2014•2015
FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

Exercise training in an animal model for acute heart failure: Effects on cardiac dimensions, cardiac function and glucose regulation.

Promotor :
Prof. dr. Bert OP 'T EIJNDE

Copromotor :
Mevrouw An STEVENS

Bart Fanton , Luk Martens
Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

universiteit hasselt
KNOWLEDGE IN ACTION

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Research context

Heart failure and its associated symptoms such as breathelessness, tiredness, fatigue, confusion, weakness and inactivity are important risk factors for early death. At present, heart failure prevalence can be estimated at 1-2% in the western world and its age-associated yearly incidence is estimated at 5-10/1000 persons (Mosterd et al.). Within the context of age-related incidence, unfortunately, numbers are expected to further increase (Bonneux et al.).

Because of these striking numbers, it is important to optimize therapy of heart failure. To date, therapy mainly consists of medication and exercise rehabilitation. The latter is considered an important tool to counteract many of the symptoms related to the heart failure induced inactivity. Examples of symptoms related to inactivity are increased blood glucose and insulin levels (McMurray et al.). The effects of exercise rehabilitation on these comorbidities have not been studied thoroughly in patients with acute heart failure. Therefore this will be investigated in an animal model for heart failure, using Dahl salt-sensitive rats. After inducing hypertension and heart failure, rats were subjected to treadmill training to assess the effects of the exercise therapy on blood glucose and insulin levels.

This master thesis is part of the PhD project entitled “Influence of exercise therapy on glucose tolerance in patients with chronic heart failure.” of Dr. A. Stevens. The experiments were performed within the framework of the Hasselt University REVAL, a rehabilitation research centre. This master thesis is part of the master program rehabilitation sciences and physiotherapy educational program of Luk Martens and Bart Fanton. Tasks in the duo-thesis were divided equally. Data-acquisition and methods-protocol were performed by Dr. A. Stevens. Structuring, processing, analysing and writing of data and methods were performed by BF and LM. The experimental protocol was designed and performed by (co)promotors.

References:

Mosterd, A. and A.W. Hoes, *Clinical epidemiology of heart failure.* Heart, 2007. **93**(9): p. 1137-46.

Bonneux, L., et al., *Estimating clinical morbidity due to ischemic heart disease and congestive heart failure: the future rise of heart failure.* Am J Public Health, 1994. **84**(1): p. 20-8.

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 Heart J, 2012. **33**(14): p. 1787-847.

Exercise training in an animal model for chronic heart failure:
Effects on cardiac dimensions, cardiac function and glucose regulation.

ABSTRACT:

Background: Comorbidity between heart failure and glucose dysregulation is common and correlated with mortality worldwide. Current study aims to investigate the effect of exercise training on cardiac dimensions, cardiac function and glucose regulation in an animal model.

Methods: 48 Dahl salt-sensitive rats received a high salt diet (8% NaCl; HSD) or a normal diet (0,3% NaCl; CD). After five weeks, groups were divided into sedentary (SED) or trained group EX. Animals in EX performed treadmill training five days/week at a speed of 18 meters/minute, 60 minutes/ a day and with an inclination of 5%. Outcome measurements were left ventricle mass, ejection fraction, strain/strain rate, fibrosis, serum glucose and insulin levels.

Results: Five weeks of HSD resulted in increased left ventricle mass and reduced cardiac deformations. Additional six weeks HSD resulted in reduced cardiac functions and cardiac dimensions. Serum glucose levels decreased in both HSD and CD groups, no differences between both were demonstrated after the first five weeks of diet. Exercise training improved left ventricle mass characteristics, radial and circumferential strain parameters and developed fibrosis. Exercise demonstrated no improvements for serum glucose and insulin levels

Discussion: The HSD induced cardiac hypertrophy and a reduced heart function, demonstrating the development of heart failure and resulting in considerable mortality rates. HSD did not result in attenuations of glucose regulation. Exercise training is able to decrease left ventricular hypertrophy, shows beneficial effects for cardiac function, and decreases the formation of fibrosis. Exercise could not prevent mortality due a HSD.

Conclusion: Beneficial effects on cardiac function were found in an animal model for heart failure after exercise intervention. Demonstrating the importance of exercise training as treatment of heart failure.

Keywords: Heart failure, glucose regulation, exercise training, Dahl salt-sensitive rats

Introduction

Worldwide, heart failure is one of the main causes of death [1]. Prevalence is estimated at 1-2% in the western world and the incidence is estimated at 5-10/1000 [2]. In the Belgian population incidence is 194/100 000, increasing with age, reaching 10% to 20% in people of 70-80 years of age [3]. Mortality rates reach 19% within six months after diagnosis and 26% within one year of diagnosis [4, 5]. Therefore, research investigating treatment for overall heart failure including rehabilitation is crucial.

Chronic heart failure can be described as a complex clinical syndrome with the incapability of the heart to function effectively as a pump and therefore failing to support proper circulation (forward failure), or to only be able to accomplish this by an abnormally increased filling pressure (backward failure) [6].

Heart failure can have various causes, i.e. loss or damage of the heart muscle tissue, acute or chronic ischemia, increased vascular resistance with hypertension, the development of a tachyarrhythmia, among others [6, 7]. Hypertension induces a higher workload of the heart leading to hypertrophy of the left ventricle and is considered to be one of the most important causes of heart failure [7, 8]. Common symptoms of heart failure are exercise intolerance, breathlessness, tiredness, fatigue, confusion, muscle weakness [7, 9] and inactivity. In other populations it has been shown that decreased physical activity leads to increased blood glucose and insulin levels which could further lead to insulin resistance [10]. As shown in literature [10, 11], insulin resistance is a very common comorbidity of chronic heart failure, resulting in increased mortality and morbidity [11]. However these parameters have not been profoundly studied in a model for acute heart failure

