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Myocardial Mitochondrial Metabolism in Diastolic Heart Failure

The Influence of Different Exercise Training Intensities

Master's Thesis in Exercise Physiology and Sport Sciences

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Abstract

Background – Diastolic heart failure (DHF) has a growing prevalence and current treatment options have not improved this prognosis. To better understand DHF, this study, as part of a multicenter study denominated OptimEx, looked into the myocardial mitochondrial metabolism in DHF in female Dahl salt sensitive (SS) rats and the influences of different exercise training intensities on this metabolism.

Methods – In a hypertension animal model, 120 female Dahl SS rats were randomized into five groups. One group was fed a normal diet (LS, n = 20) and was used as a sedentary control group. The other four groups were fed a 8 % NaCl diet. Of these four groups, one was sedentary (HS, = 40) and three were subjected to different training intensities; high intensity intervals (HIT) at a low volume of two intervals (HIT-LV, n = 20), HIT at a high volume of four intervals (HIT-HV, n = 20) and moderate continuous training (MCT, n = 20). Rats from every group were regularly controlled for blood pressure, signs of DHF and tested for oxygen uptake to adjust the exercise training protocol. Examiners were blinded. Study endpoint was DHF in HS.

Results – Exercise training decreased the limitation of oxidative phosphorylation of HIT-HV compared to LS. Further, after merging all exercise groups into one, improved mitochondrial efficiency could be seen for exercised groups compared with HS.

Conclusion – Myocardial mitochondrial metabolism is not influenced by the development of DHF. However, exercise training has positive effects on the myocardial mitochondrial efficiency and at high intensities also on the limitations of oxidative phosphorylation.

Key Words – Diastolic Heart Failure, Hypertension, Dahl Salt Sensitive Rats, Myocardial Mitochondrial Metabolism, HIT, MCT, Mitochondrial Efficiency, Oxidative Phosphorylation

Preface

This thesis has been submitted in partial fulfillment of the requirements for the master's degree at the Norwegian University of Science and Technology. The research project was supervised by Natale Pinheiro Lage Rolim and Fredrik Hjulstad Bækkerud, and was conducted as part of an European Research Council Multicenter study denominated OptimEx (the acronym for "Optimizing Exercise Training in Prevention and Treatment of Diastolic Heart Failure") coordinated by professor Ulrik Wisløff (head of the Cardiac Exercise Research Group - CERG). This thesis would not have been possible without the help of many people of which I am grateful and wish to express my gratitude to. I especially want to thank my supervisors Natale Pinheiro Lage Rolim, Fredrik Hjulstad Bækkerud and Ulrik Wisløff for their guidance and endless seeming work hours. Further, I want to thank the members of OptimEx as well as the staff at the animal facility for their help with the research project. Lastly I want to thank my friends and family for supporting me outside the professional environment and maintaining my social life.

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Abbreviations

ACE	angiotensin converting enzyme
Acetyl-CoA	acetyl-coenzymeA
ADP	adenosine-di-phosphate
Ang II	angiotensin II
ATP	adenosine-tri-phosphate
BP	blood pressure
BW	body weight
CAC	citric acid cycle
CCCP	carbonyl cyanide m-chloro phenyl hydrazone
CO	cardiac output
DHF	diastolic heart failure
EF	ejection fraction
ETS	electron transport system
E/A	ratio for early diastolic transmitral peak flow to late diastolic transmitral peak flow
E/E'	ratio for early diastolic transmitral peak flow to early diastolic velocity of the mitral annulus
$FADA_2$	flavin adenine dinucleotide
FAO	fatty acid oxidation
G	glutamate
HF	heart failure
HFNEF	heart failure with normal ejection fraction of left ventricle
HFPEF	heart failure with preserved ejection fraction of left ventricle
HFREF	heart failure with reduced ejection fraction of left ventricle
HIT	high intensity interval training
HIT-HV	intensity interval training – high volume
HIT-LV	high intensity interval training – low volume
HR	heart rate
HS	high salt
LAD	end-systolic left atrium dimension
LS	low salt
LV	left ventricle

LVEDP	left ventricular end diastolic pressure
LVEF	left ventricular ejection fraction
lvGM	left ventricle complex I respiration (with substrates glutamate + malate + ADP)
lvGM/GMS	ratio for left ventricle complex I respiration (lvGM) to left ventricle maximal respiration (lvGMS)
lvGM/Lgm	ratio for left ventricle complex I respiration (lvGM) to left ventricle complex I LEAK respiration (lvLEAKgm)
lvGMS	left ventricle maximal respiration (complex I and II; with substrates glutamate + malate + succinate + ADP)
lvGMS/CCCP	ratio for left ventricle maximal respiration (lvGMS) to uncoupled respiration
lvLEAKgm	left ventricle complex I proton slip (also called LEAK respiration, with substrates glutamate + malate; no ADP)
lvRCR	left ventricle respiratory acceptor control ratio
M	malate
MCT	moderate continuous training
NADH	nicotinamide adenine dinucleotide
NT-proBNP	NH_2-terminal portion pro-brain natriuretic peptide
O_2	oxygen
P	phosphate
PCr	phosphocreatine
S	succinate
SBP	systolic blood pressure
sig.	significant
SHF	systolic heart failure
SS	salt sensitive
SUIT	substrate-uncoupler-inhibitor titration
SV	stroke volume
VO_2	oxygen uptake
VO_2max	maximum oxygen uptake
VO_2peak	peak oxygen uptake

1. Introduction

Currently 1.9 % Americans and 1.1 % Europeans are affected by heart failure (HF) [2]. This presents with more than 50 % of patients having diastolic heart failure (DHF) compared to systolic heart failure (SHF) and more affected females and elderly people [3, 4]. An incidence of 4.4 % over eleven years has been reported for DHF [5]. Further a 23 % higher prevalence within the American population is predicted for 2030. However, even though the prevalence of DHF is higher than SHF, no treatment in the recent years was able to improve the outcome of DHF [6], and although the treatment of DHF is still needs to be improved, positive changes of DHF with exercise training have been reported [7, 8].

Some have described HF to be like an engine out of fuel [9]. More specifically, HF has been defined as a dysfunction or abnormal structure of the heart with the consequence of an insufficient delivery of substrates and oxygenated blood to the cells throughout the body [10]. Main characterisations of DHF and SHF are predominant diastolic or systolic dysfunction, respectively [11], and left ventricle (LV) hypertrophy [12], for which decreased adenosine-tri-phosphate (ATP) concentrations have been reported [13]. Further, decreases in ATP are affiliated with impaired calcium (Ca^{2+}) pumps in the muscle and can lead to impaired muscle relaxation [14-17], which in the LV is the diastole [18]. Therefore, a decrease in ATP can lead to an impaired LV relaxation, and thereby a diastolic dysfunction.

The presented facts show, that it is necessary to accumulate more knowledge about DHF. Therefore, the aim of this study was to investigate an animal model for DHF in rats and the investigation of the myocardial mitochondrial metabolism in DHF as well as the influence of different aerobic exercise intensities on this metabolism. To achieve this, a model for DHF using Dahl salt sensitive (SS) rats and a high salt (HS) diet will be used. The hypothesis for this study is that the myocardial mitochondrial metabolism is different in DHF and that this metabolism can be influenced by different exercise intensities.

To give a clear outline, the main information will be presented in the introduction. This includes topics about HF, exercise, mitochondria and animal models. After that, the methods used to conduct this study will be described. That section is followed by a part presenting the results, which are afterwards discussed. To complete this, a conclusion and limitations will be given at the end.

1.1. Heart Failure

The different types of HF are distinguished based on ejection fraction (EF). EF is the quotient of stroke volume (SV) divided by end-diastolic volume of a determined ventricle [3, 5, 19-21] and is used to separate HF into two categories; HF with preserved or normal EF of the LV (HFPEF and HFNEF) and HF with reduced EF of the LV (HFREF) [3, 19, 22].

Left ventricular ejection fraction (LVEF) is related to the two phases the heart works in, the systole and the diastole. Before and during ejection (systole), increases in contractility and pressure within the LV can be seen, while the diastolic phase is associated with relaxation and low pressure [18]. With a LVEF of < 40 %, HF patients are considered to have HFREF [3, 21], LVEF > 50 % falls under the category of HFPEF [3, 5, 19] and LVEF 41 % - 49 % is considered a borderline area and subjects within that area are either excluded from studies [5] or categorized as an extra group [3]. HFPEF and HFREF are often also termed DHF and SHF, respectively [11, 23, 24]. From now on DHF will be used to describe HF with LVEF ≥ 50 % and SHF to describe HF < 50 %.

It has been shown, that DHF patients present with diastolic dysfunctions [25]. The diastole phase of the heart beat is a phase of LV filling and systole a phase of LV ejection [26]. Therefore, it can be deducted that a diastolic dysfunction does not change the LVEF. This paradigm is discussed by Solomon, Anavekar [27]. They argue that with LVEF < 45 % risk factors for cardiac events are related to systolic dysfunction. However, that with a LVEF > 45 % diastolic dysfunction is probable because cardiac events could not be linked to systolic dysfunctions. Further, it is discussed, that the terms DHF and SHF are misleading because they are not mutually exclusive since they don't present with only diastolic or only systolic dysfunctions, respectively [22]. However, the predominant feature of DHF is diastolic dysfunction [11].

1.1.1. Diastolic Heart Failure and Hypertension

Other than women and elderly, a further risk factor for the development of DHF is hypertension [3, 4]. Chronic hypertension leads to DHF, as for example investigated in an animal model for diastolic dysfunction via hypertension [12]. Therefore, it is important to understand how blood pressure (BP) levels are regulated.

Physiologically, pressure level decreases are detected by arterial baroreceptors, leading to a release of renin from the kidney. Renin is an enzymatic activator that hydrolyses inactive

angiotensinogen from the liver to angiotensin I [16, 28]. Angiotensin I is in turn converted to angiotensin II (Ang II) by angiotensin converting enzyme (ACE) which comes primarily from epithelial cells within the lung [28], but which is also present in kidney and endothelial epithelial cells. Ang II acts on the adrenal cortex, causing it to release a steroid hormone called aldosterone. The secretion of aldosterone plays a role in the BP regulation mainly by increasing ions and water retention in the kidneys, which cause the conservation of sodium, secretion of potassium and increased blood volume and BP [16, 29]. However, Ang II also acts directly on the tubular resulting in the same activities as seen from aldosterone. Other BP increasing mechanisms that are activated by Ang II are an increased activity of the sympathetic nervous system, arteriolar vasoconstriction and antidiuretic hormone release by the pituitary gland [29].

Epstein [28] presents two ways in which this BP regulation system may have an impact on the development of HF. Firstly, it was mentioned that increased BP induced by Ang II could lead to cardiomyocyte hypertrophy. Secondly, that increased levels of aldosterone are seen in HF, which is partly responsible for fluid and salt retention.

1.1.2. Diagnostic Parameters for Diastolic Heart Failure

It has been established that elderly people and women are at higher risk for developing DHF, especially, if they have hypertension. However, risk factors are not a diagnosis. In order to diagnose DHF specific signs and symptoms of this disease have to be present. They include signs and symptoms of HF, LVEF > 50 %, increased LV end-diastolic pressure (LVEDP), increased early diastolic transmitral peak flow (E) to the early diastolic velocity of the mitral annulus (E') ratio (E/E' ratio) and other echocardiographic measurements as well as increased natriuretic peptides, as to be described in detail below.

LVEF is often used to distinguish between types of HF and is therefore an important diagnostic parameter, where LVEF > 50 is DHF [30]. This is, as presented, related to the phases of the heartbeat, and the relaxation during the diastole and is relevant, because it has been reported that increased systolic BP (SBP) could cause increased relaxation times, which could elevate LV diastolic pressures, and at rapid heart rates, lead to increases in pulmonary hypertension, cause dyspnea, and even congestive HF [31]. However, in this scenario, LVEF

should be unaltered as Paulus, Tschope [22] as well as Vasan and Levy [30] have summarized while describing different diagnostic parameters.

Other important signs of DHF as mentioned by Vasan and Levy [30] were to confirm impaired LV relaxation and filling, as well as diastolic distensibility. Important measurements that point towards these changes are increased LVEDP assessed by catheterization, E/E' from tissue Doppler and natriuretic peptides as presented in a flowchart for diagnosing DHF by Paulus, Tschope [22]. A change in the relationship of end-diastolic volume and pressure has been reported for DHF. The LVEDP is higher than normal, while the volume is smaller [24], for example, a higher LVEDP has been shown in a canine acute pressure-overload model [32].

Further, it is being reported that E/E' > 15 is a strong predictor for LV diastolic filling pressure in patients [33]. Increased LV filling pressure leads to subsequent increases of the pulmonary artery pressure and pulmonary oedema [25], this in turn leads to increased work of breathing and dyspnea [8]. Moreover, an increased E to the late diastolic transmitral peak flow (A) ratio (E/A ratio) has been shown [32]. E/A ratio can be included as an additional diagnostic parameter, although it should be used as a secondary measurement to E/E', if E/E' yields results between 8 and 15 [22]. If this is the case, it is being suggested to include measurements of natriuretic peptides as well [22].

The levels of natriuretic peptides, for example NH_2-terminal portion pro-brain natriuretic peptide (NT-proBNP), can be useful to establish a prognosis of DHF. Patients with diastolic dysfunction have increased amounts of NT-proBNP related to the severity of DHF [34]. It has been shown that BNP is not a good parameter to test when looking at mild diastolic dysfunctions, however, is recommended to detect moderate to severe diastolic dysfunctions [35]. Further, it has been suggested, that multiple parameters should be analyzed in order to better confirm the presence of DHF [22, 30].

