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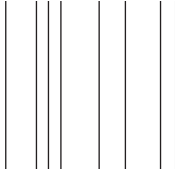
DOCTORAL DISSERTATION

# Impaired glucose regulation in heart failure: impact of exercise therapy

Doctoral dissertation submitted to obtain the degree of  
Doctor of Rehabilitation Sciences and Physiotherapy, to be defended by

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## List of abbreviations

ACT	aerobic continuous training
ADA	American Diabetes Association
ANOVA	analysis of variance
AUC	area under the curve
AWT	anterior wall thickness
AIIA	angiotensin II antagonist
BMI	body mass index
BNP	B-type natriuretic peptide
CABG	coronary artery bypass grafting
CHF	chronic heart failure
CMP	cardiomyopathy
CO	cardiac output
CON	control group
Dahl S	Dahl salt sensitive
EDV	end-diastolic volume
ESV	end-systolic volume
EX	exercise training
FI	fasting insulin
FPG	fasting plasma glucose
FS	fractional shortening
GLUT4	glucose transporter isoform 4
HbA1c	hemoglobin A1c
HF	heart failure
HFrEF	heart failure with reduced ejection fraction
HIT	high intensity interval training
HOMA	homeostasis model assessment
HR (Chapter 3)	hazard ratio
HR (Chapter 5)	heart rate
HS	high salt
HW/BW	heart weight/body weight ratio
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
LV	left ventricular
LVEDD	left ventricular end-diastolic diameter
LVESD	left ventricular end-systolic diameter
LVEF	left ventricular ejection fraction
MVI	myocardial velocity imaging
ND	not described
NGT	normal glucose tolerance
ns	not significant
NS	normal salt
NGT	normal glucose tolerance
NYHA	New York Heart Association

OGTT	oral glucose tolerance testing
OUES	oxygen uptake efficiency slope
PWT	posterior wall thickness
QoL	quality of life
RM	repetition maximum
SD	standard deviation
SEM	standard error of the mean
SED	sedentary
Scirc	circumferential strain
Srad	radial strain
SRcirc	circumferential strain rate
SRrad	radial strain rate
STE	speckle tracking echocardiography
T2DM	type 2 diabetes mellitus
VCO <sub>2</sub>	carbon dioxide output
VE	minute ventilation
VO <sub>2</sub>	oxygen uptake
VT1	first ventilatory threshold
VT2	second ventilatory threshold
WHO	World Health Organization
2D	two-dimensional
2hPG	2 hour glucose value during OGTT





## The failing heart

Heart failure (HF) is one of the most important causes of morbidity and mortality in the Western world. The incidence of HF increases rapidly with age, with a mean age for acute HF patients of 74 years.<sup>1</sup> Because populations grow increasingly older, revascularization is commonly accessible and survival following myocardial infarction is increased, the prevalence of HF will further increase amongst most European countries.<sup>2</sup>

Heart failure has been defined as “an abnormality of cardiac structure or function leading to failure of the heart to deliver oxygen at a rate commensurate with the requirements of the metabolizing tissues, despite normal filling pressures (or only at the expense of increased filling pressures)”.<sup>3</sup> It is a clinical syndrome with typical symptoms (breathlessness, ankle swelling and fatigue) and signs (elevated jugular venous pressure, pulmonary crackle, displaced apex beat).<sup>3</sup> In addition, heart failure does not only comprise cardiac malfunction, it is a syndrome with multi-organ failure. Consequently, abnormalities in lungs, kidneys, liver, hormonal balance and skeletal muscle often dominate the clinical picture.<sup>4</sup> In this respect, impaired glucose tolerance has also been described as a comorbidity in chronic HF.

There are various aetiologies of HF. Amongst others, coronary artery disease (most frequent), systemic and pulmonary hypertension, valve malfunctions, viral infection, alcohol abuse and arrhythmia can cause HF.<sup>3,4</sup> Furthermore, HF can be present with reduced or preserved systolic function. In patients with *systolic dysfunction*, the affected ventricle has a reduced capacity to eject blood because of impaired myocardial contractility (from destruction of myocytes, abnormal myocytes function or fibrosis) or because of pressure overload (excessive afterload). In patients with *preserved systolic function*, abnormalities of diastolic function cause HF: impaired early diastolic relaxation and increased stiffening of the ventricular wall can be present isolated or in combination.<sup>5</sup> However, irrespective of cause or clinical manifestation, HF patients’ functioning is expressed using the New York Heart Association (NYHA) classification, in which *subjective* exercise (in)tolerance has the leading role (Table 1.1). The *objective* assessment of exercise (in)tolerance is even more important for clinical decision making and assessing treatment effects: peak oxygen consumption, minute ventilation to carbon dioxide output slope and exertional oscillatory ventilation are important prognostic parameters derived from cardiopulmonary exercise testing.<sup>6-8</sup>

Treatment of patients with chronic HF with reduced systolic function has been described in 5 therapy goals.<sup>5</sup> (1) The underlying cause of HF is to be identified and corrected. This could involve surgery, coronary artery revascularization, hypertension treatment, and others. (2) In patients who were previously in a compensated state, acute precipitating causes of symptoms are to be removed. For example, treatment of acute infection or arrhythmia, elimination of sources of excessive salt. (3) Management of HF symptoms using sodium restriction, diuretic medication, vasodilators and positive inotropic drugs. In addition,

aerobic exercise training should be prescribed in order to improve functional capacity and symptoms.<sup>3</sup> (4) Modulation of the neurohormonal alterations which are activated in HF in response to the decreased cardiac output. This can be achieved using angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers,  $\beta$ -blockers and aldosterone antagonists. (5) Prolongation of long-term survival. Furthermore, it is recommended that multidisciplinary care is organized in order to reduce the risk of hospitalizations.<sup>3</sup> To date, no pharmacological treatment has been shown to reduce morbidity and mortality in patients with preserved systolic function. However, diuretics are used to control sodium and water retention and therefore relieve dyspnea and oedema. Also adequate treatment of hypertension and myocardial ischaemia is considered to be important.<sup>3</sup>

**Table 1.1 New York Heart Association functional classification**

<b>Class I</b>	No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue, or palpitations.
<b>Class II</b>	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
<b>Class III</b>	Marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
<b>Class IV</b>	Unable to carry on any physical activity without discomfort. Symptoms at rest can be present. If any physical activity is undertaken, discomfort is increased.

## Effects of exercise training in chronic heart failure

Heart failure was considered an absolute contra-indication for exercise training in the past. However, since the milestone publication of Coats et al in 1990, knowledge regarding the beneficial effects of exercise training in chronic HF (CHF) has evolved and exercise therapy is nowadays an established part of the treatment.<sup>3,9</sup> In this regard, exercise training is recommended for stable CHF patients in NYHA class I-III with early mobilization during hospitalization (phase I rehabilitation), ambulatory exercise therapy with gradual optimization of exercise modalities (phase II rehabilitation) and a lifelong active lifestyle (phase III rehabilitation aiming at secondary prevention).

Recently, an update of the Cochrane review investigating exercise-based rehabilitation in CHF included trials with patients with reduced as well as

preserved ejection fraction.<sup>10</sup> A non-significant trend in reduction of mortality in the long term was reported. But more convincingly, exercise training was related to reduced HF-related as well as all-cause hospitalizations. In addition, a clinically important improvement in health-related quality of life was reported after rehabilitation with exercise training.

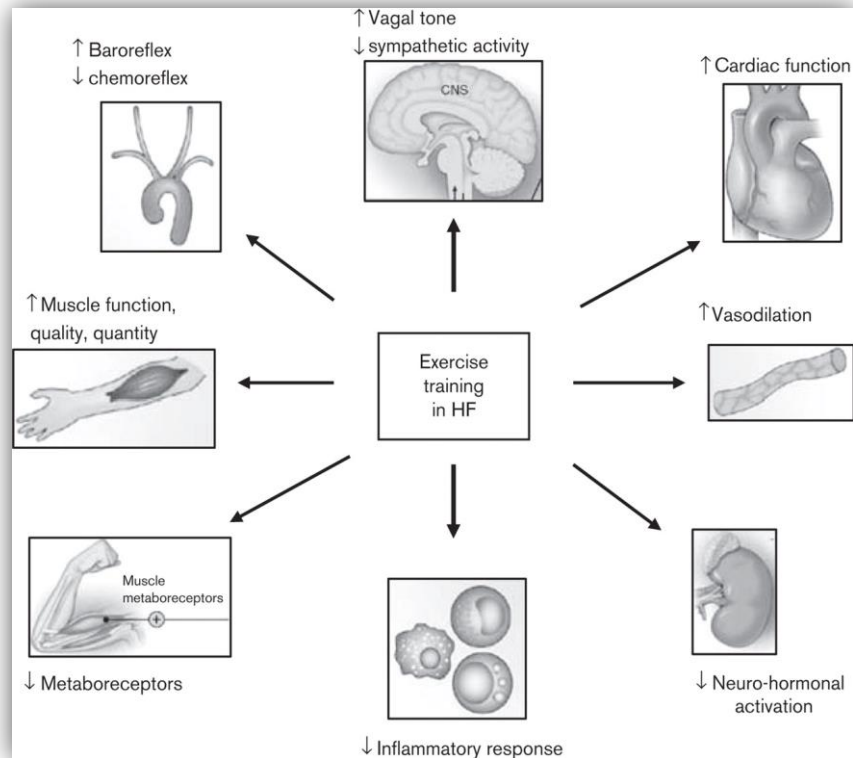
The beneficial effects of exercise training on malfunctioning central and peripheral systems in CHF are described in Figure 1.1. Exercise-induced improvements have been reported regarding peripheral arterial endothelial function, ventilatory function, neuroendocrine and autonomic nervous system activity, inflammatory state and skeletal muscle function.<sup>11-13</sup> At the site of the skeletal muscle, exercise training can improve the CHF-related myopathy by improving skeletal muscle perfusion and oxygen supply to exercising muscles through an increased capillary density, by reversing the fiber type shift towards more oxidative type I fibers, by increasing mitochondrial density and oxidative enzymes in the muscle and by reducing local inflammatory markers.<sup>11-13</sup>

However, CHF populations in trials studying the impact of exercise training are often not representative for CHF patients seen in everyday clinical practice, as patients with substantial comorbidities, women, elderly and minority groups are often excluded in these studies.<sup>14</sup> Therefore, many questions remain regarding the effects of exercise therapy in a general CHF population.

## Heart failure and impaired glucose regulation

Type 2 Diabetes Mellitus (T2DM) is frequently found in patients with HF, with reported prevalence figures varying from 8-41%, which is higher compared to the estimated 6-8% in the general population aged 20-79 years.<sup>15-18</sup> However, the exact prevalence of disturbed glucose regulation in HF is not known as many patients have undetected impaired glucose tolerance (IGT) or T2DM.<sup>16,19</sup> One study examining glucose tolerance in 413 stable CHF patients without diagnosed diabetes, found that IGT occurred in 23% and a further 18% had undiagnosed diabetes.<sup>19</sup> Possible explanations for undetected prediabetes (the presence of insulin resistance, hyperinsulinemia, increased fasting glucose or IGT) and T2DM may be the absence of routine screening for glucose regulation in HF clinics, and the absence of consensus regarding the correct assessment of glucose regulation.