Pharmacological treatment focuses on influencing the clinical manifestations due to heart failure, such as diminishing oedema and fluid retention, increasing exercise capacity, reducing fatigue, breathlessness and therefore improving the quality of life [6, 12]. Non-pharmacological treatment can include the implantation of an implantable cardioverter-defibrillator, cardiac resynchronization therapy and exercise therapy [12-14]. In patients with chronic heart failure, the impact of exercise therapy on the pulmonary, cardiovascular, neurohumoral, endothelial and anti-inflammatory system as well as on skeletal muscle functioning and metabolism have been investigated thoroughly [14]. As such exercise therapy increases exercise tolerance and quality of life, and it decreases mortality and hospitalisation rates in patients with chronic heart failure [15]. However the effects of exercise training on blood glucose and insulin levels remain unclear and further research on this topic is needed to show the impact of exercise training on blood glucose and insulin levels in this population. Nevertheless, an animal model is warranted. First, because of the lack of research considering the impact of exercise therapy on glucose regulation in this population, but also because of standardization issues (lifestyle variations, influence of medication, etc) and because of the opportunity of tissue research. An animal model provides a solution for all of these issues. Rat models are commonly used in the study of acute and chronic heart failure [16]. The Dahl salt sensitive rat model is most suited for our research purposes due to the progressive transition of hypertension into heart failure after a high-salt diet and because it is also known to have a genetic predisposition for insulin resistance [17, 18]. The dahl model has already been

used to investigate the effect of an exercise intervention on mortality, hypertension and other hypertension-related measures [17, 19-21]. Beneficial effects on mortality, pathological hypertrophy of left ventricle and pathological generated fibrosis have been showed when exercise training was initiated before or simultaneous with the high salt diet [19-21]. However the effects on glucose regulation require additional research.

In accordance with the above line of reasoning, the present study aims to explore the effects of exercise training on the clinical manifestations of acute heart failure and associated blood glucose and insulin levels in Dahl-salt sensitive rats. It is hypothesized that exercise therapy will have beneficial effects on heart function (left ventricle mass parameters, fibrosis, ejection fraction, strain, strain rate, heart rate), serum glucose levels, serum insulin levels and mortality rate after inducing heart failure.

Materials and methods

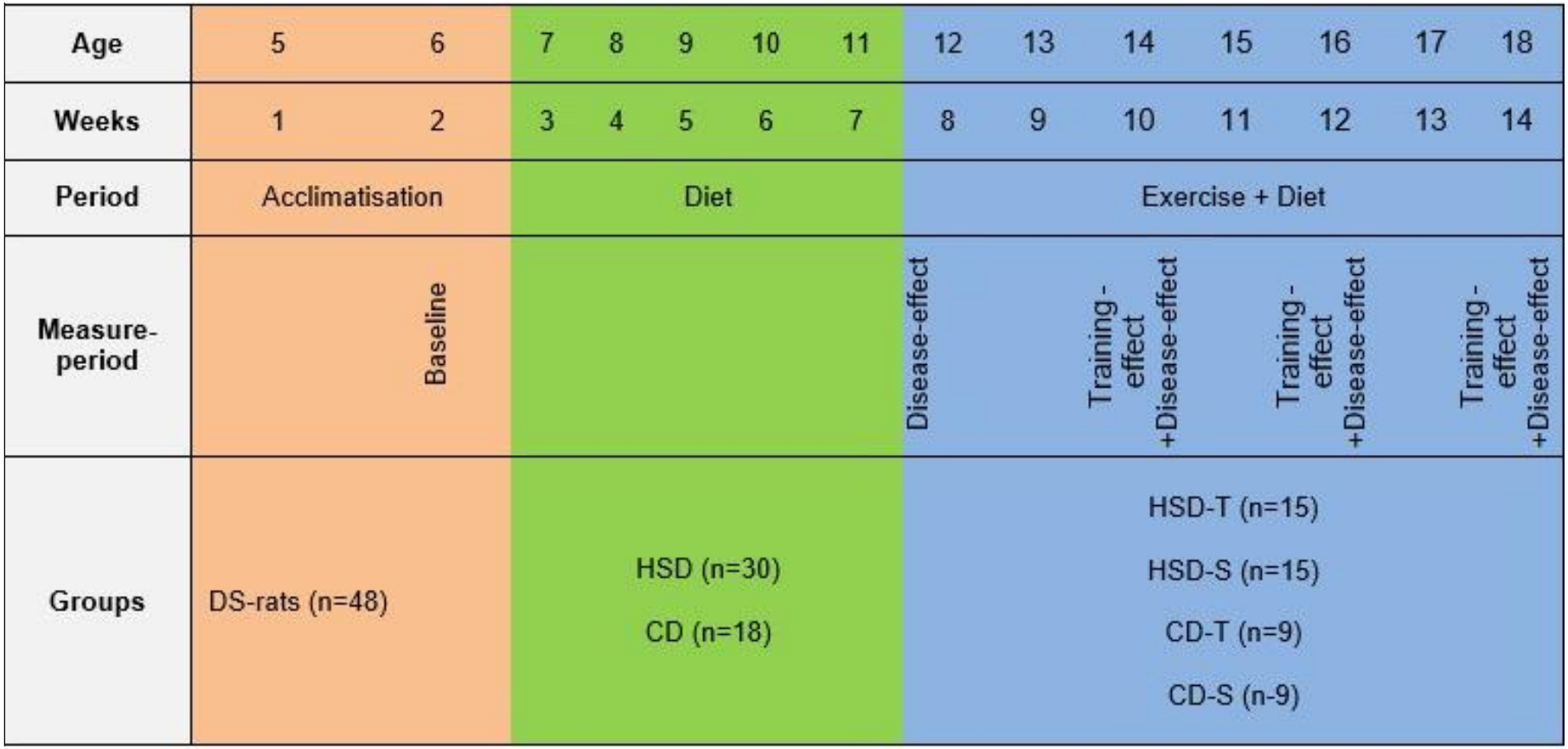
Animals

48 inbred Dahl salt-sensitive rats (DS) were obtained from Charles River at the age of five weeks. They got used of being handled by a person to diminish the stress-related hypertension during the study. Rats were weighed daily to check weight loss, which indicates illness. When animals were too ill, stopped eating or had breathing problems they received euthanasia. Furthermore rats were kept by two in a cage. The Ethical Committee for Animal Experiments of Hasselt University, Belgium approved the use of the rats in the study protocol.