In addition to the clinical signs of DHF presented above, exercise intolerance is a hallmark feature in HF [36]. Different hypotheses have been discussed as a reason for exercise intolerance of DHF. One reason for the described exercise intolerance in DHF patients [36] is perhaps dysfunction or abnormal structure of the heart with the consequence of an insufficient delivery of substrates and oxygenated blood to cells throughout the body [10]. Other reasons

might be decreases in aerobic capacity or dysfunction in skeletal muscle metabolism [37]. It seems like the Frank-Starling mechanism is impaired by the existing diastolic dysfunction in DHF [36]. However, elsewhere it is suggested, that the maladaptation during exercise might be due to other reasons such as impaired skeletal muscle metabolism and impaired perfusion. Therefore, the diastolic dysfunction seen in DHF might not be the cause for exercise intolerance [37].

1.1.3. Left Ventricular Hypertrophy

The decrease in LV volume and increases in LV pressure, that were presented previously, can be related to the type of hypertrophy apparent in DHF. Studies on rats have shown that DHF presents with LV hypertrophy [12, 38]. However, cardiac hypertrophy is not necessarily pathological, it can also occur physiologically [39] and generally present with concentric or eccentric remodelling [40, 41], which is defined by their specific geometry [41]. Concentric remodelling is initiated by pressure overload, for example through isometric strength exercise training in a physiological way as reported by Mihl, Dassen [40], or through increased levels of BP in a pathological manner as mentioned by Maillet, van Berlo [41]. In response to the increased pressure, the cardiomyocytes increase in thickness (Laplace's Law), which will increase LV wall thickness and decreases LV chamber volume [41].

Eccentric cardiac remodelling is concluded to be a response to volume overload and happens physiologically due to aerobic exercise training [40, 41]. However, it has been suggested that hearts of endurance trained athletes are subjected to a slight pressure overload as well [40]. Eccentric cardiac remodelling presents with a lengthening of cardiomyocytes, leading to thinner LV wall and increased LV chamber volume as reported by Maillet, van Berlo [41]. It can be summarized, that an increase in cardiac mass is a response to increased workloads [42] and that a physiological hypertrophy is reversible [41], while the same is not observed in concentric cardiac remodelling. Changes in cell geometry have also been demonstrated in studies using animal models showing increased cell size and ventricular mass induced by isoproterenol, with no reported changes in cell number [43].

1.1.4. Classification of Heart Failure

A classification system can be used to assess the severity of HF. According to the classification after the New York Heart Association (NYHA), class I presents with no

limitations in physical activity, class II shows minor limitations in physical activity and shows signs of fast fatigue, class III is limited in physical activity to a severity that interferes with less than normal daily tasks, and class IV may show symptoms of HF at rest and activities cannot be performed without discomfort [11, 44]. More specifically related to DHF, the grade of diastolic dysfunction has to be assessed. Grade 1 suggests a reversal of the normal E/A ratio and is considered a mild dysfunction, while a moderate dysfunction is detected with elevated left atrial filling pressures and is categorized as grade 2. Grade 3 is considered a severe diastolic dysfunction with a restrictive pattern of diastolic dysfunction that can be reversed with the Valsalva manoeuvre, whereas grade 4 is when the restrictive pattern is fixed and high E/A and E/E' ratios can be seen [45]. These classifications help understand the development and severity of the pathophysiology as well as to determine the best therapeutic strategy to prevent and treat DHF.

1.1.5. Treatment

Although treatment options for DHF exist, an improvement of the prognosis of DHF cannot be seen in the recent years [6]. Previous studies [46-48] have been searching for the efficacy of antihypertensive therapy in order to lower the systemic BP and reduce the rate of HF [49]. It is known that antihypertensive treatment is effective in decreasing hypertension and cardiac hypertrophy as well as increasing diastolic filling [46]. Although this was reported from subjects that had only mild hypertrophy and classified as functional class II or III of the NYHA. Beta-blockers, for example atenolol, were used to achieve this effect. However, it was later found that a different beta-blocker, Losartan, had a lower risk of mortality [50]. A different method to achieve a reduction in hypertension is to inhibit ACE, as it has been conducted in patients with functional class IV of ischemic HF but with LVEF > 50 % [51]. A reduction in BP levels as well as in LV mass was reported in addition to increased exercise tolerance. However, the last studies on the management of DHF have shown that although there are different possible approaches to treat DHF, the efficiency of a therapy still need improvements [49]. Exercise effects on DHF will be described under (1.2). Although treatment options with drugs exist, they do not evidently improve the outcome of DHF [49]. It is therefore important to further research DHF and its treatment and prevention possibilities.

1.2. Exercise

When addressing the general population, different aerobic endurance exercise regimens have been described in the literature. Although moderate to high intensities are recommended for healthy adults [52], it has been shown that in healthy adults, high intensity interval training (HIT) leads to larger improvements in aerobic capacity than moderate continuous training (MCT) [53]. Aerobic capacity is an important predictor for mortality in healthy subjects and patients with cardiac diseases. A better aerobic capacity was found to coincide with better survival [54].

Positive changes have also been found in DHF during exhausting exercise in mild DHF cases of NYHA class II. Although oxygen (O_2) -consumption, a-vO_2-difference, cardiac output (CO), SV and heart rate (HR) were lower in DHF patients than in healthy controls, the percentage increase from rest to peak exercise was similar [7]. Under exercise, DHF patients rely on increased a-vO_2-difference rather than increased CO to achieve a similar increase in O_2 uptake (VO_2) as healthy people. An increase in LV filling volume comparable to healthy people, requires a three times higher increase in LV filling pressure, making an increase in CO possible, but ineffective [8].

Different adaptation mechanisms during exercise have been suggested for DHF patients compared with healthy controls [7]. While an increased SV was associated with a lower HR in healthy subjects, in DHF it was associated with a higher HR [7]. Additionally, the mechanisms responsible for the changes in SV may also differ [7]. While at lower intensities, in healthy adults, both LV filling pressure and LV end-diastolic volume are raised to increase SV, only the LV filling pressure is raised at high intensities and both the end-diastolic as well as the end-systolic volumes decrease [55]. For DHF, the change in SV is reported to be dependent on LV filling instead of emptying [7]. However, it has been found that in DHF exercise has the acute effect to increase LVEDP and shorten LV relaxation time. Nonetheless, the improved relaxation was not enough to compensate for the shortening of diastolic filling time [56].

An increased peak VO_2 (VO_2peak), reverse remodelling and increased LV diastolic function as well as improvements in quality of life, were associated with three month of moderate intensity endurance and resistance training in DHF patients [57]. It is of note that this study analyzed subjects with mild HF of NYHA class II, which was improved in most of the subjects to class I in the exercise group [57]. In patients with diastolic dysfunction, 16 weeks

of moderate endurance training improved the VO_2peak and quality of life, but other parameters such as diastolic function were unchanged [58-60].

Although an improvement of endothelial function was reported in rats subjected to moderate and HIT [61], no change was found in human DHF patients [59]. For SHF subjects with moderate to severe HF, Wisloff, Stoylen [62] observed a 46 % increase in VO_2peak after HIT. Additionally, a 15 % reduction in O_2-cost and a 59 % decrease in lactate concentration at a submaximal intensity indicated improved work economy. Declines in LV end-diastolic, LV end-systolic volume as well as a decrease of LV hypertrophy were also reported. Although the previous study [62] focused on SHF, improvements in diastolic function, in addition to an improved systolic function with HIT, were visible [62]. Both immediate effects during exercise training [7] and exercise training over long periods of time [62] show improvements in DHF. It can be interesting to look upon the different training intensities, as different training intensities have different effects in healthy human [53]. In healthy mice, HIT may decrease the O_2-consumption needed for non-contractile work of the heart without changing contractile efficiency [63]. In addition, improvements in cardiomyocyte function with decreased relaxation time and contraction rates are apparent in HIT trained rats [61].

Other effects of exercise training on DHF are also described. HIT has been reported to positively influence the pathophysiology of inherited hypertension syndrome [64], and a positive effect of moderate intensity exercise has been shown for elderly hypertensive women. A decrease in resting BP was reported [65]. Considering that hypertension plays an important role during the development of DHF, it is important to consider exercise training as a possible treatment option of DHF.

This chapter about exercise shows that most of the studies that have previously conducted exercise in DHF used moderate intensity. However, different and greater improvements have been shown with HIT. It is therefore necessary to look further into HIT for DHF.

1.3. Mitochondria

Every energy-consuming process in the body requires an energy source, which is provided by an important element of the cell, the mitochondria [66]. The energy supply is generated by the conversion of chemical energy from food to ATP through mitochondrial metabolism. ATP is

an energy rich molecule and the mitochondrial metabolism follows three main steps to gain and use this energy; the utilization of substrates via glycolysis and fatty acid oxidation (FAO) [66], the electron transport system (ETS) using oxidative phosphorylation [67], and the creatine kinase system [16]. Both glycolysis and FAO supply the citric acid cycle (CAC) with acetyl-coenzymeA (acetyl-CoA). Glycolysis is the process of glucose break down into pyruvate after glucose has entered into a cell. Pyruvate then enters the mitochondrial matrix and is transformed into acetyl-CoA. Similarly FAO is the transformation of fatty acids to Acetyl-CoA within the mitochondria [66]. Already the transformation of glucose into pyruvate yields nicotinamide adenine dinucleotide (NADH) and a small amount of ATP. However, the use of acetyl-CoA in the CAC produces more NADH and in addition, flavin adenine dinucleotide ($FADH_2$) [16]. NADH and $FADH_2$ supply the ETS in the inner mitochondrial membrane with electrons by acting as electron carriers within the mitochondria [67].

The ETS consists of multiple protein complexes [66, 67]. Complex I oxidizes NADH into $NAD^+ + 1\ H^+ + 2\ e^-$ [66]. The electrons are then passed along to complexes III and IV, from lower to higher affinity, until they form water with an oxygen molecule at the end of this system. $FADH_2$ is utilized in the same manner, but the oxidation occurs at complex II [67]. This system yields excess energy at every complex. The complexes work with different substrates, where substrates with a low affinity work at complexes at the beginning of the ETS and then gradually increasing the affinity towards the end of the system with oxygen having the highest affinity [67]. The excess energy from the complexes is used to drive proton pumps, with the exception of complex II, which transport protons into the inner membrane space creating an electrochemical gradient between the inner membrane space and the mitochondrial matrix (Figure 1.1). This gradient is used by ATP-synthase, also called complex V [67] [28], to produce ATP from adenosine-di-phosphate + phosphate (ADP + P) [66, 67]. The different complexes may bind together to form supercomplexes also called respirasomes, e.g. complex I, III and IV. This is to increase the efficiency of the ETS [68]. Besides ATP, also phosphocreatine (PCr) can be used to harness the high-energy bond of phosphate to another molecule. The storage capacity for PCr is furthermore greater than for ATP. However, the splitting of PCr, even though anaerobic, is very rapid and the emptying of the storage is faster than its synthesis. The cells therefore rely on a continuous energy production, which is given with the aerobic synthesis of ATP [16].

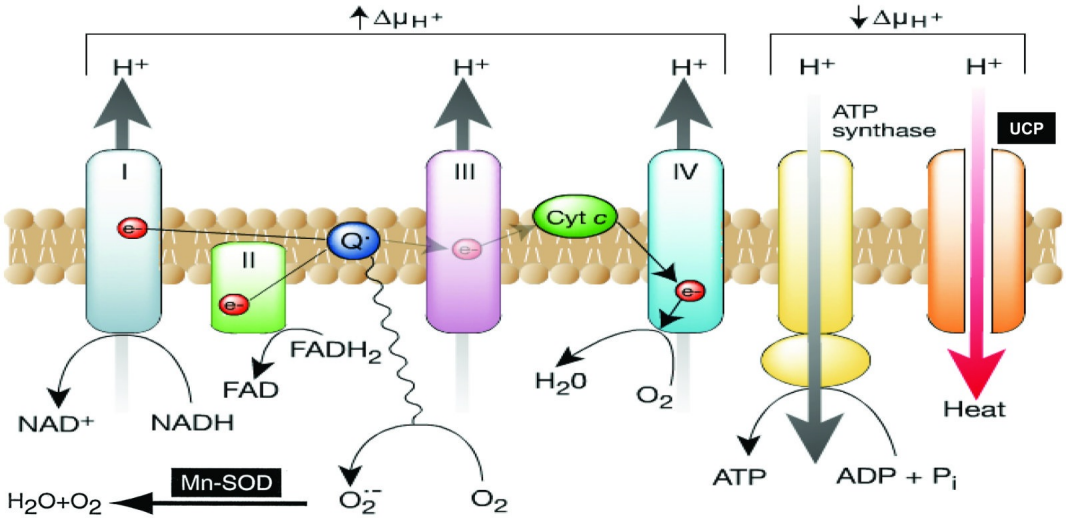

Figure 1.1 Electron Transport System

NADH = nicotinamide adenine dinucleotide; $FADH_2$ = flavin adenine dinucleotide; Cyt c = cytochrome c; P = phosphate; ADP = adenosine-di-phosphate; ATP = adenosine-tri-phosphate; UCP = uncoupling protein; the electron transport system accepts electrons from NADH at complex I and from $FADH_2$ at complex II; the electrons are transported through the system to complex IV where they bind with oxygen to form water; at complex I, III and IV protons are pumped into the mitochondria, when the gradient is high enough, they are transported across the membrane via ATP-synthase to form ATP from ADP+P; another way across the membrane is via uncoupling protein, which produces heat

Giacco and Brownlee [1]

1.3.1. Assessment of Mitochondrial Metabolism

To assess mitochondrial metabolism, respirometry can be used. Respirometry is an analysis of oxygen consumption in a controlled closed environment, which consists of a respiration medium. Permeabilized muscle tissue, in which the plasma membrane has been chemically treated so that substrates can reach the intact mitochondrial membranes, or isolated mitochondria, are added to this medium. Oxygen, ADP and complex specific substrates are then added and titrated, respectively. Complex I specific substrates are for example glutamate (G) and malate (M), whereas succinate (S) for example is complex II specific [69] [70].

