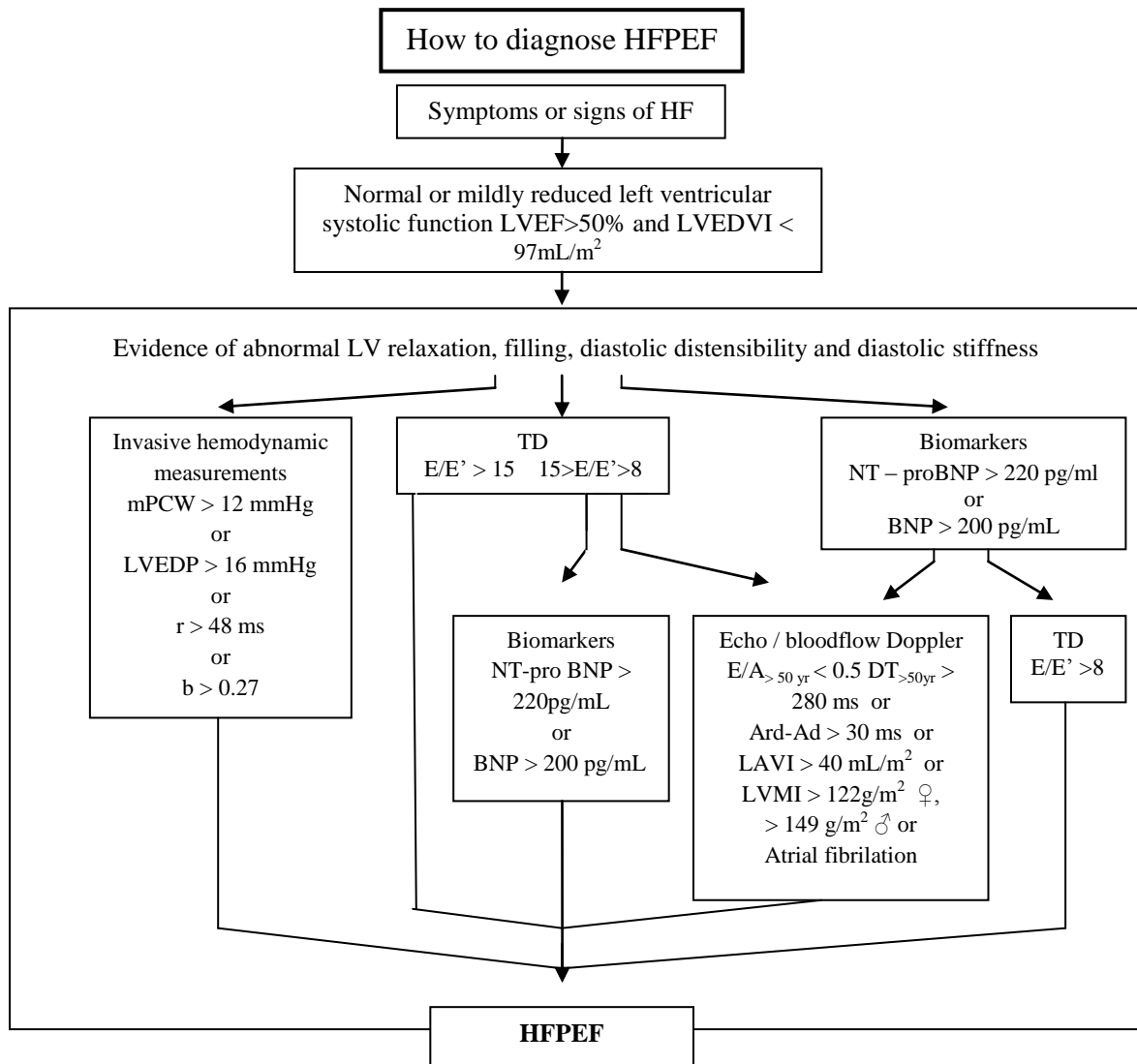## ACKNOWLEDGEMENTS

APPENDIX

Figures

Figure 1. Diagnostic flowchart on 'How to diagnose HFNEF' in patients suspected of HFNEF. LVEDVI, left ventricular end-diastolic volume index; mPCW, mean pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; t, time constant of left ventricular relaxation; b, constant of left ventricular chamber stiffness; TD, tissue Doppler; E, early mitral valve flow velocity; E', early TD lengthening velocity; NT-proBNP, N-terminal-pro brain