Study design

The study protocol is presented in Figure 1. The first two weeks after arrival consisted of an acclimatisation period. At the age of seven weeks, 30 rats were assigned to a group undergoing a high-salt diet (HSD; 8%NaCl). The other 18 rats were given a control diet (CD; 0,3% NaCl). Both received these diets during 5 weeks. Furthermore the rats received water ad libitum. At the age of twelve weeks, both of the HSD and CD groups were divided into an exercise group (HSD-T; n=15, CD-T; n=9) and a sedentary group (HSD-S; n=15, CD-S; n=9). Rats in the training condition underwent running training on an IITC treadmill, 5 times a week. During acclimatisation period, both speed and duration were systematically increased until rats could run at a speed of 18 meters per minute with an inclination of five percentage for 60 minutes a day. Rats in the sedentary condition were taken out of their cage, placed on top of the treadmill with no food or water during the same period of time. Several measurement periods were taken throughout the protocol (baseline, disease effect, and three more of which the last is used to assess training effect and late disease effect). After the last measurement, a dissection was performed to assess heart weight and to perform tissue research.

Figure 1: Study protocol



DS, dahl salt-sensitive rats, HSD, high-salt diet group; CD, control diet group; HSD-T, Trained high salt diet group; HSD-S, sedentary high salt diet group; CD-T, Trained control diet group; CD-S, Sedentary control diet group

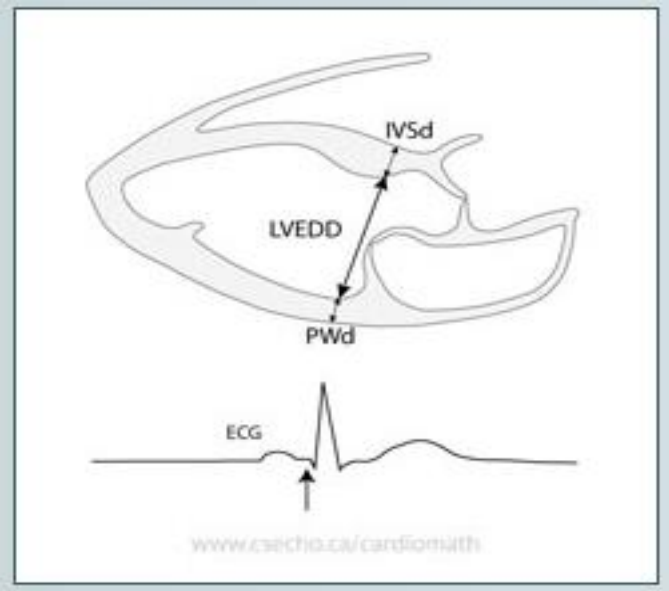
Outcome measurements

Outcome measurements that could demonstrate changes in cardiac dimensions, cardiac function and the quality of cardiac tissue were: Left ventricular mass (LVm), heart weight (g), left ventricular ejection fraction (EF), strain, strain rate and the amount of fibrosis. Furthermore, mortality rates, body weight and blood glucose and insulin levels were assessed.

A. Cardiac dimensions – left ventricular hypertrophy

Echocardiography performed using a Vivid I ultrasound machine (GE VingMed, Horten, Norway) with a 10S-RS array transducer. Rats were under 1.5 – 2% isoflurane anesthesia and placed in left lateral decubitus (maximal duration of 30min). Heart rate was monitored during echocardiography. For the measurement of the left ventricular mass (LVm) B-mode recordings were taken at midpapillary level in the parasternal using shortaxis views. Left ventricular end-diastolic diameter (mm), left ventricular posterior wall thickness at end-diastole (mm), and interventricular septal thickness at end-diastole (mm) were measured. From these measurements LVm was calculated for all of the rats using the following formula: LVm (g) = [1.04 [(LVEDd/10) + (IVSd/10) +(PWd]/10)]^3 – (LVEDd/10)^3] (Figure 2).

Figure 2: left ventricular measurements

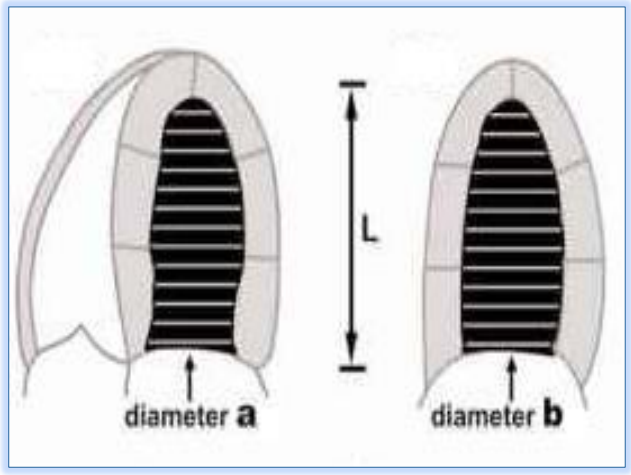


LVEDd, left ventricular end-diastolic diameter; PWd, left ventricular posterior wall thickness at end-diastole; IVSd, interventricular septal thickness at end-diastole

B. Cardiac function – left ventricular ejection fraction, strain, strain rate

For the measurement of left ventricular ejection fraction (EF) , parasternal long-axis views were taken to assess left ventricular length. Mid-ventricular short-axis views were taken to assess systolic/diastolic left ventricular diameter. According to these findings left ventricular end-systolic (ESV) and end-diastolic (EDV) volumes were calculated as following: [π ∗ left ventricular diameter * 2 ∗ (left ventricular length/6)]. Once ESV and EDV were known, EF could be calculated using the following formula: EF (%) = [(EDV − ESV) / EDV] (Figure 3).

Figure 3: Left ventricular measurements



L, left ventricular length; Diameter a, left ventricular diameter at systole; Diameter b, left ventricular diameter at diastole

For the measurement of strain and strain rate a speckle tracking imaging STE was performed on the conventional echocardiography (EchoPAC workstation (GE Vingmed Ultrasound, version 7.0.1, Horten, Norway)), as described previously. This application was used for data-analysis of strain and strain rate. Briefly, the endocardium was manually traced in an optimal frame chosen from a set of two-dimensional recordings at midventricular level. From these optimal frames a speckle-tracking region of interest was automatically selected and measurements of radial and circumferential strain and strain rate were performed. The software detected and tracked the speckle pattern subsistent to the standard two-dimensional echocardiography after separating the ventricular silhouette into 6 segments. After speckle tracking, the quality was visually inspected and needed to be satisfactory for at least five segments before being accepted.