The respiration of the mitochondria during this protocol is described with respiratory steady states. State 1 refers to the respiration with only oxygen and the respiration medium, meaning that ADP and complex specific substrates are absent. If ADP is added, the mitochondria are in state 2. However, the first two stages can only be seen in isolated mitochondria, whereas state

3 can also be seen in permeabilized tissue. In order to reach state 3, complex specific substrates must be added, with no inhibitors or uncouplers present so that oxidative phosphorylation can occur [71]. State 4 respiration is also called LEAK respiration, because complex V is inhibited by the titration of specific substrates (e.g. oligomyocin) so the proton slip across the mitochondrial membrane can be evaluated. Furthermore, it can be tested if the oxidative phosphorylation system was limited by uncoupling the respiration. This means that a protonophore substrate is added, and protons circumvent complex V without synthesizing ATP [70].

1.3.2. Mitochondrial Metabolism in Heart Failure

The description of the mitochondrial metabolism displays the physiological point of view. However, HF might affect mitochondrial metabolism, like it has been shown in rat hearts after infarction, the mitochondrial efficiency is reduced compared to a sham and training group [72]. It has further been suggested that energy depletion may have a negative influence on the contractile efficiency of the heart [9]. However, in a HF model with canine and LVEF < 50 %, oxidative phosphorylation is reduced due to a decrease in respirasomes, rather than a decrease in individual complex work [73].

These studies demonstrate that research in myocardial mitochondria has been done. However, often SHF is studied and the pathophysiology of SHF differs greatly from DHF. A change in substrate utilization on the other hand has been described for both SHF and DHF [74, 75]. For example, a reduced oxidative capacity and a decrease in glycolytic enzymes have been reported for SHF [75]. Further, both an impaired FAO and a decrease in glycolysis have been reported for rats with decreased LVEF [76]. In contrast to that, an up regulation of the glycolytic pathway is shown in DHF [74]. It is further suggested, that this alteration is because of reduced energy supplies [74], and that this, together with decreased ATP reserves in DHF [13], contributes to the fact that HF (both SHF and DHF) is affiliated with energy depletion, as suggested for SHF [9].

The decrease in available ATP could have an influence on myocardial relaxation. During a physiological muscle contraction, Ca^{2+} is released and binds to receptors on tropomyosin. This makes a contact between actin and myosin heads possible. ATP binds to these cross-bridges to break them and induce a sliding of the filaments. As long as the Ca^{2+} concentration

remains high, this process will continue. In order to achieve a relaxation the Ca^{2+} has to be pumped back into the sarcoplasmatic reticulum. This is an active process that requires ATP [16] but can delay relaxation in cardiaomyocytes when impaired [15]. In SHF this might be due to inhibited pump function or decreased pump expression [17]. However, these alterations might also have effects on relaxation [14] and therefore DHF.

1.3.3. Mitochondrial Metabolism with Exercise

Training may also alter mitochondrial metabolism. However, these changes are positive and occur both in skeletal muscle [77] and cardiac tissue [63]. Fillmore and Lopaschuk [78] concluded that a higher glycolysis and inhibited FAO may lead to increased efficiency of ATP utilization in the heart. A change towards higher glycolysis with training has recently been shown [79]. However, a higher FAO in trained compared with untrained subjects has been reported previously [80]. A decreased glycolysis and increased FAO has been shown by Burgomaster, Howarth [77] in both a MCT group and a group that exercised sprints. It was described in 1988 that glycolysis accounts for only about 20 % - 30 % of acetyl-CoA production in the heart of healthy subjects at rest [81]. However, under MCT, acetyl-CoA production increased to amounts above 50 % of the total acetyl-CoA production [81]. Contradicting these findings in substrate utilization, also a change towards higher glycolysis and lower FAO has been found for cardiac tissue in healthy mice [63]. It has also been shown that exercise influences not only substrate utilization, but also the ETS. HIT is shown to increase state 3 respiration in permeabilized cardiac tissue by 35 %, whereas MCT has no influence, with no change of LEAK respiration [63].

Although the knowledge about mitochondrial metabolism in general is vast for skeletal muscle, changes of substrate utilization or mitochondrial metabolism during exercise or DHF are still being discussed or are somewhat unexplored and need further investigation. It is therefore important to look more closely at the myocardial mitochondrial metabolism during DHF and exercise, to thereby contribute to a better understanding of DHF.

1.4. Animal Model

In order to research DHF, an animal model in which the animals develop DHF is necessary. As shown under 1.1, hypertension leads to diastolic dysfunction. This mechanism was used in an animal model to examine the development of DHF [12], which can also be seen in other

animal models for diastolic dysfunction, as for example renal wrapping in canine [82]. Although different techniques were used for the development of hypertension, the general approach towards DHF through hypertension seems valid. After hypertension and DHF have developed in rats [12] and canine [83], similar symptoms to the ones in patients (1.1) can be found. Looking deeper into the rat model used by Doi, Masuyama [12], it can be seen that hypertension was induced when rats were fed a HS diet. A linear relation of HS diet and BP has been described in rats [84]. Most of the studies [12, 38] have been using male rats, although it is well established that the prevalence and incidence of DHF is higher in female patients. The human physiology between male and female can be quite different, especially when looking on hormonal factors. Since hypertension is influenced by the renin-angiotensin-aldosterone system, which is hormone based, and because DHF has a higher incidence in women, it is important to use female rats when studying DHF.

1.5. Purpose

It is presented in this introduction, that DHF has a growing prevalence in the population with a higher incidence in women. Further, the importance of the risk factor hypertension and the mechanisms around hypertension have been explained. The signs and symptoms of DHF, possible treatment options and the importance of exercise training were presented. In addition, the energy mechanisms and changes of these mechanisms in DHF have been described. Combining these important factors, with the animal model using Dahl SS rats, the aims of this study can be shown.

The aim of this study was to investigate the myocardial mitochondrial metabolism in DHF and the influence of different aerobic exercise intensities on myocardial mitochondrial metabolism in DHF. To achieve this, female Dahl SS rats were studied in a hypertension model using a HS diet. It is hypothesized that the myocardial mitochondrial metabolism is different in DHF and that it can be influenced by exercise training during the development of DHF in rats.

2. Methods

2.1. Animals

For this study, 120 female Dahl SS rats from Charles River (Charles River Laboratories International, Inc., Connecticut, USA) were used. At seven weeks of age, the animals were split into five groups, of which four groups (n = 100) were fed with a HS diet (8 % NaCl) and a low salt group (LS, n = 20) was fed with a low salt diet (0.3 % NaCl) and used as a sedentary control group. The chow fed to the animal was produced by Special Diet Services (Witham, Essex, UK). One of the four HS dietary groups (HS, n=40) was likewise sedentary, whereas the three remaining groups were subjected to different aerobic endurance training intensities: high intensity interval training-low volume (HIT-LV, n = 20), high intensity interval training-high volume (HIT-HV, n = 20), and moderate continuous training (MCT, n = 20). All of the rats were housed under identical conditions in a 12-hour 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).

2.2. Blood Pressure

After seven days of acclimatization to control for stress, non-invasive BP measurements were performed using a commercial system for rats (CODA system, Kent Scientific Co., Torrington, CT, USA). The rats were restrained in thermic plastic chambers, which had a controlled temperature between 32 °C and 34 °C. No anaesthetics were used for the restraining. The blood volume and pressure in the tail of the animals were measured with volume pressure recordings by using tail cuffs. The SBP was detected as the mean of 10 high quality acquisitions. BP measurements were done 18 hours or more after the last exercise training to control for short-term adaptations. The measurements were done every other week with the first measurement one week prior to the start of HS diet and exercise training and the last in week 28 (Figure 2.1).

2.3. Echocardiography

Transthoracic echocardiography was performed with a 24-MHz transducer using a commercial system (Vevo 2100, VisualSonics, Ontario, Canada). Light anaesthetics (1.5 - 2 % isoflurane inhalant mixed with 1 L/min 100 % O_2) were used during the measurements, but

the rats were spontaneously breathing. The rats were placed in the supine position on a heating platform to keep the body temperature above 37 °C. During the procedure, echocardiography electrodes were used to monitor the HR and respiratory parameters. A blinded investigator placed the transducer gently in the left parasternal position. Blinding was maintained throughout the experimental phase.

End-systolic left atrium dimension (LAD) was measured in the long axis view. The M-mode curser line was placed through the left atrium walls at the level of the aortic valves. Two dimensional guidance was used. End-systolic and end-diastolic LV wall thickness and cavity diameters were measured in short axis view with the M-mode curser line perpendicular to the LV anterior and posterior walls. The curser line was placed at the level of the papillary muscles. LVEF was calculated from the LV structural parameters measured from short axis view in M-mode. The area-length method was used to estimate LV mass and LV volume [85, 86].

Doppler recordings were acquired by positioning the Doppler sample volume parallel to flow direction. This was done in the apical 4-chamber view. In this setting E and A measurements were performed and the mitral valve E/A calculated. At the level of basal septal segment, myocardial velocities were obtained from the apical view by using pulsed Doppler tissue imaging. E' was measured and E/E' ratio calculated.

Measurements were done prior to the start of HS diet and exercise training and at week 5, 7, 14, 17, 18, 19 and 28 (Figure 2.1).

All measurements were conducted and obtained in triplicate, excluding the respiration peaks. Calculations were automatically computed by the Vevo 2100 standard measurement package.

2.4. Histology

Hearts of three rats from each of the groups studied were perfusion-fixed with 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

cardiacmyocytes was determined in sagital sections (4-chamber view) of the LV after staining of plasma membrane with rhodamine-labeled (WGA) (Vector Laboratories, Inc.). The cross-sectional area was determined from four different areas of the free-wall of the LV (40x magnification), in 15 individual myocytes per frame, by digital planimetry using the ImageJ software.

2.5. Hemodynamic Measurements

Hemodynamic studies were performed as terminal procedures at each time point in each group (Figure 2.1). After induction of anaesthesia (2 % isoflurane), the cannulation of right carotid artery was performed with a conductance catheter (1.4 Fr.; Transonic Scisense Inc., London, CA) with pressure transducer that was advanced across the aortic valve into the LV. LV end-systolic and end-diastolic pressures and, after withdrawal of the catheter into the aorta, phasic and mean arterial pressures were measured. Data were recorded on LabChart 7 software (AD Instruments, UK)

2.6. Blood Measurements

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

2.7. Organ Weights

Heart and lung weights were measured immediately after their excision (Figure 2.1). The hearts were divided into four chambers. Left and right atria and left and right ventricles were weighted. LV was stored at -80 ºC 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 ºC after the first weight. Every day for four 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.

2.8. Oxygen Uptake/ Total Distance Run/ Exercise Capacity

The VO_2 was measured with a commercial system (Columbus Instruments 950 North Hauge Avenue, Columbus Ohio). The maximal VO_2 (VO_2max) was detected at the limit of the tested animals tolerance. The tests started with a 15 minute warm up at 20 ° inclination with a low speed. The treadmill band velocity was then increased by 1.8m • min^{-1} every two minutes until the animal could not run any longer. VO_2max was reached, when a levelling off of the VO_2 was detected, despite an increase in speed. This protocol has previously been described and validated in our lab [87]. During the exercise period the training speed was controlled by VO_2max tests every two weeks. The sedentary animals (LS and HS group) were tested before and after the experimental period. The total distance run was calculated considering the maximum speed and the total duration of the test.

The same speed protocol was used to measure exercise capacity. However, the test was performed on the training treadmills (Linton Instrumentation, Diss, UK). The end of the test was reached, when the rats were exhausted.

VO_2max was measured one week prior to the start of HS diet and exercise training interventions as well as in the weeks 5, 7, 17, and 21. Exercise capacity test were performed in the weeks 14, 22 and 28. Three days were between the last test and sacrifice (Figure 2.1).

2.9. Exercise Training

The rats were trained on a treadmill (Linton Instrumentation, Diss, UK) with 25 ° inclination, following three different exercise training intensities. One group was trained with a widely used model at moderate intensity consistent with 60 % of VO_2max. This training occurred five times per week for 40 minutes. The two other groups trained at high intensity interval levels, but only three times per week. Both groups trained in intervals of four minutes at 90 % of VO_2max with three-minute active rest breaks at 60 % of VO_2max. The difference between the groups was the training volume. While HIT-LV performed a low volume exercise with only two intervals, HIT-HV performed higher volume exercise with four intervals. Before each training session a warm-up of 10 minutes at 40 – 50 % of VO2max was performed in all

exercise groups. The exercise training lasted 28 weeks and the last training was done three or more days before sacrifice.