natriuretic peptide; BNP, brain natriuretic peptide; E/A, ratio of early (E) to late (A) mitral valve flow velocity; DT, deceleration time; LVMI, left ventricular mass index; LAVI, left atrial volume index; Ard, duration of reverse pulmonary vein atrial systole flow; Ad, duration of mitral valve atrial wave flow [14].
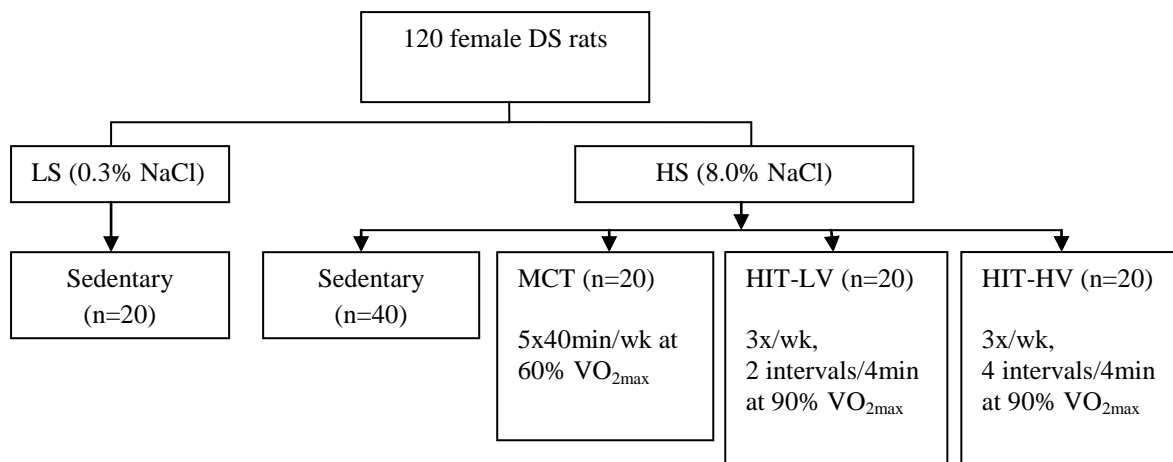
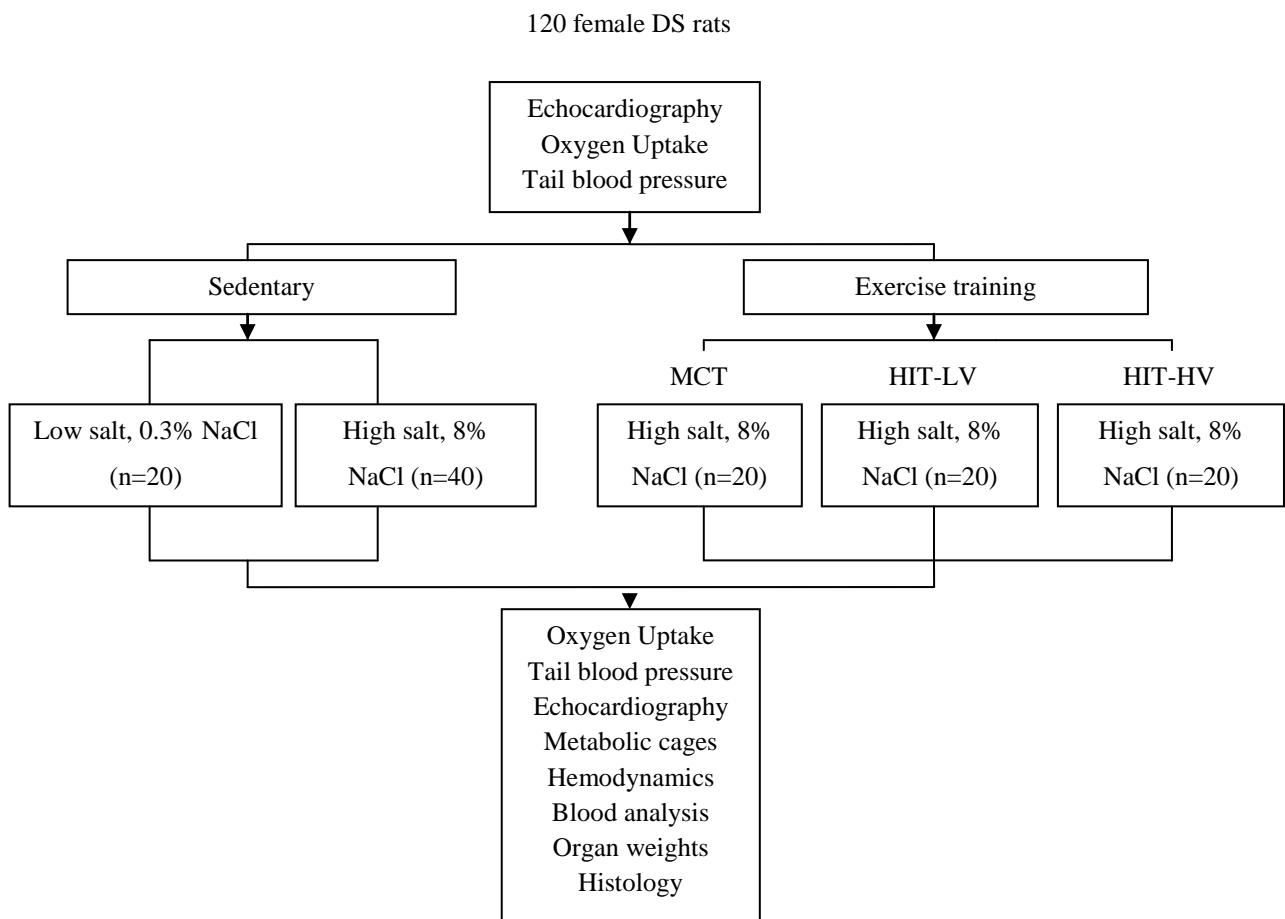**Figure 2**. Schematic diagram of experimental design and training protocol
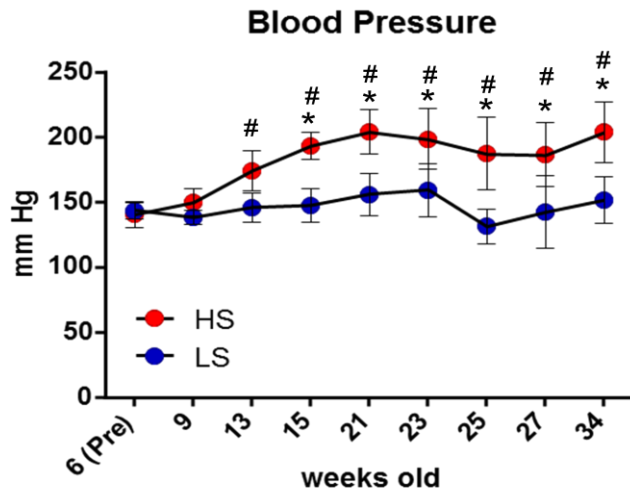