C. Quality of cardiac tissue – amount of fibrosis

At the end of the study protocol, animals were sacrificed, and the LV was blotted, weighed and frozen. Sections of 10 µm thick were obtained at midventricular level and stained using collagen-specific picrosirius red. Myocardial fibrosis was assessed in four animals per experimental group, and quantified in 4 randomly chosen fields per section, as previously described. The area of collagen deposition indicated by red staining was outlined and quantified by an automated image analysis program (Carl Zeiss, AxioVision 4.6). Blood vessels were excluded. The ratio of the area of collagen deposition to the global area was calculated and expressed as percent fibrosis.

D. Glucose regulation

Three to five days after each echocardiography, glucose tolerance was assessed using a 1h oral glucose tolerance test (OGTT). Because of repeated measurements, the OGTT was preferred over more invasive techniques. The OGTT was performed in non-anaesthetized rats (with the exception of a short 2% isoflurane anesthesia to obtain venous blood samples). After ~16h fasting, glucose (2g/kg as a 50% solution; Merck KGaA, Darmstadt, Germany) was administered into the stomach through a cannula. After glucose administration we could assess blood glucose and insulin levels by taking blood samples (25 µL) from the tip of the tail. Blood samples were centrifuged (4800 g, 6min) and serum was preserved (-80°C) until later analysis. In order to determine the blood glucose concentration these blood samples were analyzed (Analox GM7, Analis SA, Namur, Belgium) before glucose administration and after 15, 30 and 60min [22]. Glucose response was expressed as total area under the curve (AUC), calculated according to the trapezoidal rule. In order to determine the insulin level these blood samples were analyzed using an electrochemiluminescence assay (Meso Scale, Gaithersburg, MD). Measurements of increased insulin levels would indicate an increased insulin resistance because of the fact that there is a greater need of insulin to produce the needed effects in the body (insulin receptors sensitivity are therefore decreased).

Statistics

Statistical analyses were performed using JMP Pro 11.2.0 (SAS Institute Inc.). Survival rate was analyzed using the Kaplan Meier method and interpreted by log-rank test. For the analysis of measurements after five weeks of diet the HSD and CD groups have been compared using unpaired T-tests. For the analysis of measurements after an additional seven weeks of exercise intervention, as well as the analysis of fibrosis and heart weight, one-way analysis of variance (ANOVA) was used, with the Tukey-method for post-hoc comparison. For both methods mean differences have used to analyze differences. Outliers (>2,698 SD) were removed. A value of $p < 0.05$ was considered statistically significant. For some of the tests the amount of animals (n) may vary because of deceased rats or failed measurements, which were accordingly removed from statistics.

Results

1) Early disease-effects (results after a five-week high-salt diet):

At baseline, there were no differences in weight, cardiac dimensions, cardiac function nor glucose regulation between the HSD and CD groups (Table 1). However, after five weeks of diet, the HSD group showed a lower body weight when compared with the CD group ($p<0,05$). Furthermore, animals on a HSD showed a larger increase in left ventricular dimensions, assessed by echocardiography ($p<0.05$; Figure 4). When considering cardiac function, left ventricular ejection fraction, radial strain, radial strain rate nor circumferential strain rate differed between the CD and HSD group. However, circumferential strain increased more in the CD group ($p<0.05$) (Table 2).

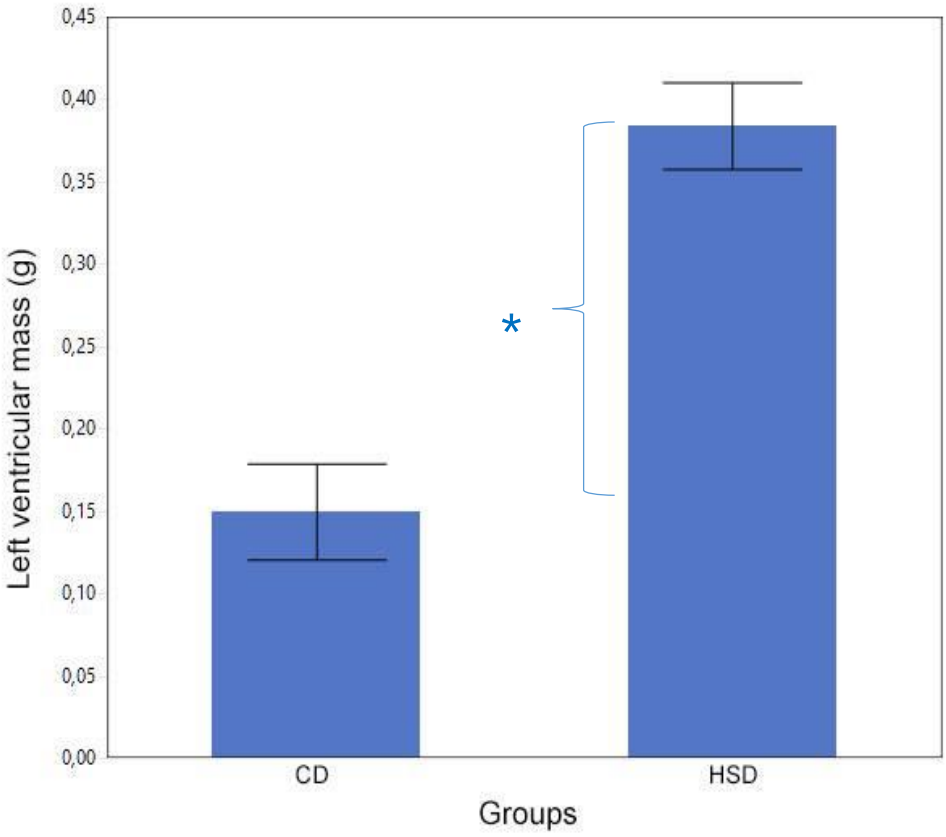
Table 1:

Baseline values of cardiac dimensions, cardiac function and glucose regulation.