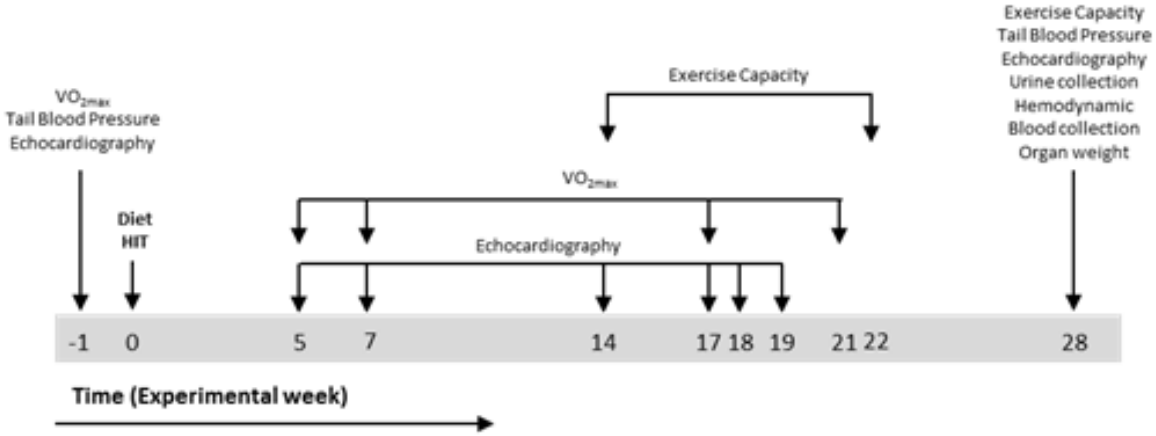

Figure 2.1 Timeline of Measurements

The timeline shown refers to the experimental week. Baseline parameters were measured one week prior (-1) to the start of the HS diet and exercise training. In week 0 of the experiment, the rats were seven weeks of age. This is when the exercise training of the different groups and the HS diet started. At week -1, 5, 7, 14, 17, 18, 19 and 28 echocardiography measures were done. VO_2max was measured in week -1, 5, 7, 17, and 21, and exercise capacity in weeks 14, 22 and 28. Week 28 was the week of the sacrifice. A minimum of three days was between the last exercise capacity test and time point of sacrifice. BP was measured at week -1 and week 28 in addition to every other week (not seen in figure). Further endpoint measurements were urine collection (data not shown), hemodynamic measurements, blood collection and measurement organ weight.

2.10. Mitochondrial Measurements

The analysis of the mitochondrial metabolism was done with the Oxigraph-2k (OROBOROS INSTRUMENTS Corp., high-resolution respirometry, Innsbruck, Austria). The biopsy was treated, prepared, weight and placed in the Oxigraph-2k chamber as described by Pesta [88]. To give a short overview, the tissue was placed on ice cold BIOPS after extraction. After that the fibres were separated and placed on Saponin for permeabilization. After 30 minutes, the sample was washed in MiR05 for ten minutes before being weight and then being placed in the chamber. Pesta [88] describe MiR06 as the respiration medium being used. However, in this study MiR05 was used instead of MiR06 and was prepared as described by Fasching [89]. The same person did the tissue preparation throughout the whole data collection. The weighing of the tissue was also done by just one person.

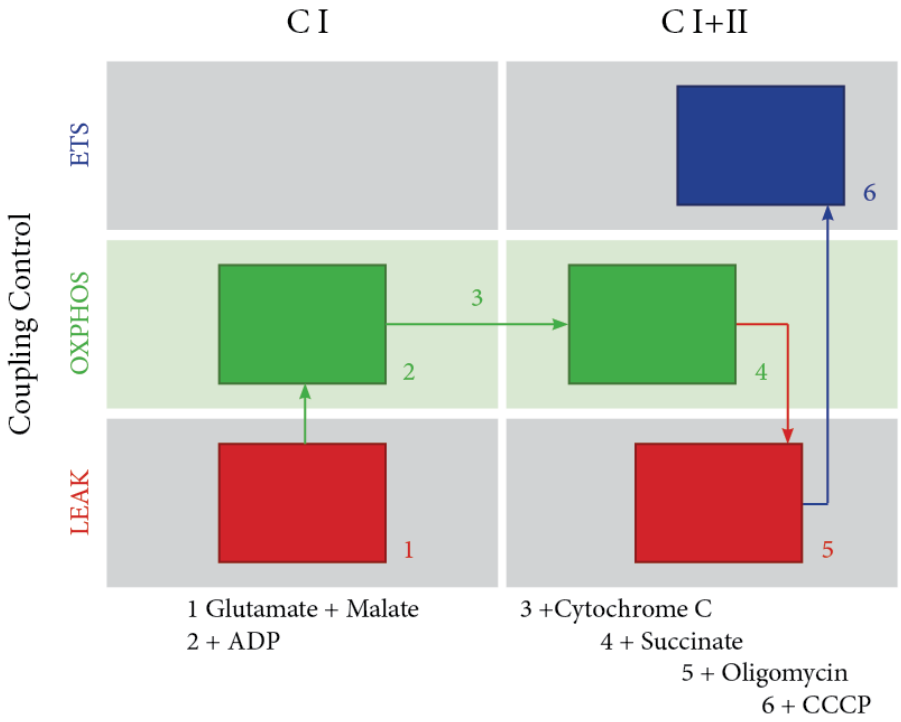

Figure 2.2 Substrate-Uncoupler-Inhibitor Titrations and Coupling Control

OXPHOS = oxidative phosphorylation; ETS = electron transport system; CI = complex I; C II = complex II; ADP = adenosine-di-phosphate; CCCP = Carbonyl cyanide m-chloro phenyl hydrazine each number corresponds to when the substrate named below the figure was titrated during the protocol; columns describe the complexes that are controlled; red fields are LEAK stages, with substrates but without ADP (1) or through inhibition of complex V (5); green fields are OXPHOS stages and describe the actual respiration of the mitochondria; the blue field is a stage after

Substrate-uncoupler-inhibitor titrations (SUIT) (Figure 2.2) (Table 2.1) started with the titration of 5 µL of a 0.8 mol Malate solution and of 10 µL of 2 mol Glutamate solution, which resulted in 2 mmol and 10 mmol concentrations in the chamber, respectively. These two titrations of substrates supplying complex I were done before the tissue was added into the chamber. It was waited for the O_2 flux (in pmol • s^{-1} • mg^{-1}) to be stable, before a next titration was done. After placing the tissue, hyper oxygenizing and the O_2 flux being stable, the proton slip for complex I (lvLEAKgm) could be assessed, and 20 µL of ADP of 0.5 mol were added resulting in a 5 mmol concentration. When O_2 flux was stable, the complex I respiration was evaluated. This was followed by 5 µL of 4 mmol Cytochrome C (final concentration: 10 µmol) to test for intact membranes, 20 µL of 1 mmol Succinate (10 mmol)

to supply complex II respiration and measure maximum respiration (lvGMS), and 1 µL of 4 mg/ml Oligomycin (2 µg/ml) to assess the proton slip of all complexes. Finally, titrations of a 1 mmol carbonyl cyanide m-chlorophenyl hydrazone (CCCP) solution were added. A 1 µL injection of that CCCP solution gave a 0.5 µmol concentration in the chamber. CCCP titrations were repeated until O_2 flux stagnated or decreased, which was interpreted as the end of the protocol. Measurements were taken when O_2 flux was stable after a substrate had been titrated. The last stable titration of CCCP was used to evaluate uncoupled respiration. The following ratios were calculated from these measurements: lvGM/Lgm from lvGM over lvLEAKgm to assess complex I efficiency, lvGM/GMS to evaluate the contribution of complex I respiration to the maximal respiration, lvRCR from lvGMS over lvLEAKgms to measure proton slip for all complexes and lvGMS/CCCP to evaluate if the oxidative phosphorylation was limited.

Table 2.1 Substrate-Uncoupler-Inhibitor Titrations

Substrate	Stock Solution Concentration	Volume Titrated (µL)	Concentration in Chamber
Malate	0.8 mol	5	2 mmol
Glutamate	2 mol	10	10 mmol
ADP	0.5 mol	20	20 mmol
Cyt C	4 mmol	5	10 µmol
Succinate	1 mmol	20	10 mml
Oligomycin	4mg/ml	1	2 µg/ml
CCCP	1 mmol	1 µL	0,5 µmol

ADP = adenosine-di-phosphate; Cyt C = cytochrome c; CCCP = carbonyl cyanide m-chloro phenyl hydrazine; Titrations in order; Tissue was added after glutamate and malate; hyper oxygenation before ADP was added, O2 flux was stable before next substrate was titrated; note that multiple titrations of CCCP were done;

2.11. Statistics

All statistics were performed with IBM SPSS Statistics (IBM, Corp., Armonk, New York, USA). Besides looking at the descriptive, a multi-comparison one-way ANOVA was used to find significant (sig.) differences between groups. If ANOVA showed sig. differences, post-hoc tests (Tukey/Games-Howell) were performed. The Kaplan-Meier method was used to estimate survival distribution and generate survival curves and Log-rank test was applied to

determine survival rates and check the difference of survival distribution between groups. Graphs were done with GraphPad Prism (GraphPad Software Inc., La Jolla, California, USA). Mean ± SD are presented and a p-value < 0.05 was considered sig.

3. Results

3.1. Animal Model

The baseline data shows no differences in BP, body weight (BW), LV Mass, and other echocardiography measurements and distance run between HS and LS group (Appendix I). However, looking at parameters for LV remodelling and LV function that have previously been mentioned as indicators for DHF (1.1), some differences can be described for the end of the experimental protocol. A higher BP in HS can be seen compared to LS group ($p < 0.05$) (Figure 3.1). Further an increased LV Mass, measured with echocardiography, and increased cardiomyocyte cross-sectional area were found in HS compared to LS group ($p < 0.05$). These parameters indicate LV remodelling (Figure 3.2 a-c). Looking at more echocardiography results, a normal systolic function, LVEF in HS > 50 % and an impaired diastolic function, increased E/E' HS ($p < 0.05$), can be seen in HS compared to LS group (Figure 3.3 a-c). Other parameters like NT-proBNP, LVEDP and pulmonary oedema that indicate DHF were also increased in HS compared to LS group ($p < 0.05$) (Figure 3.4 a-c). However the previously mentioned exercise intolerance could not be seen in HS compared to LS group (Figure 3.4 d).

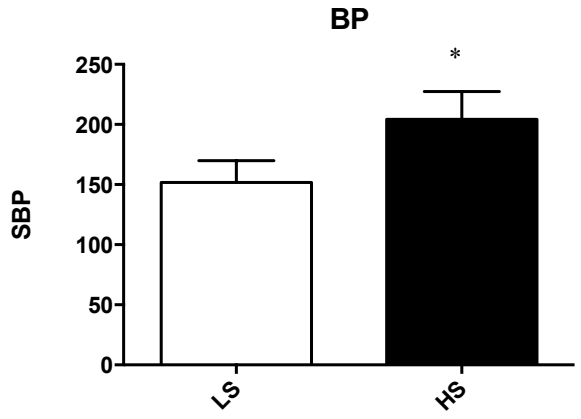

Figure 3.1 Blood Pressure at the End of Study

* = sig. $p < 0.05$ vs. LS; LS = low salt diet group; HS = high salt diet group; SBP = systolic blood pressure; a sig. increase of BP can be seen for HS at the end of study

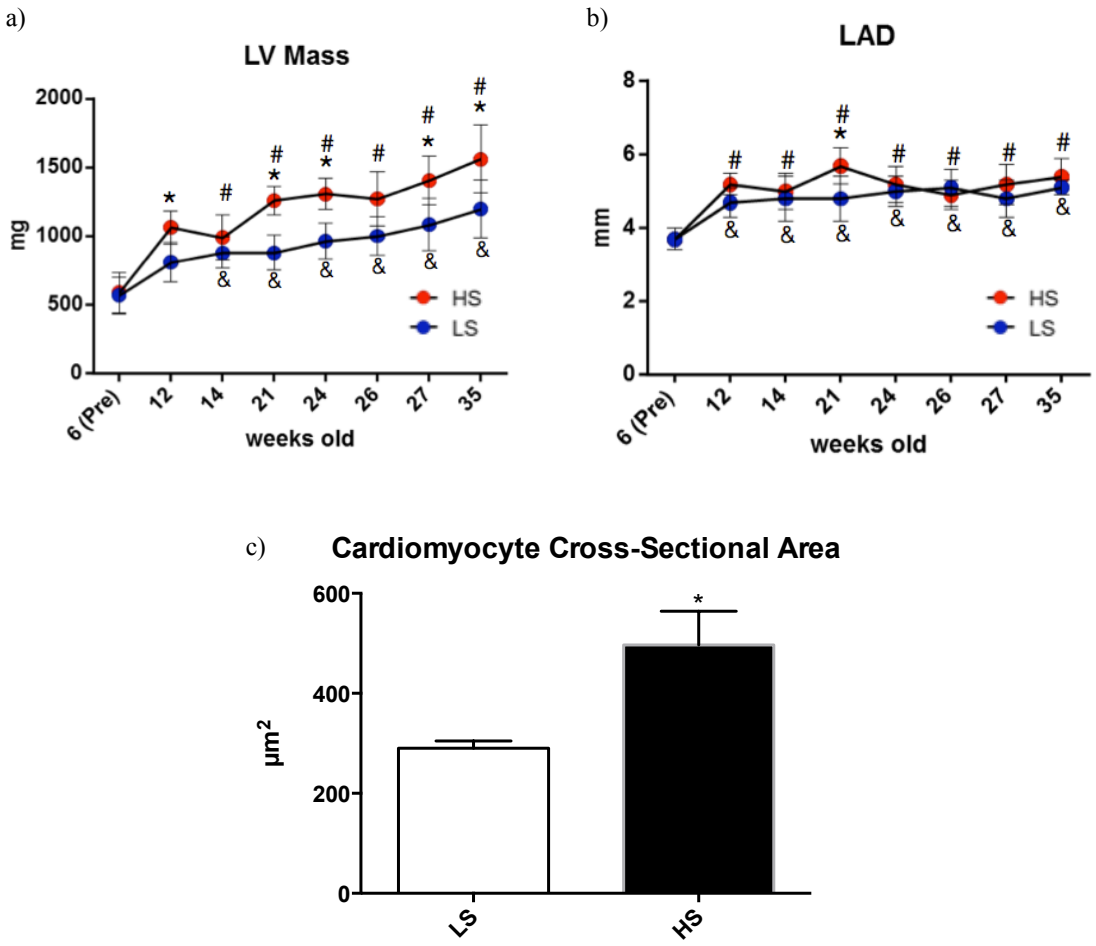

Figure 3.2 Left Ventricle Remodelling Parameters

* = sig. $p < 0.05$ vs. LS; # = sig. $p < 0.05$ vs. pre HS; & = sig. $p < 0.05$ vs. pre LS; LS = low salt diet group; HS = high salt diet group; LV Mass = left ventricular mass; LAD = left atrium dimension; LV remodelling parameters show a) increased LV Mass and b) cardiomyocyte cross-sectional area, but c) no LAD difference for HS vs. LS