Several methods can be used to investigate insulin sensitivity, insulin resistance and glucose regulation. The *euglycaemic hyperinsulaemic clamp* technique is generally considered the gold standard to assess insulin sensitivity. However, not only are clamp tests complex stress tests with insulin and glucose fluxes well outside the normal range, this method is also too expensive as well as time consuming for use in routine screening and large studies.<sup>20</sup>



**Figure 1.1 Systemic beneficial effects of exercise training in HF**

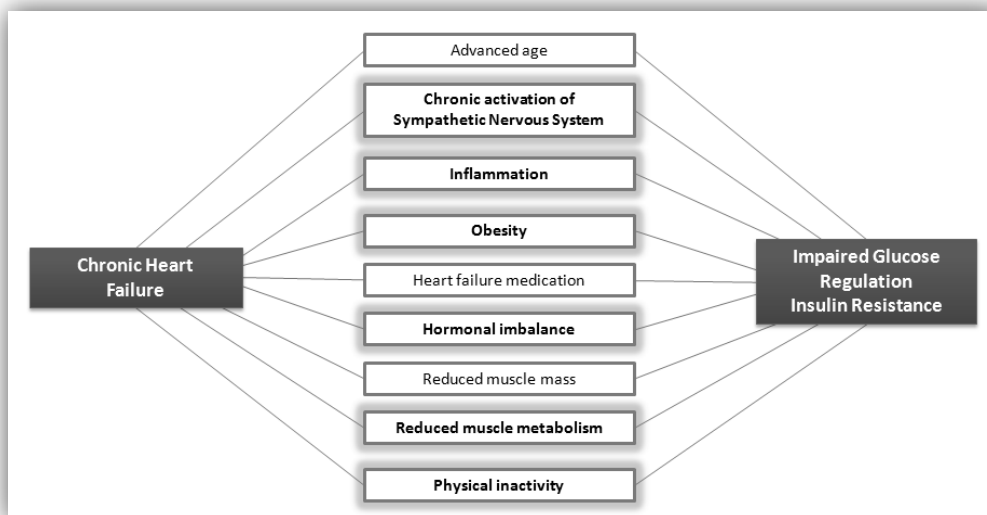
From Piepoli M et al, with permission<sup>12</sup>

On the other side, *fasting glucose* levels fail to detect a large group of patients with prediabetes and T2DM.<sup>19,21</sup> There is no consensus regarding the use and cutoff criteria of *hemoglobin A1c* (HbA1c) in the diagnosis of (pre)diabetes.<sup>22-24</sup> Finally, the *2h oral glucose tolerance test* provides information of dynamic (non-steady-state) insulin secretion and insulin sensitivity over the middle of the physiological range.<sup>20</sup> The World Health Organization (WHO) uses fasting and 2h glucose values for diagnosis of impaired glucose homeostasis and T2DM, while the American Diabetes Association (ADA) uses more stringent criteria for the latter 2 in combination with HbA1c.<sup>25,26</sup>

Both prediabetes and T2DM are not merely innocent bystanders. In patients with CHF, T2DM is an independent predictor of mortality. MacDonald et al summarized non-clinical population studies and clinical studies describing the increased risk of mortality with diabetes in patients with CHF.<sup>27</sup> Likewise, prediabetes is associated with worse prognosis, lower functional status in terms of a higher NYHA functional class and lower exercise capacity.<sup>28-33</sup>

## Why are chronic heart failure patients prone to develop impaired glucose tolerance?

From the above-mentioned studies, it has become evident that (pre)diabetes is a cause for concern in CHF patients. However, the underlying reasons have not been fully clarified yet. In literature, some mechanisms have been proposed to explain the development of (pre)diabetes in CHF patients: advanced age, sympathetic nervous system overactivity, inflammation, obesity, heart failure medication, hormonal imbalance, reduced muscle mass and altered muscle metabolism and physical inactivity (Figure 1.2). From these, heart failure medication, obesity, skeletal muscle and physical inactivity will be shortly discussed.



**Figure 1.2 Factors linking CHF and impaired glucose regulation**

*Factors that can be positively influenced by exercise training are highlighted.*

Standard medications in the treatment of HF are known to have an influence on the glucoregulatory mechanism. The increases in glycemia induced by thiazides are small and appear to attenuate over time, but opinion leaders have raised concerns about the potential for long-term adverse effects of the observed dysglycaemia.<sup>27,34,35</sup> Also most  $\beta$ -blocking agents have deleterious effects on insulin sensitivity, carbohydrate and lipid metabolism.<sup>36</sup> The newer third-generation vasodilating  $\beta$ -blockers have shown a better metabolic profile, and may reduce the risk for promoting new-onset diabetes in patients with CHF.<sup>36-38</sup> Last, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers can reduce the incidence of diabetes in CHF patients, possibly by reducing the sympathetic nervous system activation.<sup>27,38</sup>

The body mass index (BMI) has been described as a determinant of insulin resistance in the CHF population.<sup>31</sup> In one analysis, BMI could be replaced by fat mass or percentage body fat (assessed by skin fold measures), but not by fat-free mass. On the other hand, precise body composition data assessed by dual energy X-ray absorptiometry were not related to insulin sensitivity and glucose transporter isoform 4 (GLUT4) presentation in the skeletal muscle of CHF patients in another study.<sup>39</sup> In the general population, obesity, amount and distribution of fat are important regulating factors for glucose and insulin metabolism.<sup>40</sup> Fat tissue is a major player and can be considered as an endocrine organ, which produces peptide hormones and cytokines (adipokines) that influence insulin sensitivity.<sup>41</sup> Recently, research in CHF populations is also focusing on the role of adipokines as adiponectin, leptin and resistin.<sup>42,43</sup> However, we should not forget that not all CHF patients are obese, and that lean or even cachectic CHF patients present with impaired glucose regulation.

Because skeletal muscle is the major site of glucose disposal and is most affected by insulin resistance<sup>44,45</sup>, the defects that are located at the level of the skeletal muscle are of major importance to explain the elevated risk for the development of (pre)diabetes in CHF patients. First, muscle wasting is present early in the disease course and consequently a loss of muscle mass is a common problem in CHF.<sup>46</sup> Second, the relative preponderance of type II muscle fibers in CHF can lead to a decreased glucose uptake by the skeletal muscle: compared to the more oxidative type I fibers with higher capillary density, type II muscle fibers have reduced blood flow and are less sensitive for insulin.<sup>15,47</sup> In addition, the expression of GLUT4 in skeletal muscle is reduced in CHF patients when compared with healthy subjects. The degree of GLUT4 reduction directly relates to the severity of whole body insulin resistance, and the reduction in GLUT4 is parallel to the severity of CHF.<sup>39</sup> Explanations for a reduced expression of GLUT4 protein in CHF could be found in physical inactivity, reduced muscle fiber contractions (which are a stimulus for GLUT4 translocation from the inside of the cell to the plasma membrane), and tissue hypoxia (which can reduce GLUT4 translocation by 50%).<sup>48</sup> Finally, it has been proposed that the skeletal muscle can also be viewed as an endocrine organ: cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert paracrine or endocrine effects could be classified as "myokines".<sup>49,50</sup> The delicate balance between myokines is disturbed and directed towards a pro-inflammatory profile by an inactive lifestyle. Specifically, when the skeletal muscle is relatively inactive, the lack of anti-inflammatory myokines (e.g. interleukine-6) results in a decreased glucose uptake and oxidation in the skeletal muscle.<sup>50</sup>

In final, physical inactivity per se has been described as an independent predictor for insulin resistance in CHF.<sup>31</sup> Inactivity may lead, through a cascade of events, to transcriptional changes to metabolic and mitochondrial genes, thereby influencing oxidative phosphorylation.<sup>51</sup>

### **Treatment of (pre)diabetes in chronic heart failure**

Despite the high prevalence of impaired glucose regulation in patients with CHF, there is no consensus regarding treatment. According to the European Society of Cardiology guidelines on diabetes, pre-diabetes, and cardiovascular diseases “an optimal management of these co-existing conditions is still not fully evidence-based owing to a lack of clinical trials specifically addressing such patient populations”.<sup>52</sup> In fact, the use of glucose-lowering medication is not evident, as metformin and thiazolidinediones were considered contra-indicated in CHF patients because of negative effects regarding lactic acidosis and fluid retention.<sup>15</sup> Nowadays, metformin is commonly used, with possible favourable effects on outcome (hospitalizations and mortality).<sup>27,53</sup> On the other hand, treatment with thiazolidinediones is still discouraged because of an increased risk of adverse events.<sup>53</sup> The recommended non-pharmacological treatment modalities to improve glucose regulation, diet and physical exercise, are already established components of recommended CHF treatment.<sup>54</sup>

There are many reasons why exercise therapy may improve glucose regulation in CHF patients. In the general population, physical activity is a means to prevent a deterioration of glucose metabolism.<sup>55</sup> According to the Standards of Medical Care in Diabetes 2013, moderate physical activity should be performed for at least 150 minutes per week in order to prevent or delay T2DM.<sup>25</sup> These recommendations are supported by the results of large lifestyle intervention trials in populations at risk for developing T2DM, such as the Finnish Diabetes Prevention Study, the Da Qing IGT and Diabetes Study and the U.S. Diabetes Prevention Program Outcomes Study, showing that T2DM can be prevented by changes in physical activity and lifestyle.<sup>56-58</sup> The factors linking CHF and IGT, on which exercise training has a beneficial influence, are highlighted in figure 1.1.

Focusing on changes in skeletal muscles, it has been suggested that exercise training with resistance exercises in CHF may improve underlying skeletal muscle abnormalities and neuromuscular function, rather than simply increasing muscle mass.<sup>11,59</sup> First, exercise therapy enhances the trafficking and translocation of GLUT4 protein towards the cell surface and may promote a shift towards insulin-sensitive type I fibers.<sup>51,60,61</sup> Second, the expression and activity of proteins involved in insulin signal transduction are increased by exercise. Third, mitochondrial biogenesis in skeletal muscle is enhanced by exercise, thereby enhancing the oxidative capacity.<sup>51,62</sup> Last, a decrease in local muscle inflammatory factors (tumor necrosis factor- $\alpha$ , interleukine-1- $\beta$ , interleukine-6) has also been described in CHF training studies.<sup>63,64</sup>

The few studies that described effects of an exercise intervention on glucose regulation in CHF are summarized in Table 1.2.<sup>65-73</sup> In these studies, sample sizes are relatively small, and methods for assessing glucose metabolism as well as exercise capacity and exercise prescription are very heterogeneous. All studies included endurance training, but training duration varied from 20 to 90 minutes, intensity for endurance training is described in various ways and training frequency varied from 3 to 7 times a week. All studies, except from



Dylewicz et al. and Iellamo et al. included home-based training sessions.<sup>65,74</sup> The greatest training effects were found in 2 studies by Kemppainen and colleagues, using the euglycaemic hyperinsulinaemic clamp technique to assess insulin resistance, but these studies were not randomized and their results could not be confirmed by Sabelis et al.<sup>66,67,71</sup> Furthermore, training studies reporting fasting venous blood samples are inconsistent, with predominantly non-significant effects.<sup>65-67,69-73,75,76</sup>

In summary, although evidence points to a possible beneficial effect on whole body glucose uptake, no conclusive answer can be derived to the question whether exercise is an effective therapy for impaired glucose regulation in CHF patients.

### **Animal models for heart failure and impaired glucose regulation**

Because of the complexity of the clinical picture of human HF with various causes of HF, the multifactorial aspect of the advanced HF syndrome, heterogeneous life courses and various medications, the study of the disease process of acute and chronic HF also requires animal models.

Rodent models for HF are commonly used, because of the extensive knowledge of their genome, homogeneity of study population and reproducible pathological phenotypes. Interestingly, more recent technological advances in echocardiography have greatly facilitated the assessment of cardiac function in rodents, removing a significant barrier to their use in heart failure research.<sup>77,78</sup>

Induced myocardial infarction by coronary ligation in rats has been most widely used during the last decades. Rats with an induced myocardial infarction greater than 45% develop CHF after 3 weeks with elevated left ventricular (LV) filling pressures, reduced cardiac output and a minimal capacity to respond to preload and afterload stress.<sup>79</sup> Although this model mimics ischemic HF and results can carefully be translated to the human situation, it suffers from a high mortality rate within the first hours after the ligation procedure.<sup>80</sup> Another drawback for this model are the high surgical equipment costs and the extensive skills needed for the procedure. Regarding glucose regulation, normal glucose and insulin levels in a fasted state and during oral glucose tolerance testing have been reported in this model.<sup>81,82</sup>

Non-ischemic models include methods to induce pressure overload in rats. Surgical constriction of the ascending aorta in rats causes a gradual onset of myocardial pressure overload, hypertension and myocardial hypertrophy. This gradual process implies that the progression from compensated hypertrophy to decompensated HF can be studied. However, the same drawbacks regarding surgery as described in the ligation model apply here.

Pressure overload can also be induced without surgery: in the Dahl salt-sensitive (Dahl S) rat, hypertension and HF develop gradually when rats are placed on a high salt diet. When fed a high salt diet from the age of 7 weeks, Dahl S rats develop hypertension followed by cardiac hypertrophy with LV relaxation abnormalities and finally by fibrosis, myocardial stiffening and overt HF.<sup>83-85</sup> Interestingly, the combination of HF and impaired glucose regulation is

present in this model.<sup>86</sup> The congenital insulin resistance (decreased insulin-induced glucose uptake compared to the Dahl salt-resistant rat) deteriorates on a high salt diet.<sup>87</sup> Therefore, the Dahl rat provides a suitable model to study the evolution of HF in combination with the evolution of impaired glucose regulation. Finally, when exercise training is started before or simultaneously with the high salt diet in Dahl S rats, it leads to improved survival and can prevent hypertension and HF.<sup>88-91</sup> However, available data are scant regarding the impact of exercise training as a secondary prevention strategy in established hypertension and cardiac hypertrophy in this model.

In conclusion, the Dahl S model seems an appropriate model to study the evolution of diastolic into systolic HF in combination with the evolution of worsening glucose tolerance and additionally, to study the impact of exercise training on heart function and glucose tolerance.