Outcome		Baseline value (±SD)		p-value
		HSD	CD	
Cardiac dimensions	LVm (g)	0,56 (±0,07)	0,55 (±0,06)	0,59
Cardiac function	EF (%)	76 (±4)	73 (±5)	0,06
	Rad. strain (%)	45,63 (±9,15)	42,76 (±10,56)	0,38
	Rad. strain rate (sec^{-1})	9,78 (±2,86)	9,89 (±2,65)	0,91
	Circ. strain (%)	-19,51 (±2,22)	-19,17 (±4,07)	0,76
	Circ. strain rate (sec^{-1})	-5,26 (±0,88)	-5,02 (±1,01)	0,46
Glucose regulation	Glucose (AUC)	315 (±41)	341 (±44)	0,06
	Insulin 0' (µg/ml)	226 (±129)	300 (±191)	0,39
	Insulin 60' (µg/ml)	213 (±65)	364 (±127)	0,06

Legend:
Parameters are described as Mean (±SD). HSD, high-salt diet (8%NaCl); CD, control diet (0.3% NaCl); SD, standard deviation; LVm, left ventricular mass; EF, left ventricular ejection fraction; Rad. Strain, radial strain; Rad. strain rate, radial strain rate; Circ. Strain, circumferential strain; Circ. strain rate, circumferential strain rate; AUC, area under curve; Insulin 0, serum insulin levels at fasting; Insulin 60', serum insulin levels 60 minutes after glucose administration

Figure 4: Effect of a five-week high-salt diet on cardiac dimensions:
High-salt diet group clearly showing a higher increase in left ventricular hypertrophy.



CD, control diet group; HSD, high-salt diet group; = significant difference (p<0,05)*

Table 2:

The effects of a five-week high-salt diet on cardiac dimensions, cardiac function and glucose regulation.

Outcome		Group				p-value of mean difference
		HSD		CD		
		n	Mean (SD)	n	Mean (SD)	
Cardiac dimensions	LVm (g)	25	0,91 (±0,12)	18	0,68 (±0,11)	<0,0001*
Cardiac function	EF (%)	15	71 (±3)	18	69 (±5)	0,74
	Rad. Strain (%)	15	37,02 (±10,96)	18	43,53 (±13,48)	0,14
	Rad. strain rate (sec^{-1})	18	7,90 (±2,52)	18	9,01 (±0,57)	0,45
	Circ. strain (%)	18	-15,12 (±4,86)	18	-18,42 (±3,63)	<0,05*
	Circ. Strain rate (sec^{-1})	18	-4,30 (±1,25)	17	-4,71 (±1,68)	0,11
Glucose regulation	Glucose (AUC)	24	289 (±43)	17	294 (±50)	0,95
	Insulin 0' (µg/ml)	27	348 (±202)	17	886 (±92)	<0,05*
	Insulin 60' (µg/ml)	26	397 (±125)	16	547 (±129)	<0,0001*

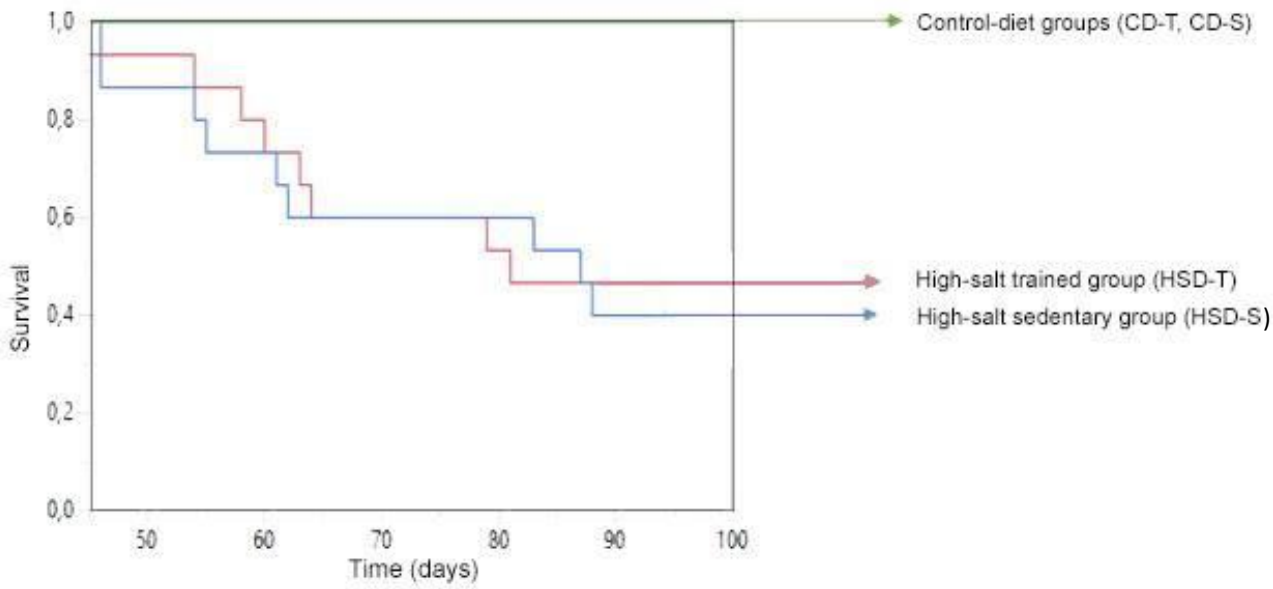
Legend:
Parameters are described as Mean (±SD). HSD, high-salt diet (8%NaCl); CD, control diet (0.3% NaCl); SD, standard deviation; LVm, left ventricular mass; EF, left ventricular ejection fraction; Rad. Strain, radial strain; Rad. strain rate, radial strain rate; Circ. Strain, circumferential strain; Circ. strain rate, circumferential strain rate; AUC, area under curve; Insulin 0, serum insulin levels at fasting; Insulin 60', serum insulin levels 60 minutes after glucose administration

A five-week diet did not result in differences in serum glucose levels between the HSD and CD group (p=0.95). The serum insulin levels at fasting and 60' after glucose administration were elevated in both groups. Slightly higher serum insulin levels at fasting (p<0,05) and profoundly higher serum insulin levels 60' after glucose administration (p<0,001) were present in the CD group.

2) Late disease-effect and training-effects (results after a seven-week training intervention with continuation of the diet):

While mortality was minimal during the first 5 weeks of diet, with one death in the HSD-T group after 45 days and two deaths in the HSD-S group after 46 days. However, mortality increased in the following weeks. All animals in CD, in the training condition as well as the sedentary condition, survived the entire protocol. In HSD, a total of eight rats deceased in the training condition and nine deceased in the sedentary condition. When comparing between HSD-T and HSD-S differences in survival were not significant (Log-Rank: p= 0,07), indicating that the exercise intervention could not decrease mortality (Figure 5).