Figure 3. Schematic diagram of experimental design

Figure 4 . Systolic blood pressure. # $p < 0.05$ vs. Pre HS; * $p < 0.05$ vs. LS
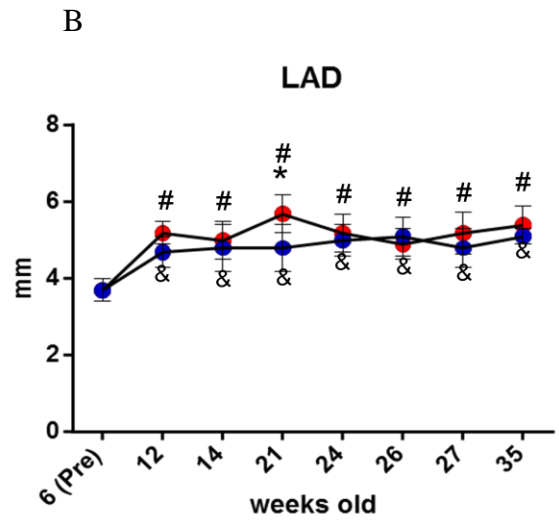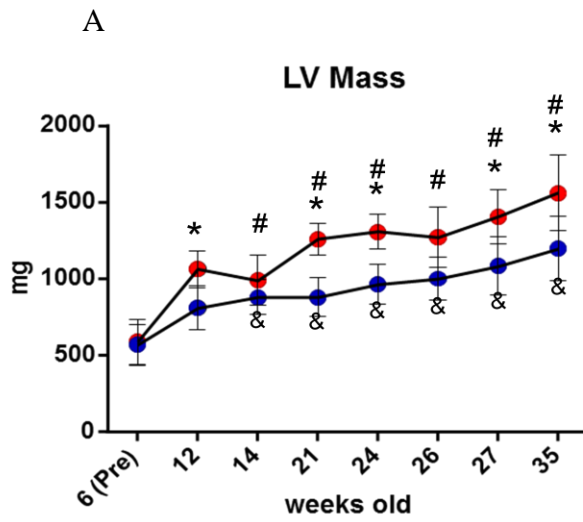
A