**Table 1.2 Studies describing the effect of exercise intervention on glucose regulation in a HF population**

Publication	Sample size** Patient population NYHA score	Method for assessing glucose metabolism	Duration (weeks)	Resistance training	Effect on glucose uptake	Effect on FPG	Effect on FI	Effect on HbA1c
<b>Dylewicz 2000</b>	20 HFrEF post-CABG	Fasting blood measurements	3	No	ND	↓	↑ ns	ND
<b>Kemppainen 2003</b>	7 vs 7 Idiopathic dilated CMP NYHA 1.4	Euglycaemic hyperinsulinaemic clamp	21	Yes	25% ↑ in whole-body insulin-stimulated glucose uptake (to level comparable with healthy control subjects)	↑ ns	↓ ns	ND
<b>Kemppainen 2003</b>	9 vs 7 Idiopathic dilated CMP NYHA 1.6	Euglycaemic hyperinsulinaemic clamp  PET scan: whole-body and regional glucose uptake	21	Yes	23%↑ in whole-body insulin-stimulated glucose uptake 53%↑ skeletal muscle glucose uptake in the resting muscle 55%↑ skeletal muscle glucose uptake in the exercising muscle	↑ ns	↓ ns	ND
<b>Stolen 2003</b>	8 vs 7 Idiopathic dilated CMP NYHA I-II	Euglycaemic hyperinsulinaemic clamp + Myocardial glucose uptake	21	yes	34 %↑ in insulin-stimulated myocardial glucose uptake	↑ ns	↓ ns	ND
<b>Sabelis* 2004</b>	36 vs 25 HFrEF NYHA II-III	Euglycaemic hyperinsulinaemic clamp  HOMA	26	Yes	Insulin sensitivity ↑ ns HOMA ↑ ns	↑ ns	↑ ns	ND

**Table 1.2 (continued)**

<b>Publication</b>	<b>Sample size** Patient population NYHA score</b>	<b>Method for assessing glucose metabolism</b>	<b>Duration (weeks)</b>	<b>Resistance training</b>	<b>Effect on glucose uptake</b>	<b>Effect on FPG</b>	<b>Effect on FI</b>	<b>Effect on HbA1c</b>
<b>Wislof 2007</b>	9 vs 9 vs 9 HFrEF postinfarct NYHA ND	Fasting blood measurements	12	no	ND	↓ in HIT	ND	ND
<b>Prescott 2009</b>	52 HFrEF NYHA II-III	Fasting blood measurements	8	Yes	ND	↓ ns	↑ ns	No effect
<b>Prescott* 2009</b>	20 vs 23 HFrEF NYHA II-IV	Fasting blood measurements	8 + 12 months	Yes	ND	Smaller ↑ in exercise group	↓ ns	Smaller ↑ in exercise group
<b>Iellamo* 2012</b>	8 ACT vs 8 HIT	Fasting blood measurements	12	no	ND	ACT = HIT ↓ (within group)	ACT ↑ ns HIT ↓ ns	ND
<b>Iellamo* 2013</b>	8 ACT vs 8 HIT	Fasting blood measurements	12	no	ND	ACT ↓ (within group) HIT ↓ (within group)	ACT ↓ (within group) HIT ↓ (within group)	ND

\* randomised controlled trial; \*\* exercise vs usual care; ACT, aerobic continuous training; CABG, coronary artery bypass grafting; CMP, cardiomyopathy; FPG, fasting glucose; FI, fasting insulin; HFrEF, heart failure with reduced ejection fraction; HIT, high intensity interval training; HOMA, homeostasis model assessment; ND: not described, ns: not significant

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## Chapter 2

### Objectives & general outline of the study



The present dissertation focuses on impaired glucose regulation in HF, in particular on the prevalence and nature of impaired glucose regulation, and on the impact of exercise training. Below, the different studies that were conducted are introduced in more detail.

From the general introduction, it has become evident that impaired glucose regulation in HF patients, although of importance for prognosis, often remains undetected. As such, the prevalence of (pre)diabetes is not known in this patient population. The **first objective** of this project was therefore to describe glucose regulation in HF patients.

In the **first study** (Figure 2.1), glucose tolerance was assessed in hospitalized HF patients on the day of discharge. Based on the hypothesis that (pre)diabetes would be the rule rather than the exception in acute HF patients, oral glucose tolerance testing was performed on the day of discharge as a clinical practice for one year. The OGTT was chosen because it is part of the diagnostic tools for (pre)diabetes (proposed by the WHO, ADA and European Society of cardiology), and because it would provide more detailed information compared to the admission and fasting blood glucose reported in available HF literature. While our goal was merely exploratory and descriptive at first, we hypothesized that glucose tolerance would be worse in hospitalized HF patients compared to chronic HF patients or compared to patients hospitalized for an acute coronary event. However, after evaluating the first results, we became interested in the prognostic impact of the different markers of prediabetes as well as T2DM observed in this population. The results of study 1 revealed that impaired glucose regulation is present in an overwhelming majority of our HF population on the day of discharge. Furthermore, our results showed that from all glycaemic markers, increased fasting blood glucose and HbA1c contain little prognostic value, while impaired glucose tolerance (elevated 2h glucose during OGTT) was the better predictor for morbidity (cardio-renal events) and mortality.

In **study 2** (Figure 2.1), the prevalence of impaired glucose regulation and the importance of the different glycaemic markers in the diagnosis of (pre)diabetes were described in patients with stable chronic HF. While the mechanisms and underlying influencing factors for impaired glucose regulation in CHF are complex and are not completely understood, many mechanisms have been suggested on different levels (sympathetic nervous system, skeletal muscle, hormones, inflammation, ...). The **second objective** of this project was to explore the relation between impaired glucose regulation and (1) severity of HF, (2) the intake and dosage of typical medical therapies for HF and (3) body composition and muscle strength in a general stable CHF population. We were particularly interested in the association between glucose regulation and exact measures of body composition (assessed by Dual Energy X-ray Absorptiometry) and muscle strength, in order to investigate exercise-induced changes in these parameters in a later stage of the doctoral project. For this purpose, a large patient group from the HF clinic of the Jessa hospital was contacted, of which 56 CHF patients were willing to participate in our study. As hypothesized, our

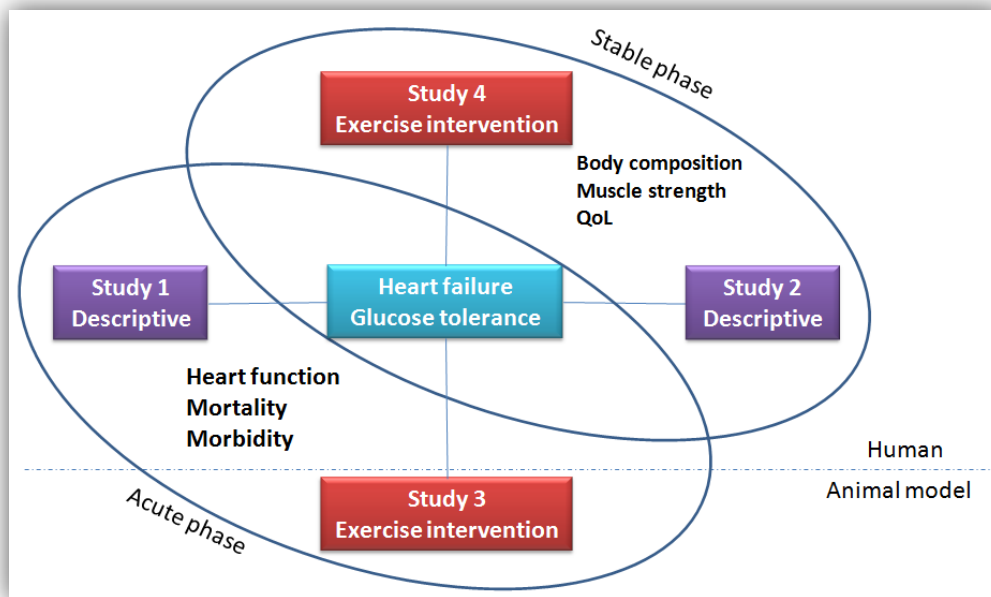
results showed that the prevalence of impaired glucose regulation was higher compared to the general population, with an important role for glucose values 2h after glucose intake in the diagnosis of (pre)diabetes. In our patient population, impaired glucose regulation could not be related to systolic function, nor to HF therapy, but the classic link of glucose intolerance with increasing obesity was confirmed. Interestingly, our results demonstrated a greater importance of fat mass and fat distribution (trunk fat/limb fat) compared to lean mass.

Available literature and official guidelines do not provide effective therapies to improve glucose regulation in HF patients. As illustrated in Table 1.2, the few exercise intervention studies have failed to give a conclusive answer whether exercise training can attenuate impaired glucose regulation in CHF patients. As described in the introduction, the studies investigating the effect of exercise therapy on whole body glucose regulation in CHF differ in patient populations as well as in methodology for training and assessment of glucose regulation, resulting in a variety of outcomes and results. Therefore, the **third objective** was to investigate the impact of exercise therapy on glucose regulation in HF and to explore the influencing parameters. This objective, which was originally the main research goal of this PhD project, was examined in **study 3** (animal model) and in **study 4** (human intervention study).

The effects of exercise therapy on the evolution into (acute) HF and impaired glucose regulation was studied in Dahl salt sensitive rats in **study 3** (Figure 2.1). This animal model for hypertrophic and diastolic HF was chosen because it is a model that does not require invasive procedures to induce HF and because the Dahl salt sensitive rat is also known to grow into insulin resistance. In this experiment, heart function and glucose tolerance were assessed longitudinally and compared between animals receiving a high salt diet and animals receiving a normal salt diet. In addition, mortality as well as fibrosis in cardiac tissue could be compared. The exercise intervention in this study was initiated after the onset of hypertrophy and signs of HF (during the acute phase of the disease), while other studies in this model initiated the exercise intervention before or simultaneous with start of the high salt diet. The exercise intervention was effective in terms of improving heart function and insulin release in response to glucose loading in our animals. However, changes in glucose regulation could not be related to changes in cardiac function. Surprisingly, exercise trained animals on a high salt diet displayed a comparable mortality rate compared to their sedentary counterparts.

In the human experiment (**study 4**, Figure 2.1), the exercise intervention was initiated in stable patients with CHF and was intended as secondary prevention through phase III cardiac rehabilitation. Our hypothesis was that exercise training improves glucose regulation in stable CHF patients. Furthermore, we wanted to investigate whether changes in glucose regulation are related to changes in exercise tolerance, skeletal muscle strength, body composition and cardiac function. The exercise prescription used in our study was consistent with current recommendations, with a combination of endurance and resistance exercises. Regarding the assessment of glucose regulation, the

same methods were used as in the descriptive studies mentioned above (OGTT in combination with HbA1c). Similar to the effects of exercise training observed in our animal model, exercise training decreased insulin release during OGTT in our CHF patient population. In addition, our data showed that changes in insulin concentrations during OGTT were related to changes in left ventricular end-diastolic pressure (E/E') and body composition (waist-to-hip fat mass ratio).



**Figure 2.1 Schematic diagram of the 4 studies**

*QoL, quality of life*



## Chapter 3

### Study 1

#### Glucose tolerance at hospital discharge as a prognostic marker in acute heart failure



Stevens A, Ignoul C, Eijnde BO, Dendale P. Glucose tolerance at hospital discharge as a prognostic marker in acute heart failure. Submitted.

## Abstract

**Background.** Because patients with heart failure (HF) and diagnosed diabetes have a poorer prognosis compared to HF patients without diabetes, it is important to understand the prognostic value of different markers for glycaemic control in this population. This study aimed to investigate the association between different markers of glycaemic control and outcome in hospitalized HF patients on the day of hospital discharge.

**Methods.** Glucose regulation (fasting plasma glucose, HbA1c, and 2h oral glucose tolerance test (OGTT)) was analyzed in combination with patient characteristics and outcome data from 192 patients consecutively hospitalized with new or worsening HF. All cause mortality combined with hospitalization for cardio-renal cause during follow-up was defined as the primary outcome.

**Results.** A history of diabetes was present in 94 patients (32%). From the remaining 98 patients, only 1% was classified as having normal glucose tolerance, 41 (13%) prediabetes and 54 (55%) newly diagnosed diabetes. During the median follow-up time of 470 days, there were 23 deaths (24%) among the previously known diabetic patients, compared to 18 (18%) in the group of (pre)diabetic patients diagnosed by OGTT. 2h glucose was a significant predictor for outcome, with a hazard ratio (95% confidence interval) of 1.08 (1.00-1.17;  $p=0.05$ ) per mmol/L increase while neither fasting plasma glucose nor HbA1c were associated with outcome.

**Conclusions.** The majority of HF patients suffer from impaired glucose regulation on the day of hospital discharge. Elevated 2h glucose during OGTT, but not fasting plasma glucose nor HbA1c, demonstrated a higher risk for worse outcome.