Figure 5: Mortality rates following a diet period, followed by an exercise intervention with continuation of diet:



CD-T, trained control diet group; CD-S, sedentary control diet group; HSD-T, trained high-salt diet group; HSD-S, sedentary high-salt diet group

Late-disease effects were analyzed comparing both sedentary groups. A lower body weight is shown in the HSD-S group when compared with CD-S group ($p<0,0001$) after an additional seven weeks of diet. Parameters considering cardiac dimensions and cardiac function have been described in table 3. As for cardiac dimensions, post-mortem heart weight was higher in the HSD-S group ($p<0,05$) consistent with the measurements of left ventricular mass derived from echocardiography, during the diet period. Regarding cardiac function, left ventricular ejection fraction ($p<0,05$) and radial strain rate ($p<0,05$) decreased in the HSD-S group concluding cardiac function was decreased.

Animals in HSD-T had a lower heart weight compared to HSD-S ($p<0.05$). In fact, heart weight was almost normalized by exercise training, as there was no difference with animals in CD-S. Assessment of training effects showed that the exercise intervention had beneficial effects on cardiac dimensions, concluded from a higher left ventricular mass in the HSD-S when compared to the HSD-T ($p<0,05$). Also cardiac function benefitted from the exercise intervention, as shown from decreased radial strain ($p<0,05$) and circumferential strain ($p<0,005$) in the HSD-S when compared to the HSD-T, whereas HSD-T showed no differences when comparing with CD-S indicating the exercise intervention normalized the effect of the high-salt feeding. The same effects were observed in left ventricular ejection fraction, radial strain rate and circumferential strain rate, all declaring the positive influence of the exercise intervention on cardiac function. Considering glucose regulation there were no differences in serum glucose levels, nor serum insulin levels at fasting or 60' after glucose administration between any of the groups (Table 3).

Table 3:

The effects of a seven-week exercise intervention in combination with a high-salt diet on cardiac dimensions, cardiac function and glucose regulation.

Outcome		Group					
		HSD-T		HSD-S		CD-S	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Cardiac dimensions	LVm (g)	9	1,01* (±0,12)	7	1,10 (±0,16)	9	0,79 (±0,06)
Cardiac function	EF (%)	8	63 (±3)	8	58 (±11)	9	68* (±2)
	Rad. strain (%)	9	45,63* (±8,58)	9	26,64 (±10,26)	9	48,73 (±14,17)
	Rad. strain rate (sec^{-1})	9	8,31 (±2,37)	9	5,79 (±1,76)	8	10,71* (±2,31)
	Circ. strain (%)	9	-16,92 o (±2,02)	9	-11,28 (±2,39)	8	-16,62 (±2,27)
	Circ. strain rate (sec^{-1})	9	-4,42 (±0,91)	9	-2,97 (±0,67)	9	-4,52 (±1,06)
Glucose regulation	Glucose (AUC)	9	273 (±40)	9	265 (±19)	9	271 (±36)
	Insulin 0' (µg/ml)	9	499 (±88)	9	257 (±121)	8	593 (±288)
	Insulin 60' (µg/ml)	9	167 (±95)	9	380 (±129)	8	627 (±171)

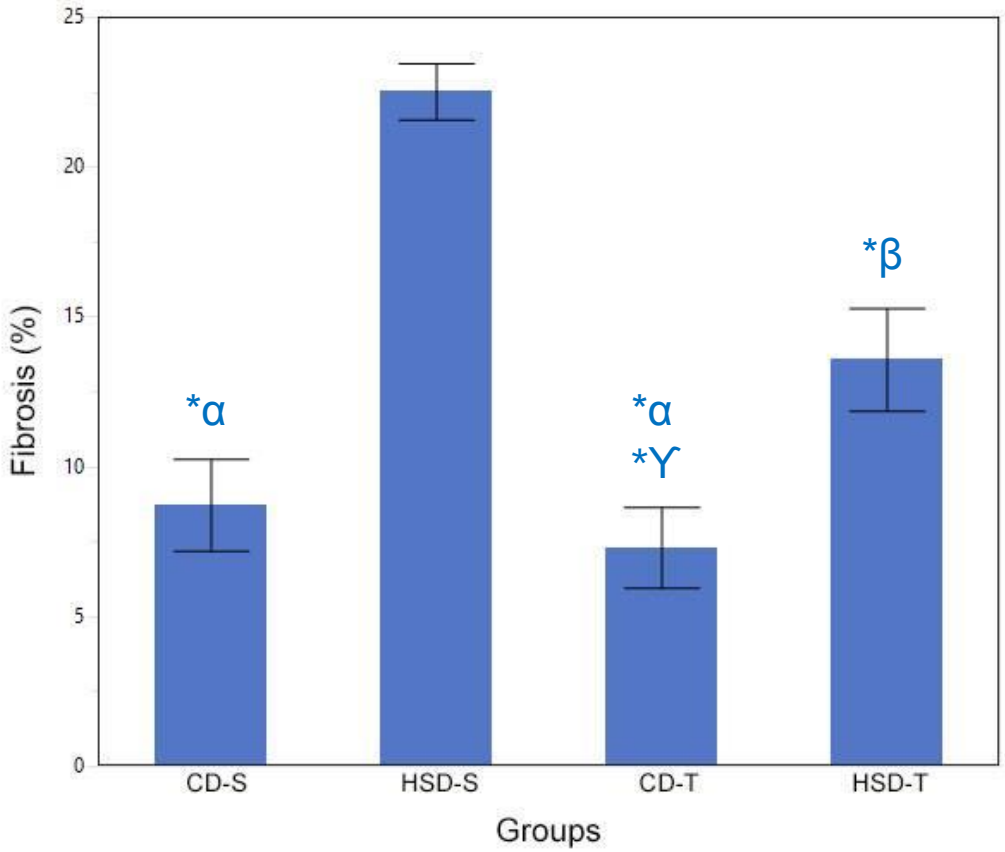
Legend:
Parameters are described as Mean (±SD). HSD-T, trained high-salt diet group (8%NaCl); HSD-S, sedentary high-salt diet group; CD-S, sedentary control diet group (0.3% NaCl); n, number of rats; standard deviation; LVm, left ventricular mass; EF, left ventricular ejection fraction; Rad. Strain, radial strain; Rad. strain rate, radial strain rate; Circ. Strain, circumferential strain; Circ. strain rate, circumferential strain rate; AUC, area under curve; Insulin 0, serum insulin levels at fasting; Insulin 60', serum insulin levels 60 minutes after glucose administration

* *= significant compared to HSD-S (p<0,05)*
o *= significant compared to HSD-S (p<0,005)*

3) Post-mortem analysis: Quality of cardiac tissue - amount of fibrosis:

More fibrotic tissue was present in the hearts of animals in HSD-S compared with CD-S ($p<0,0001$) indicating disease-effects of high-salt feeding. Furthermore, exercise training was able to prevent fibrotic processes as HSD-T showed less fibrotic tissue when compared with HSD-S ($p=0,0037$). When comparing the HSD-T group with the CD-S group however, the HSD-T group showed a higher amount of fibrosis ($p=0,1247$) (Figure 6).