B

C

D

Figure 5 . Cardiac remodeling. A: Changes over time between HS and LS in left-ventricular (LV) mass and B: left-atrial dimensions (LAD). C: Differences in cardiomyocyte cross-sectional area and D: Left-ventricular (LV) hypertrophy index. # $p < 0.05$ vs. Pre HS; * $p < 0.05$ vs. LS
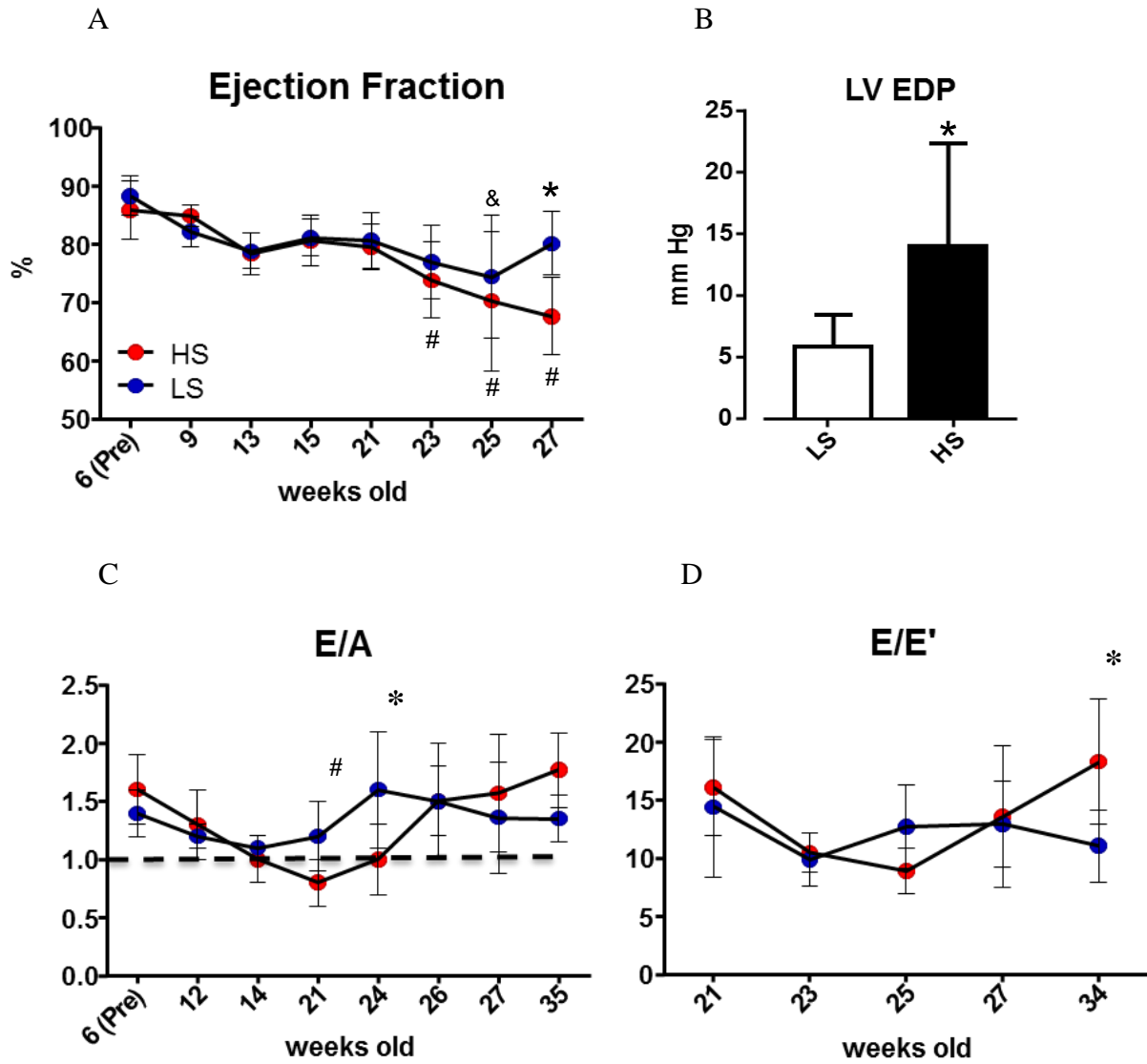
Figure 6. Diastolic function. A: left-ventricular ejection fraction. B: Left-ventricular end-diastolic pressure (LVEDP). C: E/A, E - early left-ventricular filling; A - filling after atrial contraction . D: E/E' the ratio of early mitral inflow (E) to longitudinal mitral annular early diastolic tissue velocity. # *p < 0.05* vs. Pre HS; * *p < 0.05* vs. LS; & *p < 0.05* vs. Pre LS
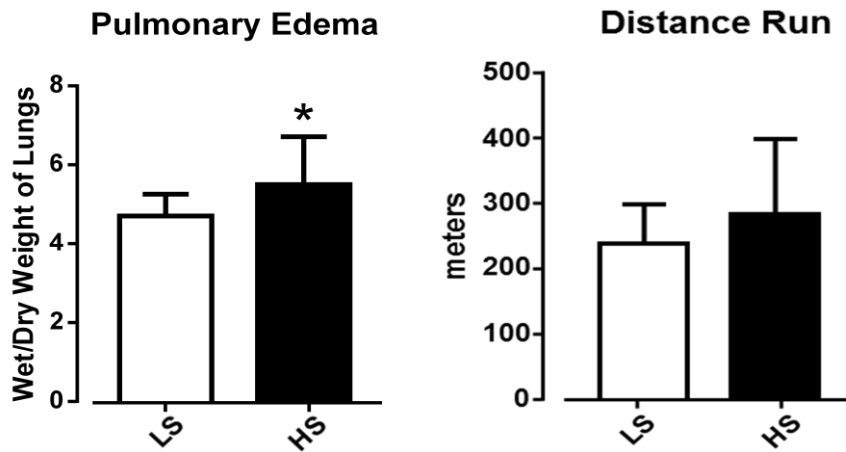
Figure 7 . Pulmonary edema and total distance run. * *p < 0.05* vs. LS

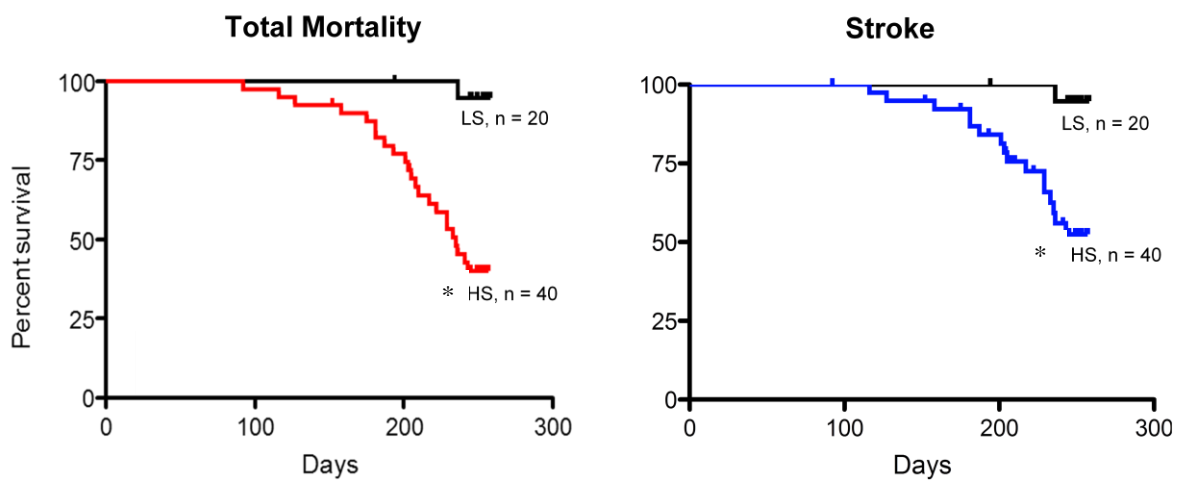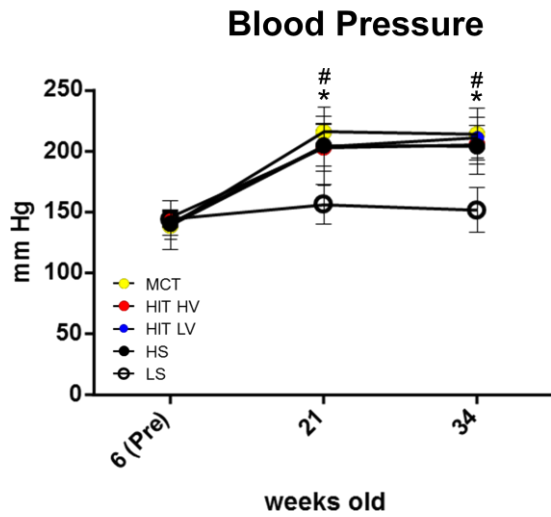**Figure 8 .** Kaplan-Meier survival curve. Total and stroke mortality for HS and LS. Log-rank test, HS vs. LS, *p < 0.05*.