## Background

Diabetes is recognized as an independent predictor of worse prognosis in patients with heart failure (HF).<sup>1</sup> In chronic HF, the reported prevalence of diabetes varies from 8% to 41%.<sup>2</sup> Similarly, in acute HF, the reported prevalence of diabetes during hospital admission varies widely (16-46%).<sup>3</sup> The large variation in prevalence of diabetes in different studies can at least partly be explained by differences in study design and inclusion criteria, geographic/ethnic characteristics of study subjects, and different diagnostic criteria for diabetes.

In non-HF populations it has been shown that the progression from prediabetes (the presence of insulin resistance, hyperinsulinemia, impaired fasting glucose or impaired glucose tolerance) to diabetes can be prevented through lifestyle changes and increased physical activity.<sup>4,5</sup> Therefore, in order to prevent diabetes and its complications in HF, early diagnosis of abnormalities in glucose regulation is important. The World Health Organization and American Diabetes Association recommend the use of fasting glucose, oral glucose tolerance testing and HbA1c in the diagnostic process.<sup>6,7</sup> Specifically for the HF population, the use of a 2 hour oral glucose tolerance test (OGTT) is promoted for diagnosing impaired glucose tolerance.<sup>8-10</sup> However, while most studies in acute HF report admission blood glucose levels<sup>11-16</sup>, only Matsue and colleagues used oral glucose tolerance testing to identify impaired glucose tolerance in relation to adverse events.<sup>17</sup> Besides a dramatic prevalence of impaired glucose regulation (63%) in patients without previously diagnosed diabetes, they reported an increased risk for adverse cardiovascular and cerebral events during follow-up in patients with known diabetes as well as in patients with impaired glucose tolerance.<sup>17</sup> However, they did not include glycated hemoglobin (HbA1c) as a marker of prolonged glycaemic state in their analyses.<sup>4</sup>

We were interested in describing the prevalence of (pre)diabetes as well as the importance of different markers for diagnosis of (pre)diabetes at the end of a hospital stay for acute HF. Moreover, this study aimed at determining the prognostic implications of the different diagnostic markers for (pre)diabetes regarding all-cause mortality and hospitalization for cardio-renal causes.

## Methods

### Subjects and study design

Between September 2012 and September 2013, all patients admitted to Jessa hospital (Hasselt, Belgium) with congestive HF were screened for diabetes and for participation in OGTT. Heart failure was diagnosed according to the European Society of Cardiology criteria.<sup>18</sup> Both patients with new-onset HF and with decompensated chronic HF were eligible for inclusion. Exclusion criteria for inclusion in the study were (1) acute myocardial infarction, (2) malignant disease (cancer) with treatment at the time of admission, (3) missing or unknown vital status. Exclusion for glucose tolerance testing were (1) corticosteroid treatment, (2) cognitive impairment (e.g. dementia), (3) unstable condition with transfer to another department (not included in flowchart) and (4) sudden discharge preventing the execution of the OGTT. Three groups were defined: patients who underwent a glucose tolerance test, patients with ongoing antidiabetic therapy and patients who fulfilled exclusion criteria for glucose tolerance testing. Ethical approval was obtained from the committees of the Jessa hospital and Hasselt University.

### Outcome parameters

Death from any cause combined with the first rehospitalization for cardio-renal causes during follow-up was defined as the primary endpoint. Other endpoints of interest were death, number of rehospitalizations, days spent in rehospitalization and days lost (for reasons of hospitalization and death). Days lost to death was calculated as follows: in case of death before the end of follow-up, the remaining days until the study end were counted as 'days lost to death'. These days were added to the number of days the patient stayed in hospital to obtain 'days lost'.

Data regarding death and hospital admissions were collected by contacting family doctors, by searching hospital files and through an online registry of death announcements from national papers. Follow-up was completed in June 2014.

### Oral glucose tolerance test and blood parameters

A 2 hour OGTT was performed immediately before hospital discharge. Following an overnight fasting period, baseline blood glucose (in fluoride-oxalate tubes) and HbA1c (in EDTA tubes) were determined via a venous blood sample. Hereafter, 75g glucose (B. Braun Melsungen AG, Melsungen, Germany) dissolved in 250mL water was ingested and venous blood samples were taken for blood glucose analysis at 30, 60, 90 and 120min. Plasma glucose was determined with an Olympus AU analyzer (Beckman Coulter, Switzerland) and HbA1c with Hi-AutoA1C Analyzer (Menarini Diagnostics, Italy). Combining the results of OGTT and HbA1c, patients were classified as having normal glucose

tolerance (NGT), prediabetes or diabetes according to the diagnostic criteria of the American Diabetes Association (see Table 3.1).<sup>4</sup>

**Table 3.1 Criteria used for glucometabolic classification**

	<b>NGT</b>	<b>Prediabetes</b>	<b>Diabetes</b>
Fasting glucose OR 2-hour glucose OR HbA1c	<5.6 mmol/L	5.6-6.9 mmol/L	≥7.0 mmol/L
	<7.8 mmol/L	7.8-11.0 mmol/L	≥11.1 mmol/L
	<5.7%	5.7-6.4%	≥6.5%

*NGT, normal glucose tolerance.*

### **Patient characteristics**

Clinical data, including body mass index (BMI), history of ischaemic heart disease (IHD) and left ventricular ejection fraction (LVEF) at the end of hospital admission were retrieved from hospital records.

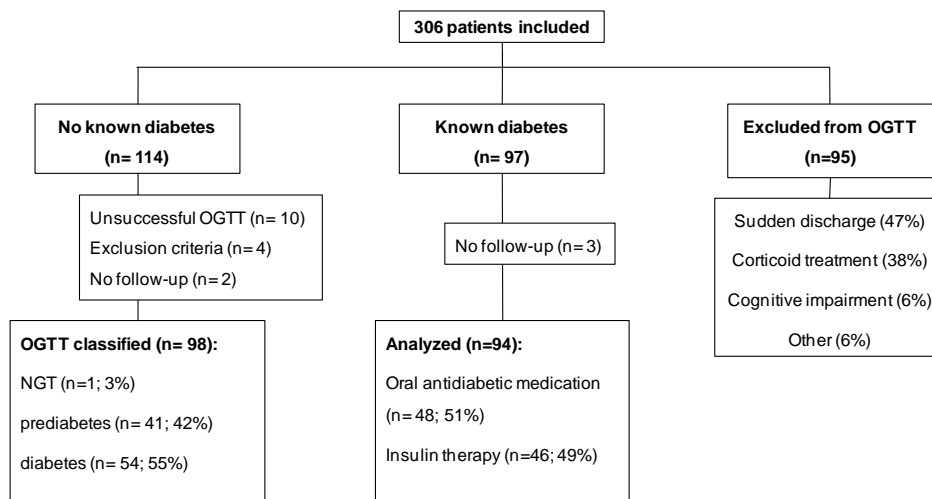
### **Statistics**

Statistical analyses were performed using SAS Enterprise Guide 4.3 and SAS 9.2 (SAS Institute Inc.) software. Patient characteristics were compared using unpaired t-tests or one-way analysis of variance (ANOVA) with Bonferroni post-hoc analysis for continuous variables and Fisher's exact test for categorical variables. The distributions of number of hospitalizations, days spent in hospital and total days lost were skewed, and therefore non-parametric ANOVA was used for these variables. Event free survival curves were constructed using the Kaplan-Meier method; differences were tested using Wilcoxon statistics because of crossing curves. Univariate and multivariate Cox proportional hazard analyses were performed to determine the independent predictors of survival and event free time. In addition to glucometabolic parameters, the following variables were tested for possible association with survival and event free time: age, gender, BMI, IHD and LVEF. Parameters significant to  $p < 0.1$  in univariate analysis were entered in the multivariate model.

Results are presented as mean  $\pm$  one standard deviation. All tests were two-sided with a P-value of 0.05 as the threshold for statistical significance.

## Results

A patient flow diagram is presented in Figure 3.1. Among 306 patients admitted with acute HF in the study period, 32% were taking anti-diabetic medication and 31% were excluded from glucose tolerance testing. Among 114 patients who underwent OGTT, ten patients could not be classified because of discomfort during glucose load (n=5) or missing results from blood analyses (n=5). From two patients in the OGTT group and three patients in the diabetic group, no follow up data after hospitalization were available. Finally, analyses were performed on 98 patients who underwent OGTT and 94 patients with known diabetes.



**Figure 3.1 Patient flow diagram**

The total cohort (n=192) had a mean age of  $74 \pm 11$  years old and consisted of 54% male patients (Table 3.2). Furthermore, mean LVEF was  $44 \pm 15\%$  with 50% of patients having a LVEF under 45%, indicating an equal proportion of patients with reduced and preserved systolic function. In patients undergoing OGTT, the median hospital duration was 7 days (range 1-109 days), compared to 9 days (range 1-70 days) in patients previously diagnosed with diabetes ( $p > 0.05$ ). According to ADA criteria, only 3 patients showed NGT, 41 patients (42%) were classified as prediabetic and 54 patients (55%) were classified as diabetic. Baseline patient characteristics of the study population stratified by glucometabolic state are presented in Table 3.2. Age, sex, BMI, LVEF and proportion of patients with IHD were comparable between patients classified in different ADA groups based on OGTT. In contrast, patients with prior diagnosis of diabetes were distinguished from (pre)diabetic patients undergoing OGTT by higher BMI and a larger proportion of patients with IHD.

**Table 3.2 Patients characteristics according to glucometabolic state**

	<b>Total group</b>	<b>NGT</b>	<b>Prediabetes</b>	<b>Diabetes</b>	<b>P*</b>	<b>Known diabetes</b>	<b>P**</b>
	<b>n=192</b>	<b>n=3</b>	<b>n=41</b>	<b>n=54</b>		<b>n=94</b>	
<b>Age (years)</b>	74 ± 11	66 ± 14	74 ± 14	78 ± 10	0.15	73 ± 11	0.09
<b>Sex (% male)</b>	103 (54%)	3 (100%)	21 (51%)	26 (48%)	0.33	53 (56%)	0.37
<b>BMI (kg/m<sup>2</sup>)</b>	28.1 ± 5.2	24.5 ± 1.9	26.1 ± 4.0	26.7 ± 4.6	0.60	29.8 ± 5.6	<b>0.0001</b>
<b>Aetiology (IHD, %)</b>	96 (50%)	1 (33%)	14 (34%)	24 (44%)	0.64	57 (61%)	<b>0.02</b>
<b>LVEF (%)</b>	44 ± 15	34 ± 23	44 ± 15	43 ± 14	0.49	45 ± 16	0.56

*NGT, normal glucose tolerance; BMI, body mass index; IHD, ischemic heart disease; LVEF, left ventricular ejection fraction. Continuous variables are presented as mean ± SD, categorical variables as number and percentage. \* denotes  $p < 0.05$  in the comparison of 3 groups with OGTT; \*\* denotes  $p < 0.05$  in the comparison of 3 groups with OGTT and patients with known diabetes.*

### Importance of OGTT in the diagnosis of prediabetes and diabetes

Mean fasting plasma glucose (FPG) from patients with a successful OGTT was  $6.0 \pm 0.7$  mmol/L, with 25% of FPG values in the normal range. Similarly, mean HbA1c was  $5.8 \pm 0.4\%$ , with 28% of HbA1c values in the normal range. As illustrated in Table 3.3, if an OGTT had not been performed, two patients in the prediabetic group (5%) and the majority of patients in the diabetic group (72%) would have been misclassified based on FPG combined with HbA1c.

**Table 3.3 Importance of 2h glucose values during OGTT for glucometabolic classification**

	Prediabetes n=41	Diabetes n=54
<b>FPG</b>	1 (2%)	1 (2%)
<b>FPG + 2hPG</b>	3 (7%)	5 (9%)
<b>FPG + Hba1c</b>	7 (17%)	-
<b>FPG + 2hPG + HbA1c</b>	15 (37%)	5 (9%)
<b>2hPG</b>	2 (5%)	39 (72%)
<b>2hPG + HbA1c</b>	10 (24%)	3 (6%)
<b>HbA1c</b>	3 (7%)	1 (2%)

*FPG, fasting plasma glucose; 2hPG, 2h plasma glucose during OGTT. Figures represent the amount of patients (%) fulfilling a certain combination of diagnostic markers for prediabetes or diabetes.*

### Outcome

The median (interquartile range) follow-up time in survivors was 470 days (384-546 days).