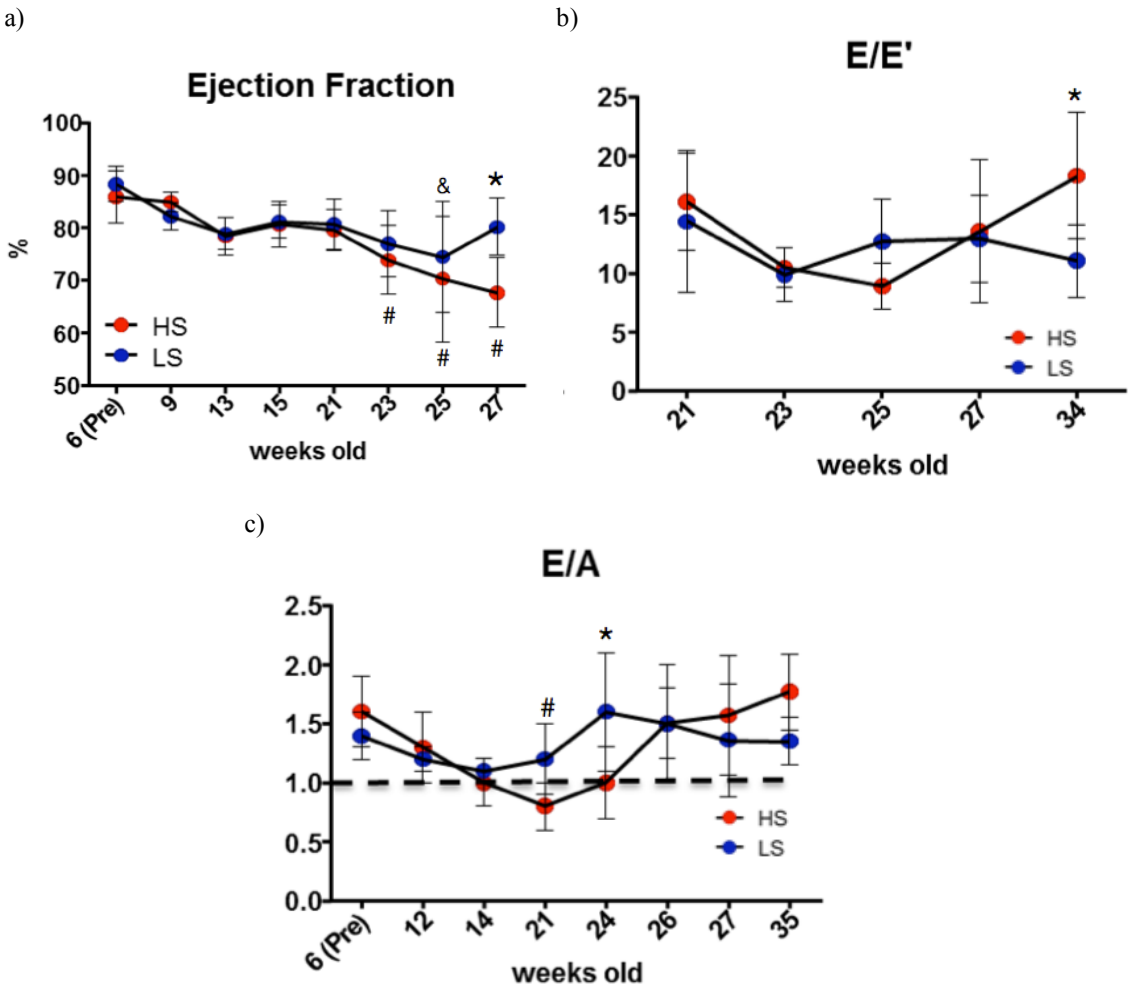

Figure 3 Left Ventricle Functional Parameter

* = sig. $p < 0.05$ vs. LS; # = sig. $p < 0.05$ vs. pre HS; & = sig. $p < 0.05$ vs. pre LS; LS = low salt diet group; HS = high salt diet group; E/E' = ratio of early diastolic transmitral peak flow (E) to the early diastolic velocity of mitral annulus (E'); E/A = ratio of early diastolic transmitral peak flow (E) to late diastolic transmitral peak flow (A); LV functional parameter show a) left ventricular ejection fraction to be lower in HS, but > 50 %, b) increased E/E' and c) normal E/A

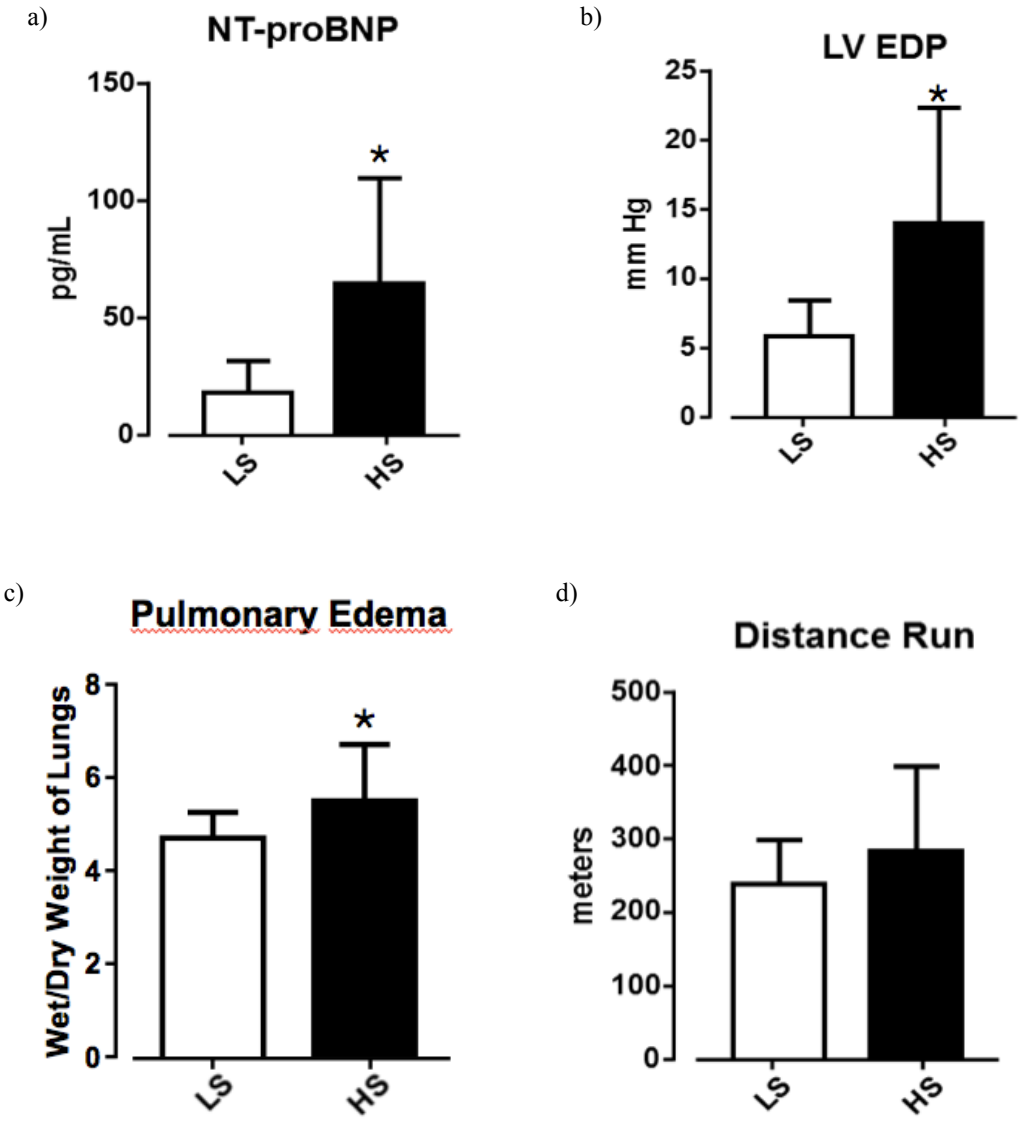

Figure 3.4 Additional Parameters for the Diagnosis of Diastolic Heart Failure

* = sig. $p < 0.05$ vs. LS; LS = low salt diet group; HS = high salt diet group; NT-proBNP = natriuretic peptide; LV EDP = left ventricular end diastolic pressure; this figure shown additional parameters that can be important for the diagnoses of diastolic heart failure; it shows a) increased NT-proBNP, b) LVEDP and c) pulmonary oedema in HS compared to LS; d) an exercise intolerance in HS could not be seen as shown by distance run

3.2. Endpoint Parameter

At the end of the study, LS presented with a lower mortality rate than the other groups ($p = 0.002$) (Figure 3.5). As seen for HS, also the exercise groups presented with a higher BP compared to LS group ($p < 0.05$) (Appendix II). The increase in LV mass that was already shown for HS, was also apparent in the exercise groups when compared to LS group ($p < 0.05$) These measurements were done in vivo with echocardiography but could be confirmed after sacrifice by weighing the tissue (Table 3.2). Further, only HIT-LV presented with the same pattern of increased E/E' ($p < 0.05$) compared to LS and normal systolic function comparable to HS group (Table 3.2). Other parameters were measured and analyzed with a multi-comparison, showed no difference, except the distance run, which increased in all training groups ($p < 0.05$) (Table 3.2).

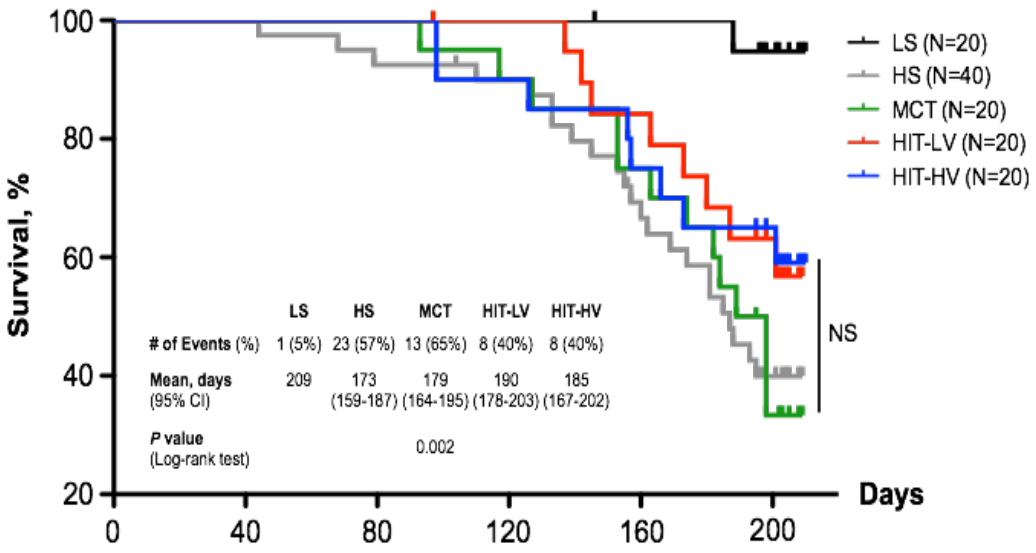

Figure 3.5 Survival of Rats in Percent Over Days

LS = low salt group; HS = high salt group; HIT-LV = high intensity interval training-low volume group; HIT-HV = high intensity interval training-high volume group; MCT = moderate continuous training group; no sig. between the high salt

Table 3.2 Endpoint Data

	LS	HS	HIT-LV	HIT-HV	MCT
BW (g)	294.5 ± 14.5	288.2 ± 18.6	278.7 ± 18.2	277.5 ± 14.5	284.4 ± 15.1
HR (bpm)	393.2 ± 29.6	414.6 ± 26.5	360.4 ± 49.9#	365.5 ± 23.5#	389.9 ± 18.5
LV Mass (echo) (mg)	1123.4 ± 135.7	1483.3 ± 283.7*	1607 ± 292.8*	1494.2 ± 107.4*	1461.1 ± 116.7*
LV Mass (weight) (g)	0.74 ± 0.04	1.04 ± 0.06*	1.14 ± 0.21*	1.09 ± 0.12*	1.04 ± 0.07*
LAD (mm)	5 ± 0.1	5.4 ± 0.6	5.3 ± 0.8	5.1± 0.5	4.9 ± 0.3
TL (mm)	41.22 ± 0.66	40.15 ± 1.18	39.45 ± 0.62	39.48 ± 0.56	39.52 ± 1.3
LV/TL (mg/mm)	17.9 ± 1.17	26.18 ± 2.43*	29 ± 5.17*	27.11 ± 3.08*	26.63 ± 2.67*
LVEF (%)	82.1 ± 2.8	68.4 ± 6.2*	63.4 ± 9*	59.7 ± 10.9*	65.7 ± 7.9*
E/E'	11.2 ± 3.7	17.8 ± 4.8	22.1 ± 13.8*	16.8 ± 7.1	13.6 ± 4.0
E/A	1.4 ± 0.2	1.8 ± 0.4	1.3 ± 0.4	1.5 ± 0.4	1.5 ± 0.7
LVEDP (mm Hg)	5.9 ± 2.5	14 ± 8.3*	11.3 ± 5.3	9.8 ± 6.3	13.9 ± 6.4
Lung weight Ratio	4.71 ± 0.14	5.49 ± 0.37	4.56 ± 0.34	5 ± 0.11	5.14 ± 0.1
Distance run (m)	238.6 ± 59.6	282.7 ± 116.1	381.4 ± 74.1*	455.3 ± 154*#	481.1 ± 82.6*#

Mean ± SD; * = sig. $p < 0.05$ vs. LS; # = sig. $p < 0.05$ vs. HS; LS = low salt group; HS = high salt group; HIT-LV = high intensity interval training-low volume group; HIT-HV = high intensity interval training-high volume group; MCT = moderate continuous training group; BW = body weight; LV mass = left ventricular mass (echocardiography); HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MP = mean pressure; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction; E/E' = ratio of early diastolic transmitral peak flow (E) to the early diastolic velocity of the mitral annulus (E'); E/A = ratio of early diastolic transmitral peak flow (E) to late diastolic transmitral peak flow (A); LAD = left atrium dimension

3.3. Mitochondrial Metabolism

The mitochondrial metabolism was analyzed after sacrifice. The analysis of all groups showed no difference for LV mitochondrial efficiency expressed by LV respiratory acceptor control ratio (lvRCR) (Figure 3.6 a). Collapsing HIT-LV and HIT-HV into one group (Figure 3.6 b), and further, collapsing all exercise groups into just one group, a difference for exercise compared to HS group can be seen ($p < 0.05$) (Figure 3.6 c).