Blood Pressure

Figure 9 . Systolic blood pressure. # $p < 0.05$ all HS groups vs. Pre; * $p < 0.05$ vs. LS

A

LV Mass

B

LAD

C

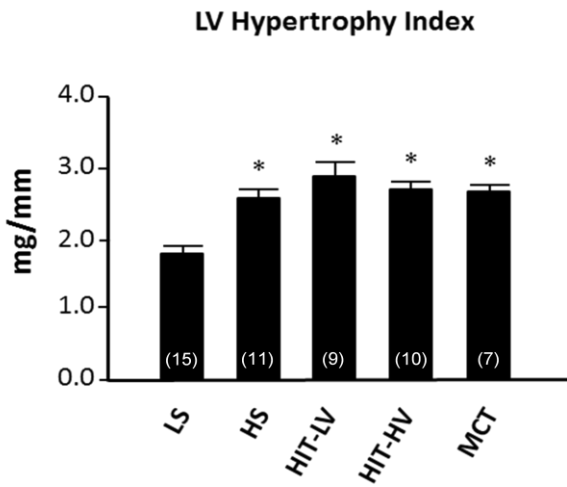**Cardiomyocyte Cross-Sectional Area**

D

LV Hypertrophy Index

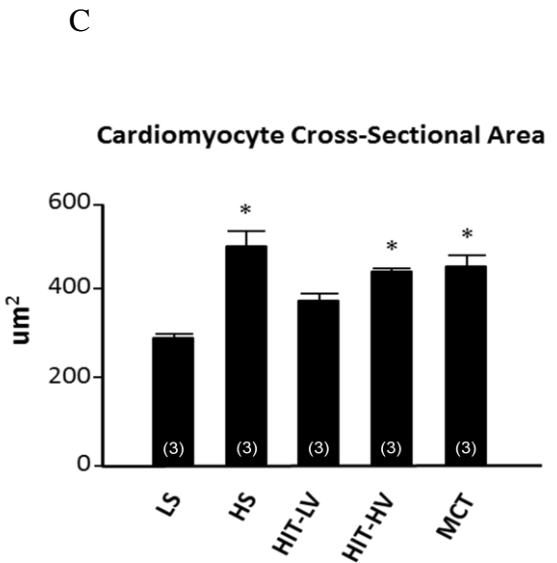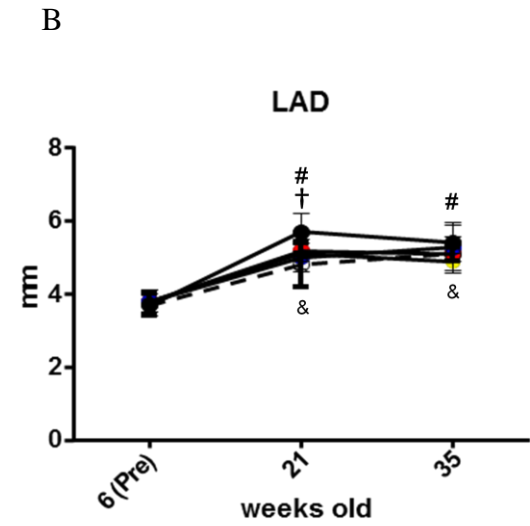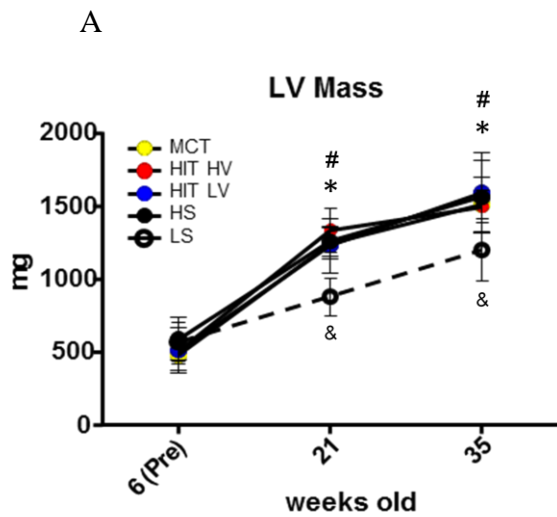**Figure 10** . Cardiac remodeling. A: Changes over time between HS groups and LS in left-ventricular (LV) mass and B: left-atrial dimensions (LAD). C: Differences in cardiomyocyte cross-sectional area and D: Left-ventricular (LV) hypertrophy index. A, B: # $p < 0.05$ HS groups vs. Pre; * $p < 0.05$ HS group vs. LS; & $p < 0.05$ LS group vs. Pre; † $P < 0.05$ HS group vs. LS. C, D: * $p < 0.05$ vs. LS