#### **Mortality combined with readmission for cardio-renal cause**

In the group of patients with previously diagnosed diabetes, 23 (24%) died during follow-up, compared with 18 (18%) in the group with no prior diagnosis of diabetes ( $p=0.13$ ). When mortality data were combined with (re)hospitalization for cardio-renal cause, Kaplan-Meier curves for the 4 groups (3 ADA groups and previously diagnosed diabetics) were comparable ( $p=0.63$ , Figure 3.2A). Also when focusing on prediabetic patients and newly diagnosed diabetic patients, there was no significant difference ( $p=0.17$ ). Looking into the separate markers for glucometabolic diagnosis, 2h plasma glucose during OGTT (2hPG) was the best predictor for outcome ( $p=0.17$ ; Figure 3.2B) compared to FPG ( $p=0.66$ ) and HbA1c ( $p=0.72$ ).



Univariate analyses of hazard ratio showed that 2hPG was a near-significant predictor for events with an increased risk of 8% per mmol increase in 2hPG ( $p=0.05$ ; Table 3.4). Further analyses searching for confounding factors revealed that age was an important confounder, next to history of IHD, while sex, BMI and LVEF were not. The first multivariate model containing 2hPG, age and IHD revealed that only age remained an independent predictor. The second model, containing only 2hPG ( $p=0.34$ ) and age ( $p=0.01$ ) confirmed that no significant relationship remained between 2hPG and mortality and readmission for cardio-renal causes.

### **Mortality**

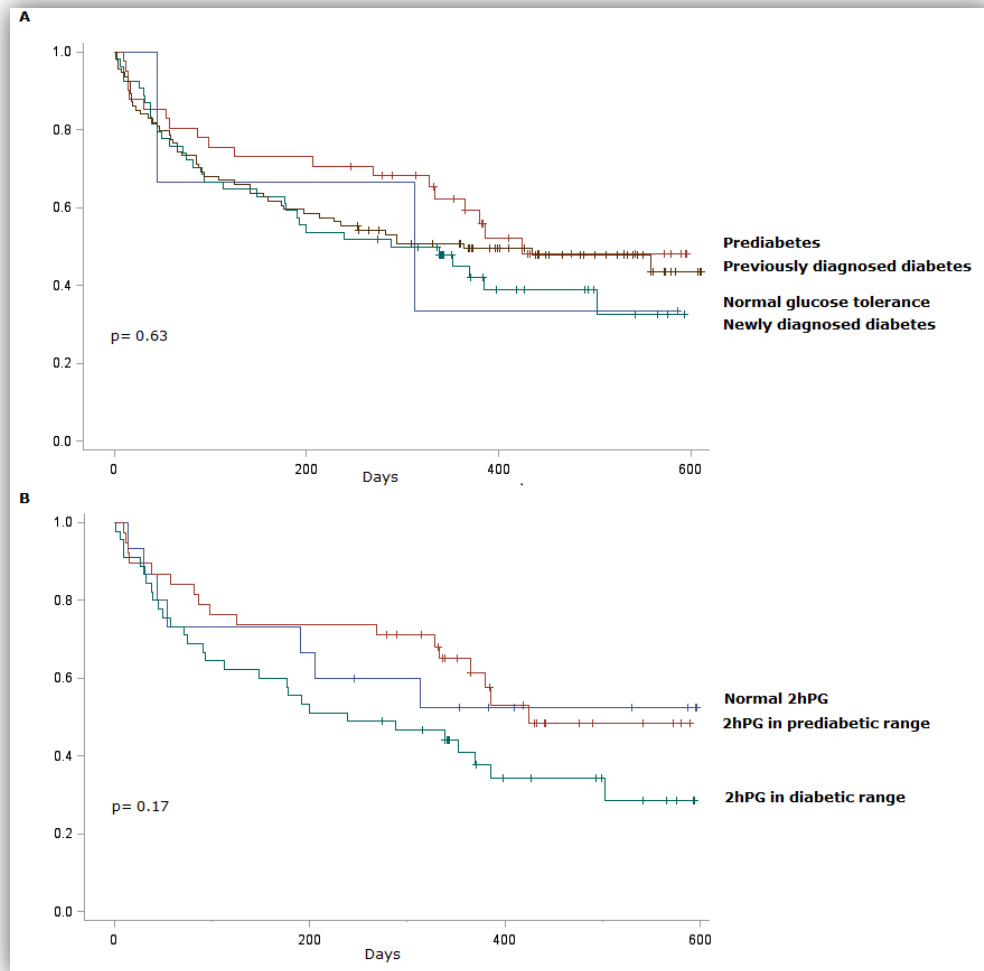
Figure 3.3A shows the Kaplan-Meier survival curves for patients stratified by ADA classification and prior diagnosis of diabetes. Although some trends are visualized (early mortality seems to be higher in patients with prior diagnosis of diabetes and late mortality seems to be higher in patients with newly diagnosed diabetes), these were not statistically significant ( $p=0.38$ ). Interestingly, there was no mortality in the group of 15 patients with normal 2hPG (Figure 3.3B) and patients with 2hPG in the diabetic range seem to have a worse outcome. However, differences were not significant ( $p=0.17$ ).

Similar to the primary outcome summarized above, univariate analyses investigating the predictive effect of glucometabolic parameters showed that only 2hPG was a predictor for mortality with hazard ratio (95% Confidence Interval)= 1.19 (1.04-1.37;  $p=0.01$ ). However, after model building to investigate the independent effects, the influence of age (hazard ratio= 1.06 (1.02-1.10);  $p<0.01$ ) reduced the significance of 2hPG ( $p=0.09$ ) as a predictor for mortality.

From the group of patients with no previous diagnosis of diabetes, survivors were younger during hospitalization compared to deceased patients ( $74\pm 13$  vs  $83\pm 6$  years old,  $p<0.05$ ), while BMI and LVEF were comparable. Surprisingly, FPG was higher in survivors ( $6.0\pm 0.7$  vs  $5.7\pm 0.5$  mmol/L,  $p<0.05$ ) and HbA1c was equal in both groups ( $5.8\pm 0.4$  vs  $5.8\pm 0.4\%$ ,  $p>0.05$ ). Again, 2hPG was able to demonstrate a difference between survivors and non-survivors ( $10.7\pm 3.2$  vs  $12.5\pm 3.2$  mmol/L,  $p<0.05$ ).

### **Rehospitalizations and days lost**

The median of the number of rehospitalizations for all reasons of the total group was one (range: 0-9), and was not different between groups. However, patients newly diagnosed with diabetes spent more days in hospital (7 [0-126]) compared to prediabetic patients (2 [0-42];  $p<0.05$ ). Furthermore, there was a trend towards more days lost in the former group (11 [0-614] vs 2 [0-499],  $p=0.07$ ).



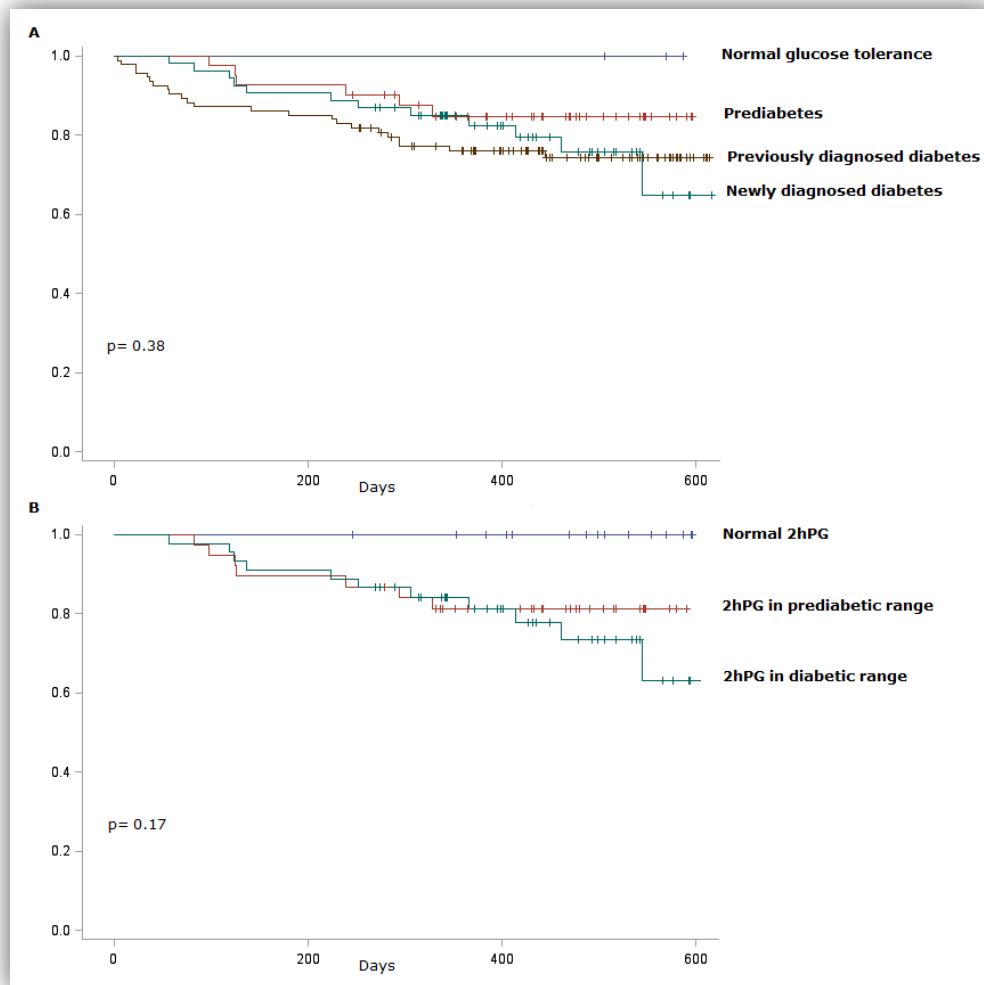
**Figure 3.2** Kaplan-meier curves for survival combined with freedom from cardiorenal hospitalization according to general ADA classification (A) and classification based on 2h glucose during OGTT only (B)

*2hPG, 2h glucose value during OGTT; | denotes a censored value*

**Table 3.4. Hazard ratios for all-cause death and CR hospitalization**

Predictor	Hazard Ratio (95% Confidence Interval)	P value
<b>According to ADA classification</b>		
<b>ADA classification</b> Prediabetes vs newly found diabetes	1.50 (0.85-2.65)	0.16
<b>Impaired fasting glucose</b> per classification level increase		0.60
<b>Impaired glucose tolerance</b> per classification level increase		0.16
<b>Elevated HbA1c</b> per classification level increase		0.59
<b>Glucometabolic parameters</b>		
<b>0 min glucose</b> per mmol/L increase	0.83 (0.55-1.26)	0.39
<b>30 min glucose</b> per mmol/L increase	1.01 (0.86-1.19)	0.88
<b>60 min glucose</b> per mmol/L increase	1.03 (0.93-1.14)	0.58
<b>90 min glucose</b> per mmol/L increase	1.04 (0.96-1.14)	0.32
<b>120 min glucose</b> per mmol/L increase	<b>1.08 (1.00-1.17)</b>	<b>0.05</b>
<b>HbA1c</b> per 0.1% increase	1.01 (0.99-1.02)	0.44
<b>Confounding factors</b>		
<b>Age</b> per year increase	<b>1.04 (1.02-1.06)</b>	<b>&lt;.0001</b>
<b>Male sex</b>	1.33 (0.90-1.97)	0.16
<b>BMI</b>	0.98 (0.94-1.02)	0.30
<b>History of IHD</b>	<b>1.59 (1.07-2.36)</b>	<b>0.02</b>
<b>LVEF</b> per 5% increase	1.01 (0.95-1.08)	0.75
<b>Multivariate models</b>		
<b>120 min glucose + Age + IHD</b>		
120 min glucose	1.04 (0.95-1.13)	0.40
Age	<b>1.04 (1.01-1.07)</b>	<b>0.02</b>
IHD	1.32(0.76-2.29)	0.33
<b>120 min glucose + Age</b>		
120 min glucose	1.04 (0.96-1.13)	0.34
Age	<b>1.04 (1.01-1.07)</b>	<b>0.01</b>

*BMI, body mass index; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.*



**Figure 3.3** Kaplan-meier curves for survival according to general ADA classification (A) and classification based on 2h glucose during OGTT only (B)

*2hPG, 2h glucose value during OGTT; | denotes a censored value*

## Discussion

Our results showed that impaired glucose regulation at hospital discharge, is omnipresent and underrecognized in patients admitted with acute HF. Furthermore, this study indicates that from all three markers for diagnosis of (pre)diabetes (fasting glucose, 2h glucose during OGTT and HbA1c), elevated 2h glucose during OGTT is the better predictor for mortality combined with rehospitalization for cardio-renal causes.