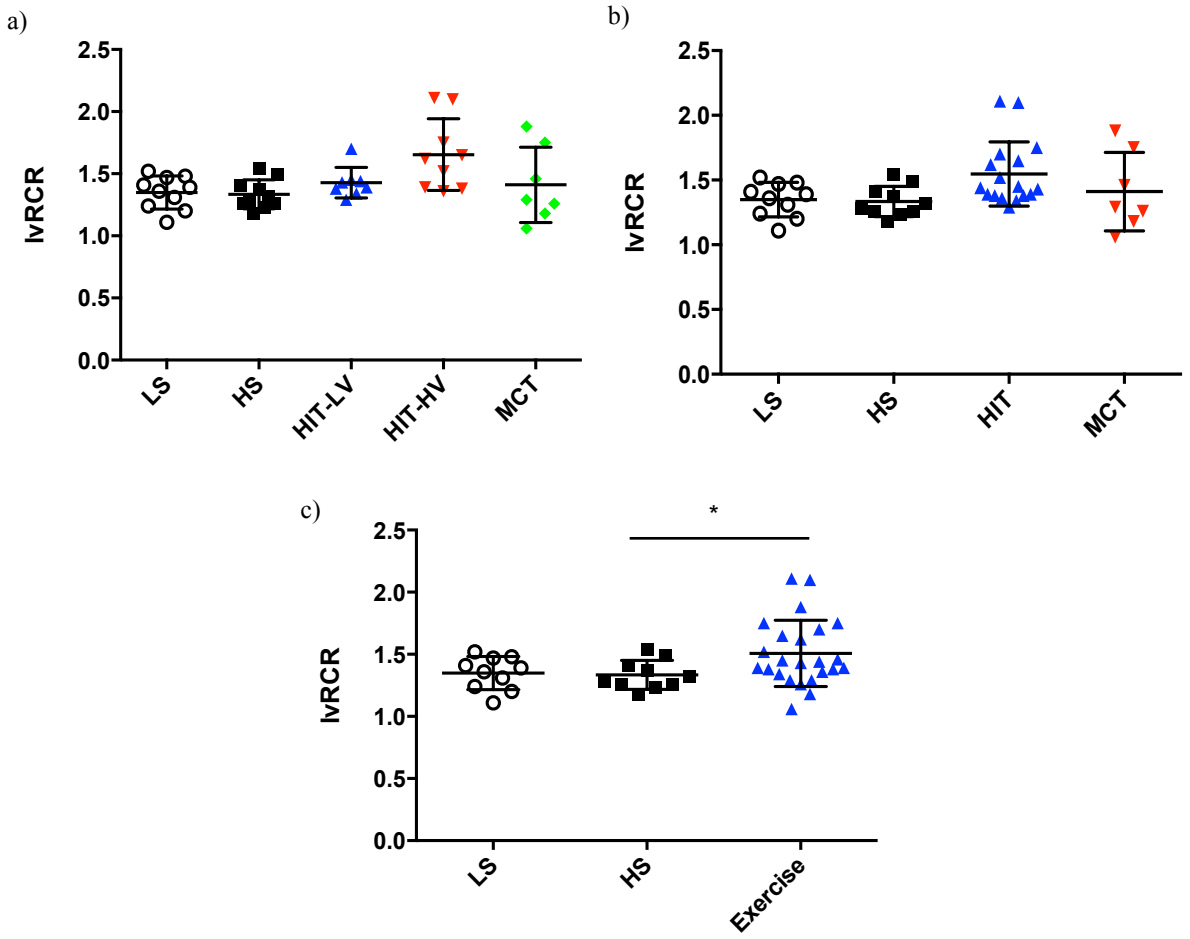

Figure 3.6 Mitochondrial Efficiency

* = sig. $p < 0.05$; lvRCR = left ventricle respirator acceptor control ratio; LS = low salt group; HS = high salt group; HIT-LV = high intensity interval training-low volume group; HIT-HV = high intensity interval training-high volume group; MCT = moderate continuous training group; HIT = combination of HIT-LV and HIT-HV; Exercise = combination of all training groups; an analysis of a) all groups and of b) collapsed HIT groups no difference was found; the analysis of c) all exercise groups collapsed into one shows increased mitochondrial efficiency of exercise compared with HS

Looking at the ratio of maximal respiration over uncoupled respiration it can be seen, that the oxidative phosphorylation system of HIT-HV is less limited than of the LS group ($p < 0.05$) (Figure 3.7).

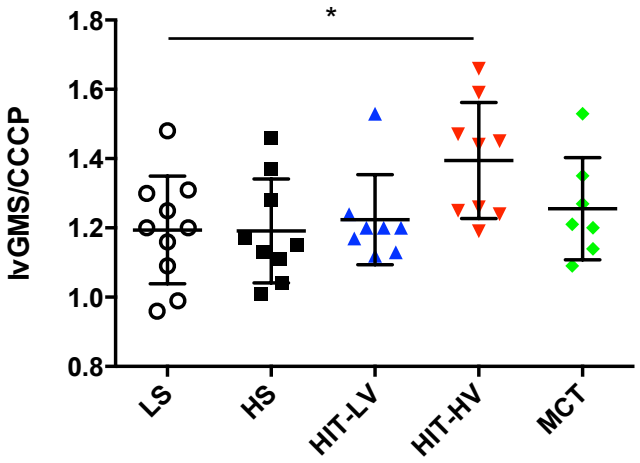

Figure 3.7 Limitation of Mitochondrial Oxidative Phosphorylation

* = sig. $p < 0.05$; lvGMS/CCCP = ratio of maximal respiration over uncoupled respiration; LS = low salt group; HS = high salt group; HIT-LV = high intensity interval training-low volume group; HIT-HV = high intensity interval training-high volume group; MCT = moderate continuous training group; HIT-HV has a smaller limitation of oxidative phosphorylation than LS group

Considering other parameters measured to evaluate the LV mitochondrial metabolism, no difference was found (Table 3.3).

Table 3.3 Left Ventricular Mitochondrial Metabolism

	LS	HS	HIT-LV	HIT-HV	MCT
lvLEAKgm	31.68 ± 6.1	28.12 ± 5.65	34.02 ± 4.98	33.17 ± 5.24	27.42 ± 8.2
lvGM	94.17 ± 44.21	70.18 ± 24.11	88.37 ± 29.04	117.77 ± 54.68	82.01 ± 50.83
lvGMS	298.66 ± 43.33	262.82 ± 48.28	266.30 ± 56.47	323.61 ± 90.59	253.39 ± 73.03
lvGM/GMS	0.32 ± 0.14	0.30 ± 0.16	0.35 ± 0.14	0.35 ± 0.13	0.31 ± 0.15
lvRCR	1.35 ± 0.14	1.33 ± 0.12	1.43 ± 0.12	1.65 ± 0.29	1.41 ± 0.3
lvGM/Lgm	2.90 ± 0.89	2.62 ± 1.18	2.65 ± 0.71	3.59 ± 1.76	2.83 ± 1.29
lvGMS/CCCP	1.19 ± 0.15	1.19 ± 0.15	1.22 ± 0.13	1.4 ± 0.17*	1.26 ± 0.15

Mean ± SD; *= sig. $p < 0.05$ vs. LS; lvLEAKgm, lvGM, lvGMS measures O^2-flux in pmol•s^{-1}•mg^{-1}; lvLEAKgm= LEAK respiration with Glutamate and Malate (no ADP); lvGM= complex I respiration; lvGMS= complex I and II respiration (max. respiration) ; lvGM/GMS= ratio of complex I over complex II respiration; lvRCR= respiratory acceptor control ratio (respiratory efficiency); lvGM/Lgm= complex I respiratory efficiency; lvGMS/CCCP= uncoupled respiration; this table shows the LV mitochondrial metabolism is not sig. influenced by DHF or training if five groups are analyzed. This is with the exception of phosphorylation system limitation, where HIT-HV was less limited compared to LS, although no group presented with a limitation of the phosphorylation system.

4. Discussion

The aim of this project was to confirm the animal model for DHF used in this study, as well as to study the mitochondrial metabolism in DHF and the influence of different exercise intensities on the metabolism. Results for both of these aspects have been found. While the evaluation of the results for the animal model lead to refined diagnostic criteria, influences of exercise training on LV mitochondrial metabolism in DHF were revealed.

The baseline measurements show no difference between groups and the end of the study period was defined by increased E/E' values in HS. This was reached after 28 weeks of intervention.

4.1. Confirming the Animal Model

This study was about DHF, therefore, first checked were whether differences between LS and HS groups could be found to confirm the animal model that was used. Looking back at the diagnostic parameters, DHF subjects should present with a normal LVEF [22, 30]. It can be seen that LVEF in HS was lower than in LS groups, but was still above 50 %, which is classified as normal (1.1.). The diastolic function on the other hand, was dysfunctional. This can be seen by looking at the E/E' ratio, which was increased in HS group and can be used as a predictor of high LV filling pressure [33]. A look at the increased LVEDV in HS group confirms this. Not only have both of these parameters been mentioned as important when diagnosing DHF [22, 30], but the findings in these parameters are also in congruence with previous studies that found a higher filling pressure in patients in DHF at rest compared with a control group [8], in rats with DHF [12] and in hypertensive male adult dogs [83].

Cardiomyocytes react to a pressure overload with hypertrophy [41]. That this is the case in DHF and furthermore, that concentric hypertrophy is probable has been mentioned (1.1.3). LV hypertrophy was confirmed by increased LV mass and especially by increased cardiomyocyte cross-sectional area of HS compared to LS group. However, an increase in LAD was not seen. Nonetheless, it can be said that a concentric hypertrophy was apparent, as has also been reported before [12]. The concentric hypertrophy is probable, because the cardiomyocytes showed increased cross-sectional growth, whereas eccentric remodelling is linked to longitudinal growth [41] and because LV filling pressures were increased.

Other parameters mentioned in the description of diagnostic criteria of DHF are NT-proBNP and E/A ratio (1.1.2). While an increase in NT-proBNP was found in this study in HS groups, it has been argued that NT-proBNP is better related to SHF than DHF [5]. However, increases of NT-proBNP could also be found in DHF [5]. Changes of E/A ratio could not be seen in this study, but were in previous studies in dogs [32] and patients [45] reported to be higher. Moreover, no exercise intolerance could be seen for HS sedentary group, since running distance was not different between LS and HS groups. This is in contrast to previous findings in patients [7, 37, 60]. Because the current study was done in female rats, it is difficult to say whether the rats present with the same level of activity, or inactivity during the day as the patients. This is important to discuss because inactivity can have severe effects on exercise capacity in patients [90].

But even though not all the results presented here match results from previous studies, the main parameter, which defines DHF, is in congruence. An important part of the data is therefore reproducible and looking at the normal LVEF, the LV hypertrophy as well as considering the presented data for E/E' ratio and LVEDP, it is evident that HS group developed DHF. However, this also shows that DHF is a disease that consists of multiple variables of which not all may be present. It is therefore necessary to further investigate DHF to increase the knowledge of this disease.

4.2. Mortality in Diastolic Heart Failure

At the end of the study, the presented data shows that all groups fed with a HS diet had an increased mortality rate compared to LS group. This is in contrast to a study arguing that low intensity exercise training improves survival in male hypertensive Dahl SS rats [91]. That is interesting, because in this study no difference in mortality rate could be seen between the high salt diet groups (Figure 3.5). Perhaps an important difference between male and female rats exists when it comes to the development of DHF and its consequences. In a previous study [12], the HS diet started at 7 week and at 8 weeks of age. The male Dahl SS rats used in that study developed DHF at 19 and 26 weeks of age, respectively [12]. That is earlier than the female rats in the present study, which developed DHF at about 32 weeks of age. This difference shows that a differentiation of male and female rats may be important when studying DHF. Implications for humans can only be assumed, but since the incidence is higher in women [3], female rats should be studied.

4.3. Exercise in Diastolic Heart Failure

When further examining the results for all groups, it is visible that LV mass was higher in all high salt diet groups compared to LS, which is a sign for cardiac hypertrophy. Interestingly, the LVEDP is not different in the exercise groups compared to LS group, even though SBP is elevated. On one hand, this supports a previous finding about decreased relaxation time in healthy female Sprague-Dawley rats through exercise [61], by showing that exercise improves LVEDP, which is associated with LV relaxation. On the other hand, this result is contradicting a finding that states exercise training improves hypertension in elderly women [65], because a decrease of BP could not be seen here.

This study also shows that exercise training, no matter the intensity applied, increased the running distance compared to LS group. This shows parallels to previous studies, in which both MCT [63] and HIT [61] had positive effects, such as an increased VO_2max, on the study subjects. Therefore, exercise training may improve the exercise intolerance observed in patients with DHF [7, 37, 60], although no exercise intolerance was found in this study using a rat model of DHF. Summarizing, these arguments shows that exercise training may prove as a good prevention and treatment method in the management of DHF.