50
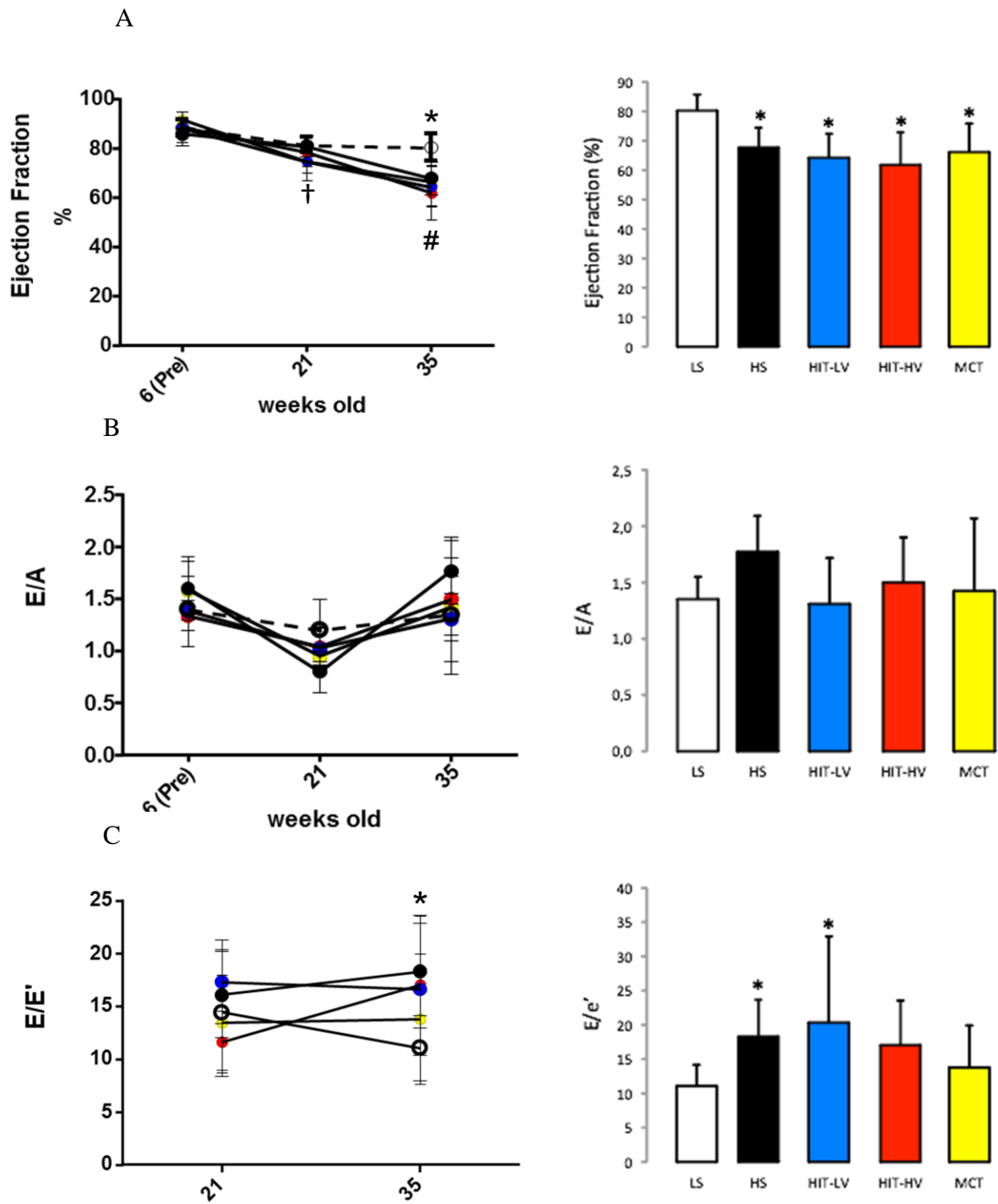
Figure 11. Diastolic function. A: left-ventricular ejection fraction. B: E/A, E - early left-ventricular filling; A - filling after atrial contraction . C: E/E' the ratio of early mitral inflow (E) to longitudinal mitral annular early diastolic tissue velocity. $* p < 0.05$ vs. LS

VO$_{2max}$ Pre diet and exercise
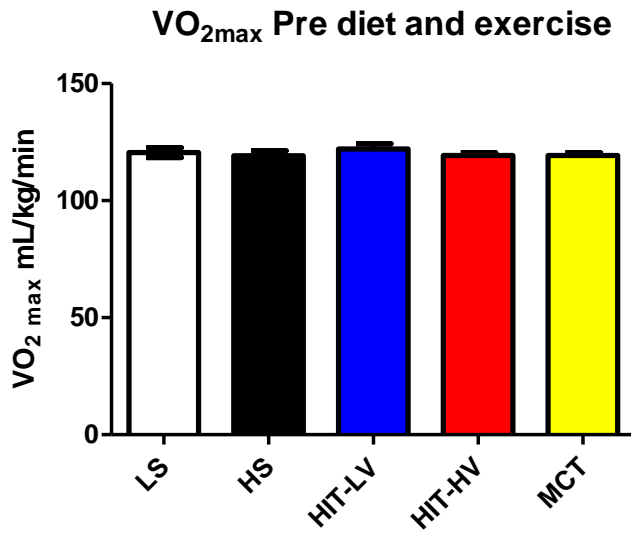
Figure 12 . VO$_{2max}$ (mL·kg^{-1}·min^{-1}), Pre diet and exercise, $p > 0.05$.