### Glucose regulation is severely disturbed in acute heart failure

In our study population, the prevalence of diabetes was 32% based on glucose-lowering therapy and known history in patient files, and was increased to 49% when the results of the OGTT and HbA1c were taken into account. A further 13% was identified as prediabetic and an insignificant proportion of patients undergoing OGTT showed normal glucose tolerance. It is safe to assume that glucose regulation is also impaired in a large proportion of the patients excluded for OGTT in this study, because this group also contains patients with corticosteroid treatment, which is known to increase glucose values.<sup>23</sup> The prevalence of impaired glucose regulation in our patient population is clearly higher compared to patients hospitalized with an acute coronary event: from 164 patients undergoing OGTT on the day of discharge in the study of Norhammar et al, classification of normal glucose tolerance-prediabetes and diabetes was assigned in 34%, 35% and 31% respectively.<sup>24</sup> Furthermore, the proportion of patients with newly diagnosed diabetes in the present patient population (55% of patients undergoing OGTT) is increased when compared to patients with stable chronic HF, as described by Egstrup et al (18%) and by our own research group (25%).<sup>8,10</sup> In comparison, in the study of Matsue et al, the OGTT on the day of discharge revealed only 9% newly diagnosed diabetics and 34% prediabetic patients. The use of the stringent ADA criteria in our study can probably partly account for the differing results.

We showed that the OGTT had a significant contribution in the diagnosis of diabetes, as the majority of the newly diagnosed diabetic patients were identified because of glucose values after glucose loading. This finding is comparable to studies in chronic HF patients.<sup>8,10</sup> Glycated hemoglobin in particular showed limited sensitivity for diagnosis of diabetes in the context of this study, which could mean that the disturbed glucose regulation is an acute reaction on illness and stress from hospitalization which is not yet reflected in this marker of prolonged glycaemia.

Elevated glucose levels at the end of a hospital admission for acute HF can have several reasons, of which three are highlighted below. (1) Hyperglycaemia and other abnormal metabolic factors are commonly found in seriously ill patients, caused by a highly complex interplay of counter-regulatory hormones such as catecholamines, growth hormone, cortisol, and cytokines.<sup>25</sup> In this respect, it is a question of debate whether elevated glucose values are

deleterious and increase mortality, or if they are rather another marker of a serious disease. (2) It is known that diuretic therapy is associated with hyperglycaemia.<sup>26</sup> Therefore, increased glucose values in acute HF patients could also be the result of the intensive diuretic therapy to relieve congestion during their hospital stay. (3) In our study, as well as in the study of Matsue et al<sup>17</sup>, patients were tested for glucose tolerance at the end of their hospital stay, which is a period of severely reduced physical activity or worse, immobilization. Hamburg and colleagues showed that even a short period of 5 days bed rest can induce insulin resistance in healthy subjects. This was illustrated by a 67% increase in the insulin response to glucose loading as well as increased glucose curves during OGTT.<sup>27</sup> Possibly, the insulin resistance resulting from bed rest during HF admissions is even worse, as the median length of hospital stay in our study was 7 days in patients undergoing OGTT and 9 days in patients previously diagnosed with diabetes. This figure is concordance with the EuroHeart Failure Study II, where the median duration of hospitalization was 9 days.<sup>28</sup> In order to prevent worsening of insulin resistance induced by bed rest during hospital admissions for HF, early mobilization should be even more emphasized and promoted.

### **Outcome**

Our data show that increased 2hPG is associated with an adverse prognosis, while FPG and HbA1c values were not able to identify patients at risk. This effect was more apparent on a continuous scale, as opposed to comparison of patients with normal-prediabetic-diabetic 2hPG values on a categorical scale. With every increase of 1 mmol/L in 2hPG, mortality risk combined with hospitalization for cardio-renal causes increased with 8% and risk for mortality with 19%. The study of Matsue and colleagues was similar to the present study in respect to patient population (inclusion of acute HF patients with reduced as well as preserved systolic dysfunction in the same age range) and assessment of glucose regulation at the day of discharge.<sup>17</sup> However, our data could not confirm the results of Matsue et al, who reported a 3-4 fold higher risk in patients classified as glucose intolerant.<sup>17</sup> Conversely, literature reporting the prognostic impact of non-fasting glucose levels at the start of the hospitalization period does not provide an unequivocal answer. A large multicentre trial showed a powerful association between admission glucose and short term (30 day) mortality.<sup>15</sup> Barsheshet and colleagues reported similar results for 60 day mortality.<sup>11</sup> Two other studies concluded that admission glucose was also an important predictor for long-term prognosis in acute HF.<sup>13,16</sup> On the other hand, the largest study of Kosiborod et al<sup>14</sup>, which included more than 50,000 elderly patients hospitalized with HF, found no association between admission glucose levels and mortality after 30 days and 1 year of follow-up. A more recent study of Barsheshet showed similar results.<sup>12</sup> As admission glucose levels reflect the glycaemic state during acute and critical illness, these findings cannot directly be compared to our findings from the time of discharge.

The predictive effect of 2hPG disappeared when corrected with age in our study population. The influence of older age on increasing short-and long term mortality has been shown by Gustafsson et al.<sup>19</sup> In addition, age has been described as an independent predictor of mortality in studies investigating the prognostic impact of known diabetes<sup>11-13,20</sup>, and admission glucose<sup>11,12,15</sup>. However, it is also known that glucose regulation is impaired in the elderly without HF, through a combination of decreased insulin secretion and increased insulin resistance.<sup>21</sup> Furthermore, in contrast with the results of Dries and colleagues in a chronic HF patient population, the negative influence of ischemic heart disease on event rate also disappeared in our multivariate model with age as the most powerful predictor.<sup>22</sup>

While an increased risk for worse outcome was described for elevated 2hPG, but not for FPG nor for HbA1c, the clinical relevance of this finding is not clear. Especially because the predictive effect was reduced when age was taken into account, our results do not promote the use of the OGTT when looking for prognostic parameters. However, it is clear that clinical follow-up is necessary in patients with elevated 2hPG in order to prevent worsening of glucose regulation as well as comorbidities associated with diabetes mellitus.

### **Limitations**

Our study did not include some factors that could add information to our predictive model. First, comprehensive echocardiographic parameters were not available for all patients. Second, severity of HF was not illustrated with BNP values, because they were not available at the moment of glucose tolerance testing.

### **Conclusions**

The majority of HF patients suffer from impaired glucose regulation on the day of hospital discharge. From all three markers for diagnosis of (pre)diabetes (fasting plasma glucose, 2h plasma glucose during OGTT and HbA1c), elevated 2h plasma glucose during OGTT is the better predictor for mortality combined with rehospitalization for cardio-renal causes.

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## Chapter 4

### Study 2

#### **Mandatory oral glucose tolerance tests identify more diabetics in stable patients with chronic heart failure: a prospective observational study**



Stevens A, Hansen D, Vandoren V, Westerlaken R, Creemers A, Eijnde BO, Dendale P. Mandatory oral glucose tolerance tests identify more diabetics in stable patients with chronic heart failure: a prospective observational study. *Diabetol. Metab Syndr.* (2014), 6: pp.44-51.

## Abstract

**Background.** Many patients with chronic heart failure (CHF) are believed to have unrecognized diabetes, which is associated with a worse prognosis. This study aimed to describe glucose tolerance in a general stable CHF population and to identify determinants of glucose tolerance focusing on body composition and skeletal muscle strength.

**Methods.** A prospective observational study was set up. Inclusion criteria were diagnosis of CHF, stable condition and absence of glucose-lowering medication. Patients underwent a 2 h oral glucose tolerance test (OGTT), isometric strength testing of the upper leg and dual energy x-ray absorptiometry. Health-related quality of life and physical activity level were assessed by questionnaire.

**Results.** Data of 56 participants were analyzed. Despite near-normal fasting glucose values, 55% was classified as prediabetic, 14% as diabetic, and 20% as normal glucose tolerant. Of all newly diagnosed diabetic patients, 79% were diagnosed because of 2 h glucose values only and none because of HbA1c. Univariate mixed model analysis revealed ischaemic aetiology, daily physical activity, E/E', fat trunk/fat limbs and extension strength as possible explanatory variables for the glucose curve during the glucose tolerance test. When combined in one model, only fat trunk/fat limbs and E/E' remained significant predictors. Furthermore, fasting insulin was correlated with fat mass/height<sup>2</sup> ( $r=0.51$ ,  $p<0.0001$ ), extension strength ( $r=-0.33$ ,  $p=0.01$ ) and triglycerides ( $r=0.39$ ,  $p<0.01$ ).

**Conclusions.** Our data confirm that a large majority of CHF patients have impaired glucose tolerance. This glucose intolerance is related to fat distribution and left ventricular end-diastolic pressure.

## Background

Chronic heart failure (CHF) is a system disease. Apart from cardiac failure, the clinical picture involves pulmonary, renal, hepatic and skeletal muscle abnormalities.<sup>1</sup> In addition, diabetes mellitus type 2 is frequently found, with percentages varying from 8-41%.<sup>2</sup> Although the suspicion arises that impaired glucose tolerance is the rule rather than the exception in this population, its exact prevalence is not known.<sup>2,3</sup>

In CHF patients with reduced systolic function, left ventricular ejection fraction (LVEF) and aetiology of CHF have been described as predictive factors for insulin sensitivity.<sup>4-9</sup> Furthermore, typical CHF medical therapies, i.e. ACE inhibitors,  $\beta$ -blockers and thiazides are believed to influence glucose tolerance.<sup>10-14</sup>

Although a higher body mass index is associated with impaired glucose tolerance and diabetes, it is also associated with better survival in CHF.<sup>15</sup> When dividing body weight into fat mass and lean mass, it could be hypothesized that a higher fat mass leads to overall detrimental effects, while a higher lean mass is associated with reduced catabolism and beneficial effects in CHF.<sup>16</sup> In addition, a higher muscle mass could lead to elevated glucose uptake. Precise measurement of body composition would therefore provide new insights in the relation between body composition and glucose tolerance in CHF.

As skeletal muscle strength is also an independent predictor for survival in CHF, it could be hypothesized that not only the quantity but also the quality of skeletal muscle plays an important role.<sup>17</sup> Still, skeletal muscle function has not been investigated in relation to glucose tolerance in CHF yet.

Following this line of reasoning, the present study aims to describe glucose tolerance in relation to (a) severity of heart failure, (b) the intake and dosage of typical CHF medical therapies and (c) body composition and skeletal muscle strength in a heterogeneous group of stable CHF patients.

## Methods

### Subjects

Patients diagnosed with CHF were recruited from the heart failure clinic of the Jessa hospital (Hasselt, Belgium). Inclusion criteria were (1) a history of CHF of at least 6 months and (2) clinically stable for more than 3 months prior to the onset of the study. Known diabetes with glucose lowering therapy, engagement in phase III rehabilitation in a hospital setting and other chronic diseases (pulmonary disease, end-stage renal disease, cancer) were the exclusion criteria. Based on a previous study, sample size was estimated on 60 patients.<sup>7</sup> All patients gave their written informed consent. Ethical approval of the study was obtained from the committees of the Jessa hospital and Hasselt University. The investigation conforms with the principles outlined in the Declaration of Helsinki.

### Study design

In this prospective observational study, patients underwent a 2 h oral glucose tolerance test (OGTT), had a late breakfast and muscle strength and body composition were assessed on a single test day. During the OGTT, health-related quality of life and physical activity level questionnaires were completed and current medical therapy was registered.

### Oral glucose tolerance test and blood parameters

Following an overnight fasting period, baseline blood glucose and insulin concentrations as well as blood lipids, HbA1c and B-type Natriuretic Peptide (BNP) were determined via a venous blood sample. Hereafter, 75 g glucose (Merck KGaA, Darmstadt, Germany) dissolved in 250 mL water was ingested and 1- and 2 hours blood samples were taken for glucose and insulin analysis. Blood samples for glucose and insulin (in serum separation tubes) and BNP (in EDTA tubes) were maintained at room temperature for 30 min, centrifuged, and the collected serum and plasma were frozen at  $-80^{\circ}\text{C}$  until analysis. Blood samples for lipids (in lithium heparin tubes) and HbA1c (in EDTA tubes) were processed on the test day. Glucose, total cholesterol and HDL cholesterol were determined with an Olympus AU analyzer (Beckman Coulter, Switzerland), insulin and BNP with ADVIA Centaur (Siemens Medical Solutions Diagnostics, Germany) and HbA1c with Hi- Auto A1C Analyzer (Menarini Diagnostics, Italy). Serum glucose was converted to plasma glucose using the following formula:  $\text{plasma glucose (mmol/L)} = -0.137 + (1.047 * \text{serum glucose (mmol/L)})$ .<sup>18</sup> Subjects were divided into 3 groups according to their glucometabolic state as recommended by the American Diabetes Association (see Table 5.1).<sup>19</sup> Reference values for insulin during OGTT were 3-28 mU/L at fasting state, 29-88 mU/L 1h after glucose load and 22-79 mU/L 2h after glucose load.<sup>20</sup>

**Table 5.1** Criteria used for glucometabolic classification

	NGT	Prediabetes	Diabetes
<b>Fasting glucose</b>	<5.6 mmol/L	5.6-6.9 mmol/L	≥7.0 mmol/L
OR			
<b>2-hour glucose</b>	<7.8 mmol/L	7.8-11.0 mmol/L	≥11.1 mmol/L
OR			
<b>HbA1c</b>	<5.7%	5.7-6.4%	≥6.5%

NGT= normal glucose tolerance.