4.4. Mitochondrial Metabolism in Diastolic Heart Failure

Since it has been suggested that the failing heart is an engine out of fuel [9], it was important to look at the mitochondrial metabolism in the animal model of DHF suggested in the present study. A possible mechanism for the reduced ATP reserves [13] could be an inefficient ETS. However, no difference for mitochondrial efficiency was reported in this study. A higher myocardial mitochondrial efficiency, expressed by lvRCR was evident in HS trained groups compared to the HS sedentary group, only after collapsing the two HIT groups into one and then furthermore, collapsing all exercise groups together (Figure 3.6). An effect of exercise in general on mitochondrial efficiency can be seen, but no distinction as to which type of exercise intensity is most effective can be made.

For healthy mice, it has been presented that HIT increases state 3 respiration [63]. That is conflicting with the data presented in this study, where no sig. difference in respiration can be seen. This might be due to an effect of DHF on mitochondria, since it has been established that if sedentary, the HS groups develop DHF. Therefore, it could be hypothesized that

exercise has an influence on the myocardial mitochondria during the development of DHF. However, further studies need to test this hypothesis. Furthermore, a depletion of ATP reserves in DHF was presented [13]. A possible reason for this could be that more ATP is used in the heart than produced by myocardial mitochondria. This could be a future study possibility since the myocardial mitochondrial efficiency that was assessed in this animal model of DHF was normal in HS group. The LV mitochondrial oxygen phosphorylation on the other hand seems to be positively influenced by HIT (Figure 3.7). This again shows that exercise has a positive influence on DHF.

During the SUIT protocol, cytochrome c was added to check for mitochondrial membrane integrity. An increase in respiration after titration is interpreted as membrane damage [92]. It has been reported that higher cytochrome c concentrations can be measured in rats and patients with HF [93]. It would therefore be critical to exclude an animal with a cytochrome c effect since an effect seen after titration might be due to HF.

5. Conclusion

Concluding this study, it can be said that the hypothesis was found to be partly true. While the myocardial mitochondrial metabolism was not different in DHF, influences of exercise were found in this study. Additionally, previously defined diagnostic parameters for DHF have been confirmed and were refined. Novel findings on myocardial mitochondrial metabolism have been presented and can be used in the future treatment and prevention management of DHF. However, both the general research on DHF and the study of myocardial mitochondria, especially in connection with DHF are still lacking and are in need of additional research to compile more knowledge. Therefore, it is important to continue with this research on DHF in the future.

6. Limitations

Although important results such as criteria and reference values for future studies were established with this pilot study, some limitations apply. A critical point is the parameter used to decide when DHF is developed, because DHF may present with multiple signs of which not all are severely expressed. However, since this is a pilot study and it was able to show that DHF was in fact developed by female Dahl SS rats fed with a high salt diet, it was possible to use the collected data to establish new and better guidelines for the diagnosis of DHF in animals. After all the analysis done in this pilot study, it seems that the 75th percentile of LS can be used as a cut-off value for three echocardiographic parameters, such as E/E', E/A as well as LAD/BW. If an animal of a HS group presents with values for all three parameters above the 75th percentile of LS, this animal is considered to have DHF. This is of importance when it comes to secondary prevention and treatment studies.

Another limitation might have been the O_2 availability during SUIT. Usually, values for lvGMS/CCCP cannot be higher than 1.0 since adding CCCP uncouples the mitochondria and creates a circumvention of ATP-synthase that leads to respiration without ATP production. There exist two reasons why this might have happened. One, because the CCCP was titrated too late in the protocol and O_2 was no longer available in sufficient amounts because of the closed chamber, and was thereby limiting. The second possible reason is that the titration of oligomycin before CCCP may have interfered with the effect CCCP is supposed to have on the mitochondria. However, since the same protocol was used in every animal, a comparison between groups should be valid.

7. Literature

1. Giacco, F. and M. Brownlee, *Oxidative stress and diabetic complications.* Circ Res, 2010. **107**(9): p. 1058-70.
2. Braunschweig, F., M.R. Cowie, and A. Auricchio, *What are the costs of heart failure?* Europace, 2011. **13 Suppl 2**: p. ii13-7.
3. Gurwitz, J.H., et al., *Contemporary Prevalence and Correlates of Incident Heart Failure with Preserved Ejection Fraction.* Am J Med, 2013.
4. Vasan, R.S., et al., *Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction: prevalence and mortality in a population-based cohort.* J Am Coll Cardiol, 1999. **33**(7): p. 1948-55.
5. Brouwers, F.P., et al., *Incidence and epidemiology of new onset heart failure with preserved vs. reduced ejection fraction in a community-based cohort: 11-year follow-up of PREVEND.* Eur Heart J, 2013.
6. Paulus, W.J. and J.J. van Ballegoij, *Treatment of heart failure with normal ejection fraction: an inconvenient truth!* J Am Coll Cardiol, 2010. **55**(6): p. 526-37.
7. Haykowsky, M.J., et al., *Determinants of exercise intolerance in elderly heart failure patients with preserved ejection fraction.* J Am Coll Cardiol, 2011. **58**(3): p. 265-74.
8. Abudiab, M.M., et al., *Cardiac output response to exercise in relation to metabolic demand in heart failure with preserved ejection fraction.* Eur J Heart Fail, 2013. **15**(7): p. 776-85.
9. Neubauer, S., *The failing heart--an engine out of fuel.* N Engl J Med, 2007. **356**(11): p. 1140-51.
10. McMurray, J.J., et al., *ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC.* Eur J Heart Fail, 2012. **14**(8): p. 803-69.
11. Zile, M.R. and D.L. Brutsaert, *New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function.* Circulation, 2002. **105**(11): p. 1387-93.
12. Doi, R., et al., *Development of different phenotypes of hypertensive heart failure: systolic versus diastolic failure in Dahl salt-sensitive rats.* J Hypertens, 2000. **18**(1): p. 111-20.
13. Bache, R.J., et al., *Myocardial oxygenation at high workstates in hearts with left ventricular hypertrophy.* Cardiovasc Res, 1999. **42**(3): p. 616-26.
14. Hasenfuss, G., *Alterations of calcium-regulatory proteins in heart failure.* Cardiovasc Res, 1998. **37**(2): p. 279-89.
15. Kass, D.A., J.G. Bronzwaer, and W.J. Paulus, *What mechanisms underlie diastolic dysfunction in heart failure?* Circ Res, 2004. **94**(12): p. 1533-42.
16. McArdle, W.D., F.I. Katch, and V.L. Katch, *Exercise Physiology: nutrition, energy, and human performance*. 7 ed. 2010, Baltimore: Lippincott Williams & Wilkins.
17. Meyer, M., et al., *Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy.* Circulation, 1995. **92**(4): p. 778-84.
18. Gillebert, T.C., A.F. Leite-Moreira, and S.G. De Hert, *Load dependent diastolic dysfunction in heart failure.* Heart Fail Rev, 2000. **5**(4): p. 345-55.
19. Dickstein, K., et al., *ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of*

Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J, 2008. **29**(19): p. 2388-442.
20. Sakata, Y., et al., *Left Ventricular Stiffening as Therapeutic Target for Heart Failure With Preserved Ejection Fraction.* Circ J, 2013.
21. Cleland, J.G., et al., *The EuroHeart Failure survey programme-- a survey on the quality of care among patients with heart failure in Europe. Part 1: patient characteristics and diagnosis.* Eur Heart J, 2003. **24**(5): p. 442-63.
22. Paulus, W.J., et al., *How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology.* Eur Heart J, 2007. **28**(20): p. 2539-50.
23. Burkhoff, D., M.S. Maurer, and M. Packer, *Heart failure with a normal ejection fraction: is it really a disorder of diastolic function?* Circulation, 2003. **107**(5): p. 656-8.
24. Maurer, M.S., et al., *Left heart failure with a normal ejection fraction: identification of different pathophysiologic mechanisms.* J Card Fail, 2005. **11**(3): p. 177-87.
25. Zile, M.R., C.F. Baicu, and W.H. Gaasch, *Diastolic heart failure--abnormalities in active relaxation and passive stiffness of the left ventricle.* N Engl J Med, 2004. **350**(19): p. 1953-9.
26. Wiggers, C.J., *STUDIES ON THE CONSECUTIVE PHASES OF THE CARDIAC CYCLE*, ed. C.J. Wiggers. Vol. 56. 1921. 439-459.
27. Solomon, S.D., et al., *Influence of ejection fraction on cardiovascular outcomes in a broad spectrum of heart failure patients.* Circulation, 2005. **112**(24): p. 3738-44.
28. Epstein, R.J., *Human Molecular Biology. An introduction to the Molecular Basis of Health and Disease*. 1st ed. 2003, Cambridge: Cambridge University Press.
29. Rad, A., *Renin-angiotensin-aldosterone system*, in *Xara X[1]*, R.-a.-a. system.png, Editor 2006.
30. Vasan, R.S. and D. Levy, *Defining diastolic heart failure: a call for standardized diagnostic criteria.* Circulation, 2000. **101**(17): p. 2118-21.
31. Zile, M.R. and W.H. Gaasch, *Mechanical loads and the isovolumic and filling indices of left ventricular relaxation.* Prog Cardiovasc Dis, 1990. **32**(5): p. 333-46.
32. Rivas-Gotz, C., et al., *Time interval between onset of mitral inflow and onset of early diastolic velocity by tissue Doppler: a novel index of left ventricular relaxation: experimental studies and clinical application.* J Am Coll Cardiol, 2003. **42**(8): p. 1463-70.
33. Ommen, S.R., et al., *Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study.* Circulation, 2000. **102**(15): p. 1788-94.
34. Tschope, C., et al., *The role of NT-proBNP in the diagnostics of isolated diastolic dysfunction: correlation with echocardiographic and invasive measurements.* Eur Heart J, 2005. **26**(21): p. 2277-84.
35. Redfield, M.M., et al., *Plasma brain natriuretic peptide to detect preclinical ventricular systolic or diastolic dysfunction: a community-based study.* Circulation, 2004. **109**(25): p. 3176-81.

36. Kitzman, D.W., et al., *Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism.* J Am Coll Cardiol, 1991. **17**(5): p. 1065-72.
37. Haykowsky, M.J., et al., *Impaired Aerobic Capacity and Physical Functional Performance in Older Heart Failure Patients With Preserved Ejection Fraction: Role of Lean Body Mass.* J Gerontol A Biol Sci Med Sci, 2013.
38. Klotz, S., et al., *Development of heart failure in chronic hypertensive Dahl rats: focus on heart failure with preserved ejection fraction.* Hypertension, 2006. **47**(5): p. 901-11.
39. Weeks, K.L. and J.R. McMullen, *The athlete's heart vs. the failing heart: can signaling explain the two distinct outcomes?* Physiology (Bethesda), 2011. **26**(2): p. 97-105.
40. Mihl, C., W.R. Dassen, and H. Kuipers, *Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes.* Neth Heart J, 2008. **16**(4): p. 129-33.
41. Maillet, M., J.H. van Berlo, and J.D. Molkentin, *Molecular basis of physiological heart growth: fundamental concepts and new players.* Nat Rev Mol Cell Biol, 2013. **14**(1): p. 38-48.
42. Dorn, G.W., 2nd, *The fuzzy logic of physiological cardiac hypertrophy.* Hypertension, 2007. **49**(5): p. 962-70.
43. Soonpaa, M.H. and L.J. Field, *Assessment of cardiomyocyte DNA synthesis during hypertrophy in adult mice.* Am J Physiol, 1994. **266**(4 Pt 2): p. H1439-45.
44. Hurst, J.W., D.C. Morris, and R.W. Alexander, *The use of the New York Heart Association's classification of cardiovascular disease as part of the patient's complete Problem List.* Clin Cardiol, 1999. **22**(6): p. 385-90.
45. Chapman, C.B., et al., *Classification of left ventricular diastolic function using American Society of Echocardiography Guidelines: agreement among echocardiographers.* Echocardiography, 2013. **30**(9): p. 1022-31.
46. Schulman, S.P., et al., *The effects of antihypertensive therapy on left ventricular mass in elderly patients.* N Engl J Med, 1990. **322**(19): p. 1350-6.
47. Brooks, W.W., et al., *Effect of angiotensin-converting enzyme inhibition on myocardial fibrosis and function in hypertrophied and failing myocardium from the spontaneously hypertensive rat.* Circulation, 1997. **96**(11): p. 4002-10.
48. Weber, K.T. and C.G. Brilla, *Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system.* Circulation, 1991. **83**(6): p. 1849-65.
49. Haq, M.A., et al., *Therapeutic interventions for heart failure with preserved ejection fraction: A summary of current evidence.* World J Cardiol, 2014. **6**(2): p. 67-76.
50. Dahlof, B., et al., *Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol.* Lancet, 2002. **359**(9311): p. 995-1003.
51. Aronow, W.S. and I. Kronzon, *Effect of enalapril on congestive heart failure treated with diuretics in elderly patients with prior myocardial infarction and normal left ventricular ejection fraction.* Am J Cardiol, 1993. **71**(7): p. 602-4.
52. Garber, C.E., et al., *American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise.* Med Sci Sports Exerc, 2011. **43**(7): p. 1334-59.