Distance Run

Figure 13. Total distance run. # $p < 0.05$ vs. Pre; * $p < 0.05$ vs. LS and HS

Figure 14. Kaplan-Meier survival curve. Percentage number of adverse events and mean survival days (95% CI) are given. Number of subjects at risk represents the number of alive animals at a given time point. *p-value* shows difference in cumulative survival HS vs. LS

Tables

Table 1. Tissue weights

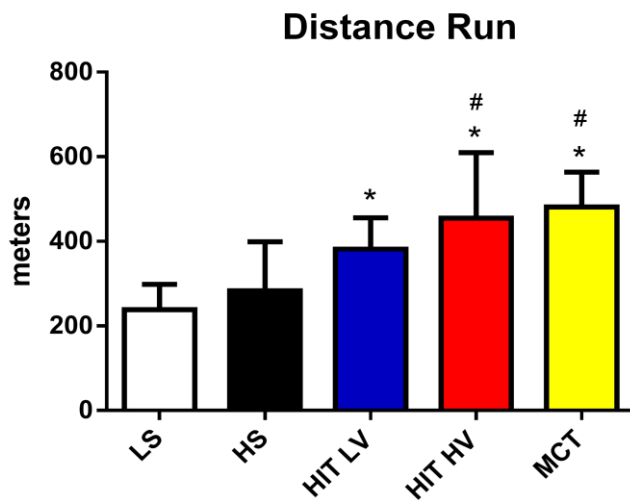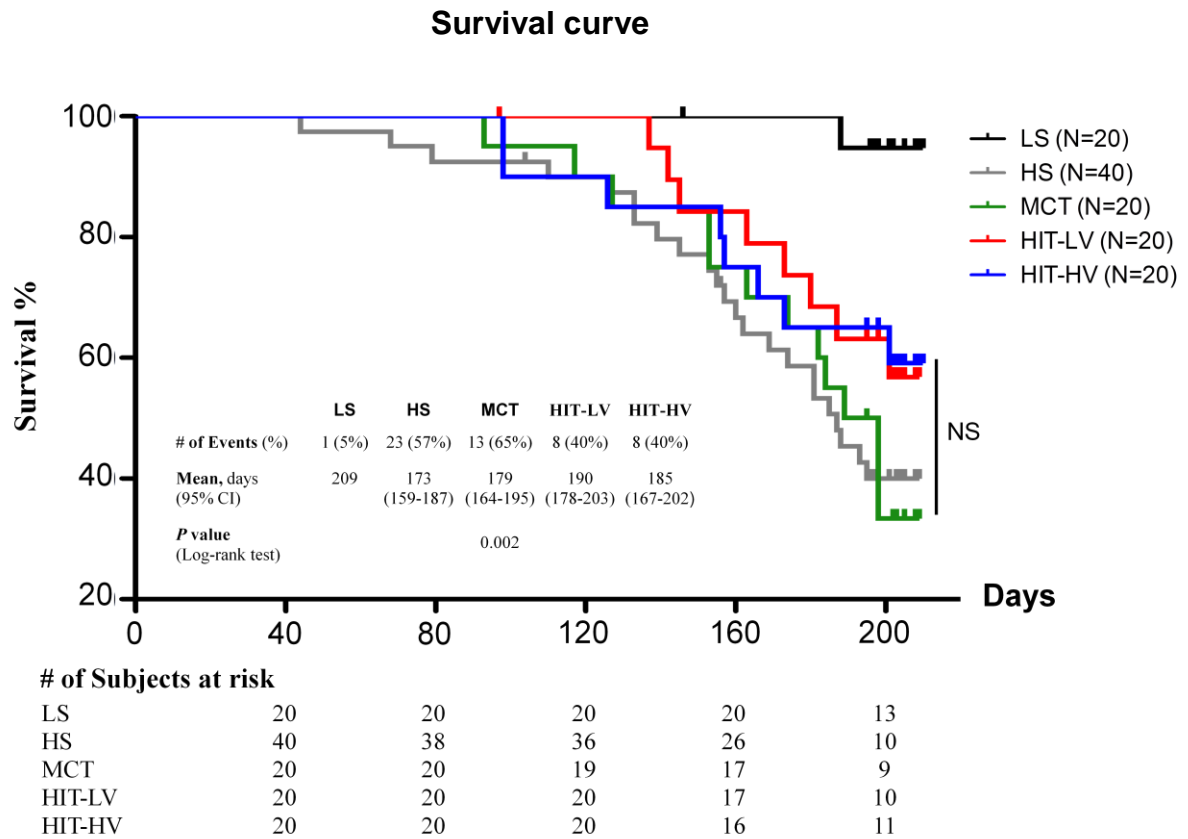| Variable (g)	LS	HS	MCT	HIT-LV	HIT-HV
BW	294 ± 14.5	288 ± 18.6	284 ± 15.1	279 ± 18.2	278 ± 14.5
LV	0.74 ± 0.04	1.04 ± 0.06*	1.05 ± 0.07*	1.14 ± 0.2*	1.07 ± 0.1*
RV	0.156 ± 0.01	0.175 ± 0.02	0.161 ± 0.02	0.178 ± 0.03*	0.172 ± 0.02
LA	0.023 ± 0.005	0.04 ± 0.01*	0.04 ± 0.01	0.051 ± 0.03*	0.04 ± 0.02
RA	0.019 ± 0.004	0.02 ± 0.01	0.047 ± 0.07	0.029 ± 0.01	0.026 ± 0.01
Heart	0.94 ± 0.04	1.282 ± 0.06*	1.293 ± 0.1*	1.401 ± 0.3*	1.308 ± 0.1*
Lung:					
Wet	0.1267 ± 0.06	0.1538 ± 0.07	0.1163 ± 0.08	0.0994 ± 0.05	0.124 ± 0.05
Dry	0.0269 ± 0.01	0.0283 ± 0.01	0.023 ± 0.02	0.0213 ± 0.01	0.0254 ± 0.01
Ratio	4.71 ± 0.5	5.49 ± 1.2*	5.14 ± 0.3	4.56 ± 1.02	5.0 ± 0.3
% H20	78.38 ± 3.5	81.23 ± 2.9	80.51 ± 0.9	76.6 ± 7.4	79.91 ± 1.5
TL (mm)	41.2 ± 2.5	40.2 ± 3.9	39.5 ± 3.4	39.5 ± 1.9	39.5 ± 1.8
LV/BW	2.52 ± 0.1	3.63 ± 0.3*	3.69 ± 0.3*	4.11 ± 0.7*	3.95 ± 0.6*
LV/TL	1.79 ± 0.1	2.62 ± 0.2*	2.66 ± 0.3*	2.9 ± 0.5*	2.71 ± 0.3*
LA/BW	0.08 ± 0.02	0.15 ± 0.04*	0.14 ± 0.05	0.18 ± 0.1*	0.17 ± 0.07*
LA/TL	0.54 ± 0.1	1.07 ± 0.3*	1.01 ± 0.3	1.29 ± 0.9*	1.04 ± 0.42