Figure 6: Effect of a five-week diet and an exercise intervention on quality of cardiac tissue: *These differences show a higher amount of fibrosis in the HSD groups, indicating high-salt feeding decreases quality of cardiac tissue. However the trained high-salt diet group shows less fibrosis indicating an exercise program is beneficial for quality of cardiac tissue.*



*CD-S, sedentary control diet group; HSD-S, sedentary high-salt diet group; CD-T, trained control diet group; HSD-T, trained high-salt diet group, *α = significant compared to HSD-S ($p<0,0001$; *β = significant compared to HSD-S ($p<0,05$); *Ɣ = significant compared to HSD-T ($p<0,05$)*

Discussion

The Present study aimed to explore the effects of exercise training on the clinical manifestations of acute heart failure and associated blood glucose and insulin levels in Dahl-salt sensitive rats. Our hypothesis was that exercise therapy has a positive influence on mortality rate, pathological hypertrophy, heart function characteristics (ejection fraction, strain and strain rate), fibrosis, serum glucose levels, and serum insulin levels after inducing acute heart failure. Our results demonstrated that exercise training resulted in improvements in cardiac dimensions, parameters reflecting heart function and reduced fibrosis in this hypertrophic acute heart failure model.

Early Disease effects (results after a five-week high-salt diet):

High-salt diet causes hypertension and eventually induces heart failure in Dahl salt-sensitive rats as described in literature. Apart from mortality after one to five weeks of HSD, aggravation in left ventricular mass, systolic blood pressure, fibrosis and ejection fraction evidences these findings in literature [9, 19]. After five weeks of high salt diet did not show mortality in both groups. Thereby, mortality showed no significant differences after five weeks of high-salt diet, corresponding with findings in the studies of Miyachi et al. and Libonati et al. after five weeks of a HSD [19, 20].

The Present study shows an early disease effect following five weeks of high salt feeding in Dahl Salt-Sensitive rats. High salt diet showed a significantly lower increase of bodyweight, which indicates an early stage of disease effect.

Here above, findings are further indicated by significant increase in left ventricular mass due to the high salt diet, indicating hypertrophy due high-salt diet as expected. However, after a five-week HSD diet ejection fraction, radial strain, radial strain rate and circumferential strain rate were not yet impaired. Nevertheless, circumferential strain was increased at this stage, indicating subtle changes in an early stage of the disease process. Doi et al. described changes of heart function and left ventricular mass parameters after six weeks of high-salt diet [23]. These findings suggest that after six weeks of high-salt diet rats start to develop a disease effect.

Alterations of hypertensive heart failure characteristics due to high salt diet have been described in literature. Left ventricle mass (LVm) alterations due to changes of wall thickness, fibrosis and ejection fraction (EF) all indicate heart failure due a high salt diet [9, 19, 24]. In the present study, echocardiography showed an increase of left ventricle mass after five weeks of high salt diet (Figure 4). Several studies show a high-salt diet influences glucose and insulin levels in heart failure along with Dahl salt-sensitive rats [18, 25]. During a study by Ogihara et al, using the hyperinsulinemic euglycemic clamp technique to assess insulin resistance, Dahl salt-sensitive rats developed significant insulin resistance due a high-salt diet indicated by a decrease in glucose utilization [18, 25]. Comparatively with current study, rats were fed high-salt diet at the age of four weeks, which may indicate induce of insulin resistance contributes earlier in the study of Ogihara et al. [25]. The Current study demonstrates no disease effects in glucose levels between HSD and CD group. Results of insulin levels in 60 minutes after glucose administration show an increase in both groups. Strangely, animals on a normal diet had

higher insulin levels. Fasting insulin levels of the normal diet group were increased in comparison with the high salt diet group. These findings were not expected; especially because the Dahl rats are known to induce insulin resistance due a high salt diet while hypertension is as well is developed. Although, Shehata et al. described lean Dahl Salt-Sensitive rats react different on insulin signalling, which influences insulin sensitivity and might have an impact on the result as seen in this study [18]. Furthermore, we observed that standard deviations are very large in the insulin measurements as seen in table 1 and the use of OGTT for testing glucose and insulin levels differ from the study of Ogihara et al., which could suggest the difference in observed results for glucose regulation [25].

Further development disease effect (after seven additional weeks of high salt diet):

Several parameters show the further development of the disease effect during seven additional weeks of a high salt diet. Demonstrated by significant lower bodyweight of rats due to continue of the high-salt diet. The sedentary high-salt diet group will furthermore develop heart failure demonstrated by a decrease in bodyweight. Several studies demonstrated these findings in bodyweight due initiation of disease effect due a high-salt diet [19, 23]. Further findings were higher post-mortem weight of the heart and lower ejection fraction and radial strain rate due seven additional weeks of high-salt diet. Additionally, current study shows an increase in percentage fibrosis indicating normal diet is more beneficial than high-salt diet, even when a high-salt diet is combined with an exercise intervention. This is comparable with findings demonstrated in the study of Doi et al. [23] These findings suggest that the development of heart failure continues due additional weeks of high-salt diet.

Training effect (results after a seven-week training intervention):

Fifteen rats deceased during the study. Eight rats of the trained high-salt diet group deceased and nine rats of the sedentary, high-salt group had deceased. Normal diet groups did not show mortality. Suggesting that induced heart failure in Dahl salt-sensitive rats therefor results in a reduced chance of survival. Training intervention has no beneficial effects on survival, although several studies shown that survival rates increase due a training intervention in a Dahl Salt-Sensitive rat population [19-21].