### Muscle strength

Maximal voluntary unilateral strength of the upper leg was evaluated in a seated position on an isokinetic dynamometer (System 3; Biodex Medical Systems, New York, USA). The rotational axis of the dynamometer was aligned with the transverse knee joint axis and connected to the distal end of tibia. Subjects performed 2 maximal isometric knee extensions and flexions at knee angles of 45° and 90°. Maximal contractions (4 s) were interspersed by 30 s rest intervals. The highest isometric extension and flexion torques (Nm) at each knee angle were selected as peak torque. Maximal strength was expressed as peak torque relative to lean tissue of the right leg.

### Body composition

To determine body composition, a Dual Energy X-ray Absorptiometry scan (Hologic, Vilvoorde, Belgium) was performed. Fat tissue mass and lean tissue mass were obtained for the whole body and for the following separate regions: legs, trunk, gynoid and android region. From these findings, the following indices were calculated: waist-to-hip fat mass ratio (android fat (g)/gynoid fat (g) ratio), fat of the trunk / fat mass of the limbs ratio.

### Medical history and echocardiography

Hospital records were retrospectively reviewed for aetiology of heart failure (ischaemic versus non-ischaemic), and for LVEF in the most recent echocardiography. In 25 patients, echocardiography was performed in the month following OGTT, with determination of E/E'.

### Health related quality of life and physical activity

The EQ-5D was used to evaluate health related quality of life. It is a standardized, non-disease-specific instrument for describing and valuing health, which is limited in length (5 short questions and a visual analogue scale).<sup>21,22</sup>

Daily physical activity was assessed using the International Physical Activity Questionnaire.

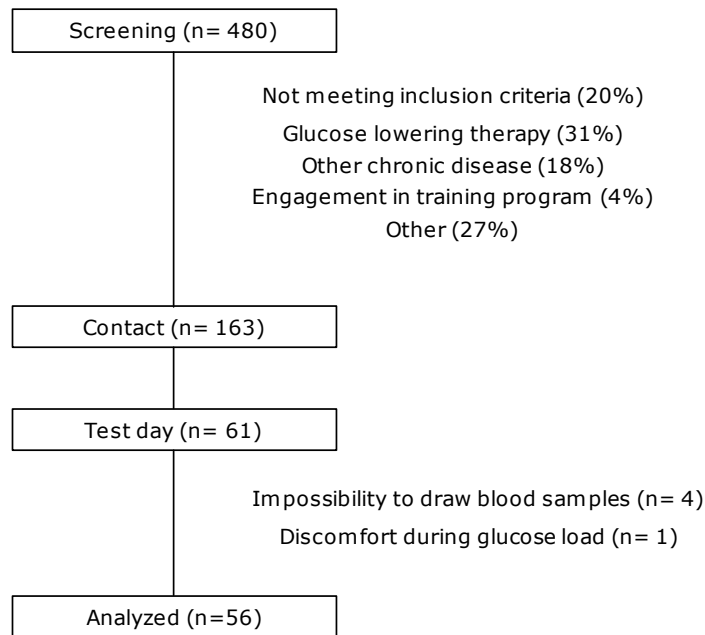
### **Statistical analysis**

Statistical analyses were performed using SAS Enterprise Guide 4.3, SAS 9.2 (SAS Institute Inc., Cary, NC) and R2.10.1 software. All measures are presented as mean  $\pm$  SD. Continuous data were compared using nonparametric one way ANOVA and *post hoc* multiple comparison procedures were performed using Wilcoxon rank sum test with Bonferroni correction. Categorical data were compared using Fisher exact test. Because the glucose curve consists of longitudinal data (3 time points for each patient), relations between the glucose curve and other patient characteristics (explanatory variables) were investigated with mixed model analysis. Multiple imputation (number of imputed datasets = 5) was performed for E/E' based on the glucose levels during OGTT.<sup>23</sup> Bivariate correlation (Spearman) was performed between fasting insulin and predictive variables. All tests were two-sided with a P-value of 0.05 as threshold for statistical significance.



## Results

From March 2011 to March 2012, a total of 480 patients were screened. A patient flow diagram is presented in Figure 5.1.



**Figure 5.1 Patient flow diagram**

### Oral glucose tolerance test

Despite near-normal mean fasting glucose in the total group ( $5.7 \pm 0.6$  mmol/L), 14 patients (25%) were classified as having newly diagnosed diabetes, 31 (55%) as having prediabetes and 11 (20%) as having NGT. Overt diabetes was diagnosed because of 2 h glucose values only in 79% of patients, while only 3 patients could be diagnosed because of fasting glucose values and none because of HbA1c (Table 5.2). Glucose and insulin curves during OGTT are presented in Figure 5.2. In all 3 groups, mean fasting insulin levels were in the normal range but increased above reference values 1 h after glucose load. Two hours after glucose load, insulin curves did not decline in prediabetics and diabetics.

### Patient characteristics and body composition

Table 5.3 shows patient characteristics of the 3 groups. Differences in New York Heart Association class distribution and BNP values between groups were not significant. Medical treatment, LVEF, age, gender, whole body weight and fat mass were comparable between groups. Fat distribution expressed as fat trunk / fat limb ratio tended to be higher in diabetics compared to prediabetics and NGT ( $p < 0.03$ ). However, these differences did not reach significance after Bonferroni correction.

### **Muscle strength**

Maximal isometric strength was comparable between groups.

### **Health related quality of life and physical activity**

The total score of the EQ5D was similar in all 3 groups (data not shown), as well as overall self-rated health status (NGT:  $7.0 \pm 1.0$ , prediabetics:  $6.7 \pm 1.9$ , diabetics:  $6.4 \pm 2.0$ ;  $p = 0.90$ ). Also reported daily physical activity did not differ between groups.

### **Contributors to glucose tolerance**

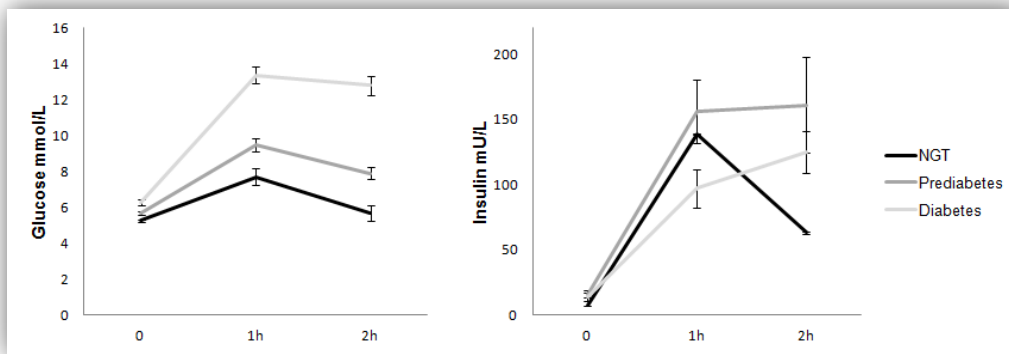
Univariate mixed model analysis revealed 6 possible explanatory variables for the glucose values during OGTT: ischaemic aetiology, daily physical activity, E/E', android/gynoid fat ratio, fat trunk /fat limbs and knee extension strength at 90°. Android/gynoid fat ratio was excluded from the final model building because of interference with the other fat distribution variable. When the remaining 5 variables were combined in one model with random intercept, and after manual backward selection procedure, fat distribution showed a main effect for overall glucose values. Furthermore, E/E' and fat distribution also showed an interaction effect with glucose curve over time. Multiple imputation analysis produced the same results.

Because insulin release after glucose loading was decreased in the diabetic group, and the insulin curves therefore did not follow the same increasing trend as the glucose values, mixed model analysis was not performed for the insulin curves. Instead, fasting insulin was found to correlate moderately with body mass index ( $r = 0.51$ ,  $p < 0.0001$ ) and fat mass/height<sup>2</sup> ( $r = 0.51$ ,  $p < 0.0001$ ). Fasting insulin was also slightly related with total body fat (%;  $r = 0.49$ ,  $p = 0.0001$ ), triglycerides ( $r = 0.39$ ,  $p < 0.01$ ) and quadriceps strength at 45° ( $r = -0.33$ ,  $p = 0.01$ ).

**Table 5.2 Importance of 2h glucose values during OGTT for glucometabolic classification**

	<b>Prediabetes</b> <b>n=31</b>	<b>Diabetes</b> <b>n=14</b>
<b>FPG</b>	5 (16%)	1 (7%)
<b>FPG + 2hPG</b>	4 (13%)	2 (14%)
<b>FPG + Hba1c</b>	6 (19%)	-
<b>FPG + 2hPG + HbA1c</b>	3 (10%)	-
<b>2hPG</b>	4 (13%)	11 (79%)
<b>2hPG + HbA1c</b>	6 (19%)	-
<b>HbA1c</b>	3 (10%)	-

*FPG, fasting plasma glucose; 2hPG, 2h plasma glucose during OGTT. Figures represent the amount of patients (%) fulfilling a certain combination of diagnostic markers of glucose regulation.*



**Figure 5.2 Glucose and insulin concentrations during 2h OGTT**

*NGT: normal glucose tolerance. Data are shown as mean ± SE.*

**Table 5.3 Comparison of patients characteristics according to glucometabolic state**

	<b>NGT</b> <b>n=11</b>	<b>Prediabetes</b> <b>n=31</b>	<b>Diabetes</b> <b>n= 14</b>	<b>p</b>
<b>Age (years)</b>	64 ± 17	69 ± 11	70 ± 11	.58
<b>Sex (% male)</b>	91	58	64	.15
<b>BMI (kg/m<sup>2</sup>)</b>	25.1 ± 3.7	27.6 ± 5.5	29.9 ± 9.7	.39
<b>Aetiology (% IHD)</b>	27	32	57	.22
<b>NYHA class (% I-II-III)</b>	55-36-9	23-48-29	21-57-21	.34
<b>LVEF (%)</b>	43 ± 13	42 ± 12	40 ± 12	.71
<b>E/E' (n= 5, n=13, n=7)</b>	21.4 ± 19.4	21.2 ± 11.0	15.0 ± 8.7	.33
<b>ACE-inhibitor or AIIA (%)</b>	100	74	86	.16
<b>% optimal daily dosage</b>	73 ± 26	96 ± 32	88 ± 61	.15
<b>Selective β-blocker (%)</b>	73	77	86	.75
<b>% optimal daily dosage</b>	41 ± 17	52 ± 27	52 ± 25	.56
<b>Non-selective β-blocker (%)</b>	18	13	7	.75
<b>% optimal daily dosage</b>	150 ± 71	137 ± 75	50 ± 0	.40
<b>Diuretic (%)</b>	64	84	71	.32
<b>% usual daily dosage</b>	113 ± 67	103 ± 73	129 ± 106	.75
<b>BNP (pg/mL)</b>	167 ± 120	177 ± 207	293 ± 383	.39
<b>HbA1c (%)</b>	5.3 ± 0.2	5.7 ± 0.3	5.9 ± 0.3	<b>.01*</b>
<b>Total cholesterol (mg/dL)</b>	176 ± 39	173 ± 45	169 ± 53	.81

**Table 5.3 (continued)**

	<b>NGT</b> <b>n=11</b>	<b>Prediabetes</b> <b>n=31</b>	<b>Diabetes</b> <b>n= 14</b>	<b>p</b>
<b>HDL cholesterol (mg/dL)</b>	43 ± 11	50 ± 15	49 ± 15	.39
<b>LDL cholesterol (mg/dL)</b>	107 ± 29	93 ± 32	88 ± 42	.25
<b>Triglycerides (mg/dL)</b>	131 ± 35	159 ± 101	160 ± 88	.94
<b>Body weight (kg)</b>	76.5 ± 15.8	76.5 ± 16.5	82.9 ± 30.0	.10
<b>Fat mass (%)</b>	31.1 ± 6.9	35.2 ± 9.1	34.8 ± 9.2	.52
<b>Lean mass (kg)</b>	52.2 ± 9.3	49.3 ± 9.7	52.2 ± 12.4	.62
<b>Fat trunk/fat limb ratio</b>	1.28 ± 0.39	1.39 ± 0.26	1.63 ± 0.35	<b>.03**</b>
<b>Extension strength (Nm/kg)</b>				
<b>45°</b>	16.8 ± 6.1	13.9 ± 2.9	13.7 ± 5.1	.32
<b>90°</b>	16.6 ± 3.0	15.4 ± 4.1	15.3 ± 6.4	.58
<b>Flexion strength (Nm/kg)</b>				
<b>45°</b>	9.0 ± 2.8	8.2 ± 2.2	7.8 ± 3.4	.89
<b>90°</b>	7.5 ± 2.2	6.7 ± 1.9	6.2 ± 2.4	.60
<b>Self-reported physical activity (MET minutes/day)</b>	1921 (533-4313)	1872 (1789-4047)	1219 (664-5686)	.73

*BMI, body mass index; BNP, B-type natriuretic peptide; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction; NGT, normal glucose tolerance; NYHA, New York heart association functional class. Extension and flexion strength are expressed as peak torque relative to lean tissue of the right leg. Continuous variables are presented as mean ± SD, physical activity is presented as mean and 95% confidence interval. Categorical variables are presented as number and percentage. \*NGT versus prediabetes and diabetes, \*\*post hoc differences not significant after Bonferroni correction.*

## Discussion

Despite near-normal fasting glucose values, our data show that the majority of stable CHF patients have impaired glucose tolerance. This was related to body composition and left ventricular end-diastolic pressure, but not to severity of heart failure symptoms, severity of left ventricular dysfunction, nor typical medical therapies.