53. Helgerud, J., et al., *Aerobic high-intensity intervals improve VO2max more than moderate training.* Med Sci Sports Exerc, 2007. **39**(4): p. 665-71.
54. Myers, J., et al., *Exercise capacity and mortality among men referred for exercise testing.* N Engl J Med, 2002. **346**(11): p. 793-801.
55. Higginbotham, M.B., et al., *Regulation of stroke volume during submaximal and maximal upright exercise in normal man.* Circ Res, 1986. **58**(2): p. 281-91.
56. Borlaug, B.A., et al., *Diastolic relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction.* Heart, 2011. **97**(12): p. 964-9.
57. Edelmann, F., et al., *Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study.* J Am Coll Cardiol, 2011. **58**(17): p. 1780-91.
58. Smart, N., et al., *Exercise training in systolic and diastolic dysfunction: effects on cardiac function, functional capacity, and quality of life.* Am Heart J, 2007. **153**(4): p. 530-6.
59. Kitzman, D.W., et al., *Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial.* J Am Coll Cardiol, 2013. **62**(7): p. 584-92.
60. Kitzman, D.W., et al., *Exercise training in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial.* Circ Heart Fail, 2010. **3**(6): p. 659-67.
61. Kemi, O.J., et al., *Moderate vs. high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function.* Cardiovasc Res, 2005. **67**(1): p. 161-72.
62. Wisloff, U., et al., *Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study.* Circulation, 2007. **115**(24): p. 3086-94.
63. Hafstad, A.D., et al., *High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart.* J Appl Physiol, 2011. **111**(5): p. 1235-41.
64. Ciolac, E.G., et al., *Effects of high-intensity aerobic interval training vs. moderate exercise on hemodynamic, metabolic and neuro-humoral abnormalities of young normotensive women at high familial risk for hypertension.* Hypertens Res, 2010. **33**(8): p. 836-43.
65. Braz, N.F., et al., *Influence of aerobic training on cardiovascular and metabolic parameters in ederly hypertensive women.* Int J Prev Med, 2012. **3**(9): p. 652-9.
66. Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., *Essential Cell Biology*. 4th ed. 2014, New York: Garland Science.
67. Pollard, T.D., Earnshaw, W. C., *Cell Biology*. 1st ed. 2002, London: Sounders.
68. Schagger, H., *Respiratory chain supercomplexes.* IUBMB Life, 2001. **52**(3-5): p. 119-28.
69. Pesta, D. and E. Gnaiger, *High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle.* Methods Mol Biol, 2012. **810**: p. 25-58.
70. Gnaiger, E., *Mitochondrial Pathways and Respiratory Control. An Introduction to OXPHOS Analysis*. 3rd ed. Mitochondrial Physiology Network. 2012, Innsbruck: OROBOROS INSTRUMENTS Corp.

71. Chance, B. and G.R. Williams, *Respiratory enzymes in oxidative phosphorylation. III. The steady state.* J Biol Chem, 1955. **217**(1): p. 409-27.
72. Kraljevic, J., et al., *Aerobic interval training attenuates remodelling and mitochondrial dysfunction in the post-infarction failing rat heart.* Cardiovasc Res, 2013. **99**(1): p. 55-64.
73. Rosca, M.G., et al., *Cardiac mitochondria in heart failure: decrease in respirasomes and oxidative phosphorylation.* Cardiovasc Res, 2008. **80**(1): p. 30-9.
74. Nascimben, L., et al., *Mechanisms for increased glycolysis in the hypertrophied rat heart.* Hypertension, 2004. **44**(5): p. 662-7.
75. Kalsi, K.K., et al., *Energetics and function of the failing human heart with dilated or hypertrophic cardiomyopathy.* Eur J Clin Invest, 1999. **29**(6): p. 469-77.
76. Doenst, T., et al., *Decreased rates of substrate oxidation ex vivo predict the onset of heart failure and contractile dysfunction in rats with pressure overload.* Cardiovasc Res, 2010. **86**(3): p. 461-70.
77. Burgomaster, K.A., et al., *Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans.* J Physiol, 2008. **586**(1): p. 151-60.
78. Fillmore, N. and G.D. Lopaschuk, *Targeting mitochondrial oxidative metabolism as an approach to treat heart failure.* Biochim Biophys Acta, 2013. **1833**(4): p. 857-65.
79. Ferreira, R., et al., *Lifelong exercise training modulates cardiac mitochondrial phosphoproteome in rats.* J Proteome Res, 2014. **13**(4): p. 2045-55.
80. van Loon, L.J., et al., *Effect of training status on fuel selection during submaximal exercise with glucose ingestion.* J Appl Physiol, 1999. **87**(4): p. 1413-20.
81. Gertz, E.W., et al., *Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments.* J Clin Invest, 1988. **82**(6): p. 2017-25.
82. Dubi, S. and Y. Arbel, *Large animal models for diastolic dysfunction and diastolic heart failure-a review of the literature.* Cardiovasc Pathol, 2010. **19**(3): p. 147-52.
83. Hart, C.Y., et al., *Load versus humoral activation in the genesis of early hypertensive heart disease.* Circulation, 2001. **104**(2): p. 215-20.
84. Meneely, G.R., et al., *Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure.* J Exp Med, 1953. **98**(1): p. 71-80.
85. Kiatchoosakun, S., et al., *Assessment of left ventricular mass in mice: comparison between two-dimensional and m-mode echocardiography.* Echocardiography, 2002. **19**(3): p. 199-205.
86. Collins, K.A., et al., *Accuracy of echocardiographic estimates of left ventricular mass in mice.* Am J Physiol Heart Circ Physiol, 2001. **280**(5): p. H1954-62.
87. Wisloff, U., et al., *Intensity-controlled treadmill running in rats: VO(2 max) and cardiac hypertrophy.* Am J Physiol Heart Circ Physiol, 2001. **280**(3): p. H1301-10.
88. Pesta, D., Gnaiger, E., *Preparation of permeabilized muscle fibers for diagnosis of mitochondrial respiratory function.* Mitochondrial Physiology Network, 2011. **14.14: 1-5**.
89. Fasching, M., Fontana-Ayoub, M., Gnaiger, E., *Mitochondrial Respiration Medium.* Mitochondrial Physiology Network, 2014. **14**(13): p. 1-4.
90. McGuire, D.K., et al., *A 30-year follow-up of the Dallas Bedrest and Training Study: I. Effect of age on the cardiovascular response to exercise.* Circulation, 2001. **104**(12): p. 1350-7.

91. Libonati, J.R. and J.P. Gaughan, *Low-intensity exercise training improves survival in Dahl salt hypertension.* Med Sci Sports Exerc, 2006. **38**(5): p. 856-8.
92. Kuznetsov, A.V., et al., *Mitochondrial defects and heterogeneous cytochrome c release after cardiac cold ischemia and reperfusion.* Am J Physiol Heart Circ Physiol, 2004. **286**(5): p. H1633-41.
93. Chen, L., et al., *Mitochondrial OPA1, apoptosis, and heart failure.* Cardiovasc Res, 2009. **84**(1): p. 91-9.

Apendix I

Table Appendix I Baseline Data

	LS	HS	HIT-LV	HIT-HV	MCT
BW (kg)	0.134 ± 0.01	0.14 ± 0.01	0.122 ± 0.01	0.128 ± 0.01	0.127 ± 0.01
LV mass (mg)	569.4 ± 133.3	589.52 ± 147.1	510.33 ± 154.78	511.10 ± 88.39	480.11 ± 105.52
HR (bpm)	452.4 ± 22	460.5 ± 15.3	422.3 ± 52.7	445.7 ± 38.7	460.3 ± 36.9
SBP (mmHg)	144 ± 6.4	140.9 ± 9.9	139.4 ± 11.5	145.9 ± 6.3	139.1 ± 20.1
DBP (mmHg)	124.6 ± 10.5	123.9 ± 22.9	116.1 ± 16.1	130.7 ± 23.3	127.4 ± 24
MP (mmHg)	134.9 ± 8.8	135.7 ± 22.2	126.2 ± 15.9	143.4 ± 24.8	135.2 ± 24.3
LVEF (%)	88.33 ± 3.4	85.91 ±5	88.22 ± 3.23	88.30 ± 6.27	91.44 ± 3.21
E/A	1.42 ± 0.2	1.61 ± 0.3	1.38 ± 0.34	1.34 ± 0.34	1.58 ± 0.28
LAD (mm)	3.73 ± 0.32	3.7 ± 0.34	3.84 ± 0.27	3.82 ± 0.27	3.77 ± 0.34
Distance run (m)	352.7	423.9	535.6	348.1	627.9

Mean ± SD; LS = low salt group; HS = high salt group; HIT-LV = high intensity training group with low trainings volume; HIT-HV = high intensity training group with high trainings volume; MCT = moderate continuous training group; BW = body weight; LV mas s= left ventricular mass; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MP = mean pressure; LVEF = left ventricular ejection fraction; E/A = ratio of early diastolic transmitral peak flow (E) to late diastolic transmitral peak flow (A); LAD = left atrium dimension;

Apendix II

Table Appendix II Endpoint Data All

	LS	HS	HIT-LV	HIT-HV	MCT
LV mass (echo) (mg)	1123.4 ± 135.7	1483.3 ± 283.7*	1607 ± 292.8*	1494.2 ± 107.4*	1461.1 ± 116.7*
LV mass (weight) (g)	0.74 ± 0.04	1.04 ± 0.06*	1.14 ± 0.21*	1.09 ± 0.12*	1.04 ± 0.07*
RV mass (weight) (g)	0.16 ± 0	0.18 ± 0	0.18 ± 0.01	0.17 ± 0	0.16 ± 0.01
LA mass (weight) (g)	0.02 ± 0	0.04 ± 0	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
RA mass (weight) (g)	0.02 ± 0	0.02 ± 0	0.03 ± 0	0.03 ± 0	0.05 ± 0.03
Heart mass (g)	0.94 ± 0.01	1.28 ± 0.02*	1.4 ± 0.09*	1.31 ± 0.04*	1.29 ± 0.04*
BW (g)	294.5 ± 14.5	288.2 ± 18.6	278.7 ± 18.2	277.5 ± 14.5	284.4 ± 15.1
LV/BW	2.53 ± 0.15	3.63 ± 0.29*	4.11 ± 0.69*	3.95 ± 0.58*	3.69 ± 0.32*
LA/BW	0.08 ± 0	0.15 ± 0.01	0.18 ± 0.04*	0.17 ± 0.02	0.14 ± 0.02
TL (mm)	41.22 ± 0.66	40.15 ± 1.18	39.45 ± 0.62	39.48 ± 0.56	39.52 ± 1.3
LV/TL (mg/mm)	17.92 ± 1.17	26.18 ± 2.43*	29 ± 5.17*	27.11 ± 3.08*	26.63 ± 2.67*
LA/TL (mg/mm)	0.54 ± 0.03	1.07 ± 0.1	1.29 ± 0.29*	1.04 ± 0.13	1.01 ± 0.13
Lung wet (g)	0.13 ± 0.01	0.15 ± 0.02	0.1 ± 0.02	0.12 ± 0.02	0.12 ± 0.03
Lung dry (g)	0.03 ± 0	0.03 ± 0	0.02 ± 0	0.03 ± 0	0.02± 0.01
Ratio	4.71 ± 0.14	5.49 ± 0.37	4.56 ± 0.34	5 ± 0.11	5.14 ± 0.1
% H_2O	78.38 ± 0.90	81.23 ± 0.87	76.60 ± 2.45	79.91 ± 0.47	80.51 ± 0.37
HR (bpm)	393.2 ± 29.6	414.6 ± 26.5	360.4 ± 49.9#	365.5 ± 23.5#	389.9 ± 18.5
SBP (mmHg)	147.4 ± 15.9	208.6 ± 23.9*	209 ± 28.3*	202.6 ± 25.1*	212.1 ± 12.6*
DBP (mmHg)	104.7 ± 11.3	149.9 ± 17.4*	147 ± 22.3*	13.7 ± 16.7*	150.4 ± 8.7*
MP (mmHg)	128.5 ± 13.3	178.8 ± 19.7*	166.7 ± 44.3*	172 ± 17.8*	180.7 ± 12.9*
LVEDP (mmHg)	5.9 ± 2.5	14 ± 8.3*	11.3 ± 5.3	9.8 ± 6.3	13.9 ± 6.4
LVEF (%)	82.1 ± 2.8	68.4 ± 6.2*	63.4 ± 9*	59.7 ± 10.9*	65.7 ± 7.9*
E/A	1.4 ± 0.2	1.8 ± 0.4	1.3 ± 0.4	1.5 ± 0.4	1.5 ± 0.7
E/E'	11.2 ± 3.7	17.8 ± 4.8	22.1 ± 13.8*	16.8 ± 7.1	13.6 ± 4.0

LAD (mm)	5 ± 0.1	5.4 ± 0.6	5.3 ± 0.8	5.1± 0.5	4.9 ± 0.3
Distance run (m)	238.6 ± 59.6	282.7 ± 116.1	381.4 ± 74.1*	455.3 ± 154*#	481.1 ± 82.6*#

Mean ±SD; sig.: * = $p < 0.05$ vs. LS; # = $p < 0.05$ vs. HS; LS = low salt group; HS = high salt group; HIT-LV = high intensity training group with low trainings volume; HIT-HV = high intensity training group with high trainings volume; MCT = moderate continuous training group; LV mass = left ventricular mass; RV mass = right ventricular mass; LA mass = left atrium mass; RA mass = right atrium mass; Heart mass = total heart mass; LV, RV, LA, RA and heart mass are tissue weights at sacrifice; BW = body weight; LV/BW = left ventricle mass per body weight; LA/BW = left atrium mass per body weight; TL = tibia length; LV/TL = left ventricular mass per tibia length; LA/TL = left atrium mass per tibia length; Lung wet = wet lung weight; Lung dry = dry lung weight; Ratio = ratio of wet to dry lung weight ratio; $\%H_2O$ = percentage of water in lung tissue; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MP = mean pressure; LVEDP = left ventricular end diastolic pressure; LVEF = left ventricular ejection fraction; E/A = ratio of early diastolic transmitral peak flow (E) to late diastolic transmitral peak flow (A); E/E' = ratio of early diastolic transmitral peak flow (E) to the early diastolic velocity of the mitral annulus (E'); LAD = left atrium dimension