Body weight (BW), Left-ventricle (LV), Right-ventricle (RV), Right-atrium (RA), Tibial-length (TL, mm). Tissue weighs are given in grams. Data are means ± SD. *$p < 0.05$ compare to control (LS).

Table 2. Diastolic function and structure

Variable	LS	HS	MCT	HIT-LV	HIT-HV
E/A	1.4 ± 0.2	1.8 ± 0.4	1.5 ± 0.7	1.3 ± 0.4	1.5 ± 0.7
E/E'	11.2 ± 3.7	17.8 ± 4.8*	13.6 ± 4.0	22.1 ± 13.8*	16.8 ± 7.1
EF	82.1 ± 2.8	68.4 ± 6.2*	65.7 ± 7.9*	63.4 ± 9.0*	59.7 ± 10.9*
LVEDP	5.9 ± 2.5	14.0 ± 8.3*	13.9 ± 6.4	11.3 ± 5.3	9.8 ± 6.3
LV mass	1123.4 ± 135.7	1483.3 ± 283.7*	1461.1 ± 116.7*	1607 ± 292.8*	1494.2 ± 107.4*
LAD	5 ± 0.1	5.4 ± 0.6	4.9 ± 0.3	5.3 ± 0.8	5.1 ± 0.5
CSA	290 ± 14.1	497 ± 67.4*	466 ± 25.3*	381 ± 18.5	444 ± 7.2*
NT-proBNP	17.7 ± 13.5	64.4 ± 45.1*	66.2 ± 29.7*	79.7 ± 41.1*	69.7 ± 43.9*

(E/A), E - early left-ventricular filling; A - filling after atrial contraction . (E/E') the ratio of early mitral inflow (E) to longitudinal mitral annular early diastolic tissue velocity, left-ventricular ejection fraction (EF, %), left-ventricular end-diastolic pressure (LVEDP, mmHg), left-ventricular mass (LV mass, mg), left-atrial diameter (LAD, mm), Cardiomyocyte cross-sectional area (CSA, μm2) NT-pro brain natriuretic peptide (NT-proBNP, pg/mL). Data are means ± SD, *$p < 0.05$ compare to control (LS).

Table 3. Renal function

Variable	LS (N=9)	HS (N=11)	MCT (N=7)	HIT-LV (N=11)	HIT-HV (N=12)
Body Weight, g	291 ± 16	293 ± 11	282 ± 16	287 ± 17	273 ± 24
BP, mm Hg	147 ± 55	209 ± 80*	212 ± 34*	209 ± 84*	203 ± 79*
Urine output, μl.min^{-1}.kg^{-1}	23 ± 2	285 ± 96*	181 ± 12*	181 ± 13*	170 ± 18*
Microalbuminuria, mg.24h^{-1}	0.65 ± 0.34	95 ± 42*	76 ± 13*	84 ± 47*	72 ± 37*
Creatinine, μmol/L	31 ± 3	39 ± 19	35 ± 5	43 ± 15	35 ± 6
Albumin/Creatinine ratio, mg/ μmol/L	7.9 ± 3.9	1137 ± 460*	946 ± 175*	1147 ± 523*	1043 ± 513*
Creatinine clearence, ml.min^{-1}	1.91 ± 0.58	1.73 ± 0.85	1.72 ± 0.50	1.45 ± 0.52	1.60 ± 0.66

*$p < 0.05$ compare to control (LS).

Table 4. Mortality rates

		LS	HS	MCT	HIT-LV	HIT-HV	Overall
Total N		20	40	20	20	20	120
Censored		19	17	7	12	12	67
Total N of events	N	1	23	13	8	8	53
	Percent	5.00%	57.50%	65.00%	40.00%	40.00%	44.20%
Death by stroke	N	1	16	12	7	8	44
	Percent	5.00%	40.0%*	60.0%*	35.0%*	40.0%*	36.60%

Data expressed as a number of study subjects (N) and percent mortality in total and by stroke in each group. *$p < 0.05$ compare to control (LS).

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