Left ventricle mass due training intervention, even that severe that LVm parameters restore due a training intervention. Literature is not supporting these findings, Miyachi et al. demonstrated unchanged LVm in a swimming exercise protocol [19]. This exercise protocol is different opposed to a treadmill protocol and could have had an influence on the results of LVm. Results of radial (increase) and circumferential (decrease) strain were significantly improved due to an exercise intervention. This indicates that heart function deformation, developed by high salt diet, improves due to training intervention in Dahl Salt-sensitive rat population. Improvement of contractility is in current study associated with a decrease in the pathological generated fibrosis (Figure 6). Suggesting that fibrosis is seemed to restore after exercise intervention, which is comparable with literature [19]. The importance of exercise training in heart failure is demonstrated by these findings.

Furthermore literature suggests that exercise training positively affects glucose regulation [26]. In current study training intervention did not influence glucose and insulin levels. Fasting insulin levels are even more increased in the trained high salt diet group in comparison with the sedentary high salt diet

group. These findings were not to be expected in this study protocol. As seen in table 2, standard deviations are very large and of due data-extraction many zero-values were administrated. During the OGTT rats received a small anaesthesia to obtain venous blood samples. Which may all could have an influence on the results in this study. Additional research on these parameters is required.

Side notes: Diet

Start of the diet: Literature study has indicated the importance of the age at which the diet starts. Theory suggests there is an important difference between starting the diet at an age of seven weeks or eight weeks. First of all it influences the onset of symptoms, being respectively 19 and 26 weeks. Secondly it results in another clinical picture, as the start of the diet on an age of seven weeks results in diastolic heart failure, whereas the start of the diet at an age of eight weeks results in systolic heart failure [23]. Because of the nature of our study and because of the lower amount of studies in which the onset of the diet has found place at seven weeks, we will use this principle in our study.

Conclusion

Several parameters clearly showed that acute heart failure was induced in present study. Current study shows the differences between high-salt diet group and control diet group, namely an increase of left ventricle mass, a higher amount of fibrosis, a decrease of bodyweight and an increase of the circumferential strain in the high-salt diet group. These findings indicate an early stage of development of heart failure after a five-week high-salt diet of 8% NaCl. Therefore, this study is representative for a population with acute heart failure. Seven weeks of exercise training seemed to affect the disease effect, suggesting that exercise has beneficial effects on the development of heart failure. Training intervention positively influenced left ventricular mass parameters, radial and circumferential strain and fibrosis suggesting that exercise training has beneficial effects on pathological hypertrophy, heart functions and heart tissue adaptation in heart failure.

References

1. *World Health Organization*.
2. Mosterd, A. and A.W. Hoes, *Clinical epidemiology of heart failure.* Heart, 2007. **93**(9): p. 1137-46.
3. P. Van royen, S.B., P. Chevalier, G. Dekeulnaer, M. Goossens, P. Koeck, M. Vanhalewyn, P. Van Den Heuvelp, *Chronisch Hartfalen.* 2011: p. 32.
4. Bonneux, L., et al., *Estimating clinical morbidity due to ischemic heart disease and congestive heart failure: the future rise of heart failure.* Am J Public Health, 1994. **84**(1): p. 20-8.
5. Sanderson, J.E. and T.F. Tse, *Heart failure: a global disease requiring a global response.* Heart, 2003. **89**(6): p. 585-6.
6. National Collaborating Centre for Chronic, C., *National Institute for Health and Clinical Excellence: Guidance*, in *Chronic Heart Failure: National Clinical Guideline for Diagnosis and Management in Primary and Secondary Care*. 2003, Royal College of Physicians (UK) Royal College of Physicians of London.: London.
7. 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.
8. Dahlstrom, U., *Frequent non-cardiac comorbidities in patients with chronic heart failure.* Eur J Heart Fail, 2005. **7**(3): p. 309-16.
9. 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.
10. AlZadjali, M.A., et al., *Insulin resistance is highly prevalent and is associated with reduced exercise tolerance in nondiabetic patients with heart failure.* J Am Coll Cardiol, 2009. **53**(9): p. 747-53.
11. Deaton, C., et al., *Glucose and insulin abnormalities in patients with heart failure.* Eur J Cardiovasc Nurs, 2011. **10**(2): p. 75-87.
12. 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 Heart J, 2012. **33**(14): p. 1787-847.
13. O'Connor, C.M., et al., *Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial.* Jama, 2009. **301**(14): p. 1439-50.
14. Piepoli, M.F., *Exercise training in chronic heart failure: mechanisms and therapies.* Neth Heart J, 2013. **21**(2): p. 85-90.
15. Yancy, C.W., et al., *2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines.* J Am Coll Cardiol, 2013. **62**(16): p. e147-239.
16. Hasenfuss, G., *Animal models of human cardiovascular disease, heart failure and hypertrophy.* Cardiovasc Res, 1998. **39**(1): p. 60-76.
17. Fregly, M.J., *Effect of an exercise regimen on development of hypertension in rats.* J Appl Physiol Respir Environ Exerc Physiol, 1984. **56**(2): p. 381-7.
18. Shehata, M.F., *Genetic and dietary salt contributors to insulin resistance in Dahl salt-sensitive (S) rats.* Cardiovasc Diabetol, 2008. **7**: p. 7.
19. Miyachi, M., et al., *Exercise training alters left ventricular geometry and attenuates heart failure in dahl salt-sensitive hypertensive rats.* Hypertension, 2009. **53**(4): p. 701-7.
20. 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.

21. Shepherd, R.E., et al., *Attenuation of blood pressure increases in Dahl salt-sensitive rats by exercise.* J Appl Physiol Respir Environ Exerc Physiol, 1982. **52**(6): p. 1608-13.
22. Le Floch, J.P., et al., *Blood glucose area under the curve. Methodological aspects.* Diabetes Care, 1990. **13**(2): p. 172-5.
23. 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.
24. Ishizu, T., et al., *Left ventricular strain and transmural distribution of structural remodeling in hypertensive heart disease.* Hypertension, 2014. **63**(3): p. 500-6.
25. Ogihara, T., et al., *High-salt diet enhances insulin signaling and induces insulin resistance in Dahl salt-sensitive rats.* Hypertension, 2002. **40**(1): p. 83-9.
26. Qin, B., et al., *Voluntary running improves in vivo insulin resistance in high-salt diet fed rats.* Exp Biol Med (Maywood), 2007. **232**(10): p. 1330-7.

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