### Description of glucose tolerance

In our study population, 80% showed an abnormal reaction to glucose intake. This was easily detected using a 2 h oral glucose tolerance test, as proposed by the American Diabetes Association.<sup>19</sup> In fact, only 21% of patients with diabetes would be correctly classified using fasting glucose values only, and none using HbA1c.

Glucose values were in the prediabetic range in 55% of the patients, and in the diabetic range in 25%. The proportion of prediabetes and undetected diabetes is much higher in this study when compared to the findings of other studies, reporting 22-23% prediabetes and 18% newly diagnosed diabetes in a selected population of CHF patients with reduced LVEF.<sup>3,9</sup> This may be due to the heterogeneous study population irrespective of LVEF. Furthermore, it is related to the use of stricter glucose cutoff values in addition to HbA1c values for diagnosis of (pre)diabetes in the present study.

The prognostic impact of this finding is enormous. A stepwise increasing mortality rate with increasing glucose intolerance assessed by OGTT was found by Egstrup et al.<sup>3</sup> Consequently, 80% of the study population, which represented the general CHF population without glucose lowering therapy in the heart failure clinic, is at higher risk for mortality compared to other CHF patients with normal glucose tolerance. Therefore, the transition into overt diabetes and worse prognosis should be prevented. Treatment of (pre)diabetic CHF patients with glucose lowering medication is not evident, as they may be contra-indicated in this population.<sup>24</sup> Therefore, diet counseling and exercise therapy are the preferred treatment methods. Although evidence points to a possible beneficial effect of exercise therapy on whole body glucose uptake in CHF, results are not conclusive. More studies using standardized glucose tolerance assessment and supervised exercise interventions are needed.<sup>25</sup>

### Glucose tolerance in relation to severity of heart failure

Previous studies describing LVEF as a determinant for glucose tolerance were performed in CHF patients with reduced LVEF.<sup>4,7,26</sup> In addition, an association between diastolic function and impaired glucose tolerance in CHF patients with preserved EF has been reported.<sup>27-29</sup> Because we included patients with reduced as well as preserved LVEF, we expected to find a relation between glucose tolerance and EF with worse glucose tolerance in patients with preserved LVEF. However, mixed model analysis showed no influence of LVEF on overall glucose

values or shape of the glucose curve. Likewise, BNP was not associated with glucose curve. Also, a stepwise increase along the diabetic continuum as shown by Stahrenbergh et al. and Dinh et al. was not present in this study population.<sup>28,29</sup> On the other side, E/E', a marker of left ventricular end-diastolic pressure, appeared to be a contributing factor for glucose response during OGTT.

### **Glucose tolerance in relation to typical CHF medical therapies**

Almost all patients in the study group were optimally treated, and therefore pharmacological treatment between groups was similar. Our hypothesis that glucose tolerance is related to the intake and dosage of typical CHF medical therapies could therefore not be confirmed.

### **Glucose tolerance in relation to body composition and skeletal muscle strength**

The classic link of glucose intolerance with increasing obesity was confirmed. Interestingly, a greater importance of fat mass and fat distribution (trunk fat/limb fat) was shown compared to lean mass. The ideal body weight for CHF patients has been the subject of debate. On the one hand, a low body mass index is a risk factor for mortality in CHF while the presence of obesity (body mass index 30-35 kg/m<sup>2</sup>) is associated with lower mortality.<sup>15</sup> On the other hand, more detailed body composition parameters may give another view on the beneficial effects of obesity in CHF. Oreopoulos et al. suggested that higher lean mass is protective in CHF, while fat mass is associated with detrimental effects as higher fasting glucose.<sup>16</sup> As higher fat mass and fat distribution around the trunk were predictors for glucose response in the present study, our data agree with this logic. However, although lean mass was quantitatively not different between groups, it is probable that muscle quality and function are decisive factors for glucose tolerance. In this respect, Doehner et al have showed the decreased glucose transporter protein type 4 in skeletal muscle of CHF patients, independent of body composition.<sup>5</sup>

Muscle function in terms of extension strength of the upper leg was related to overall glucose values, but was not a predictor for glucose tolerance when combined with other variables. In addition, extension strength was negatively correlated with fasting insulin values. This confirms our hypothesis that higher skeletal muscle strength is associated with better glucose tolerance. Although we believe that higher muscle strength is a reflection of increased levels of physical activity, this was not confirmed by the results of physical activity assessment with the International Physical Activity Questionnaire.

This study has some limitations. Echocardiographic data were retrieved from hospital records and were not prospectively assessed. Physical activity was assessed by questionnaire, although it does not reflect true physical activity as compared to pedometers and accelerometers. Furthermore, cardiopulmonary exercise tolerance and isokinetic strength endurance assessment could have

added valuable information to the predictive model. Finally, the study did not include healthy controls.

### **Conclusions**

The proportion of glucose intolerant CHF patients is alarmingly high, and is underestimated when screening only fasting glucose and HbA1c. Our data did not show an association between glucose tolerance and LVEF, New York Heart Association class, nor medication use. However, glucose tolerance was associated with left ventricular end-diastolic pressure and body fat distribution.

### **Authors' contributions**

AS conceived of the study, performed the data collection, statistical analysis, interpretation of data and drafted the manuscript. DH participated in interpretation of data and drafting the manuscript. VV and RW helped setting up the study, performed data collection and statistical analyses. AC gave statistical counseling and performed statistical analyses (mixed models). BO and PD were involved in the conceiving of the study and in the interpretation of data as well as drafting the manuscript. All authors read and approved the final manuscript.

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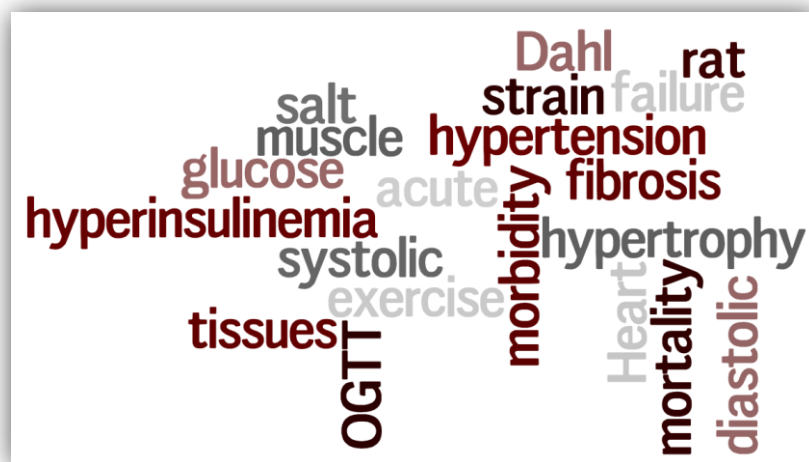
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## Chapter 5

### Study 3

#### Exercise improves cardiac function and insulin resistance in Dahl salt-sensitive rats



Adapted from:

Stevens A, Ferferieva V, Bito V, Wens I, Verboven K, Deluyker D, Voet A, Vanhoof J, Dendale P, Eijnde BO. Exercise Improves Cardiac Function and Insulin Resistance in Dahl salt-sensitive Rats. Under review.

## Abstract

**Purpose.** The development of heart failure (HF) secondary to hypertension is a complex process related to a series of physiological and molecular factors including glucose dysregulation. The overall objective of this study was to investigate whether exercise training could improve cardiac function and insulin resistance in a rat model of hypertensive HF.

**Methods.** Seven weeks old Dahl salt-sensitive rats received either 8% NaCl (high salt, HS; n=30) or 0.3% NaCl (normal salt, NS; n=18) diet. After 5-weeks diet, animals were randomly assigned to exercise training (treadmill running at 18m/min, 5% inclination for 60 minutes, 5 days/week) or kept sedentary for 6 additional weeks. 2D echocardiography was used to calculate left ventricular (LV) dimensions, volumes and global functional parameters. LV global deformation parameters were measured with speckle tracking echocardiography. Insulin resistance was assessed using 1h oral glucose tolerance testing.

**Results.** 12 weeks of HS diet led to cardiac hypertrophy and HF, characterized by increased anterior and posterior wall thickness (respectively  $2.12 \pm 0.06$ mm and  $2.23 \pm 0.05$ mm in HS vs  $1.58 \pm 0.02$ mm and  $1.72 \pm 0.05$ mm in NS;  $p < 0.05$ ) and LV volumes as well as reduced radial and circumferential strain and strain rate. In addition, high salt diet was associated with the development of insulin resistance evidenced by increased insulin values in fasted state and after glucose loading. Exercise training improved cardiac function (e.g. radial strain  $45.6 \pm 2.8\%$  in trained vs  $26.6 \pm 3.4\%$  in sedentary animals on HS diet,  $p < 0.05$ ), reduced the extent of interstitial fibrosis ( $13.6 \pm 1.7\%$  in trained vs  $22.5 \pm 0.9\%$  in sedentary animals on HS diet,  $p < 0.05$ ) and reduced insulin levels 60 minutes post-glucose administration.

**Conclusions.** Even not fully reversed, exercise training in HF animals improved cardiac function and insulin resistance. Adjusted modalities of exercise training might offer new insights not only as a preventive strategy, but also as a treatment for HF patients.

## Background

The development of heart failure (HF) due to hypertension is a complex process that relates to a series of physiological and molecular factors, characterized by structural and functional disorders that still remain incompletely understood. Glucose dysregulation and insulin resistance are comorbidities commonly observed in acute and chronic HF. Both features play a major role in increasing HF risk and are considered as early pathophysiological processes involved in the worsening of the patient outcome.<sup>1</sup>

It is commonly admitted that exercise training, as adjuvant non-pharmacological therapy, is a valuable tool for primary and secondary prevention of HF and its consequences. As such, exercise therapy reduces (re)hospitalizations and increases health-related quality of life.<sup>2</sup> Physical training has been shown to have beneficial effects on neurohumoral, inflammatory, metabolic and central hemodynamic responses as well as on endothelial, skeletal and cardiovascular function, leading to improvement of cardiac performance.<sup>3</sup> Because regular exercise can improve insulin sensitivity as well as insulin independent glucose uptake in non-HF populations<sup>4,5</sup>, the use of exercise in HF patients to improve insulin sensitivity and glucose regulation appears to be a promising strategy to ameliorate patient outcome. However, in a clinical setting, the effect of endurance exercise alone on global cardiac performance and glucose tolerance is difficult to determine due to many confounding factors (e.g. coexisting cardiovascular disease and medication). In that scope, animal models have much to offer in the better understanding of the underlying (patho)physiological mechanisms and potential treatments of HF. During the last decades, the Dahl salt-sensitive rat model has been extensively used for the investigation of myocardial hypertrophy and its transition to HF.<sup>6-8</sup> Despite growing evidence that physical activity has a substantial beneficial effect on cardiac function in this model<sup>9</sup>, the effect of exercise training used as a treatment rather than a preventive strategy still remains unclear.

Two-dimensional (2D) speckle tracking echocardiography (STE) is a newly developed technique that quantifies myocardial deformation by tracking the motion of speckles throughout the cardiac cycle. Several studies have recently applied the speckle tracking approach on different small animal models of left ventricular (LV) dysfunction demonstrating that STE-derived strain/strain rate measurements accurately reflect pathology and time course of HF development in these animals.<sup>10-12</sup>

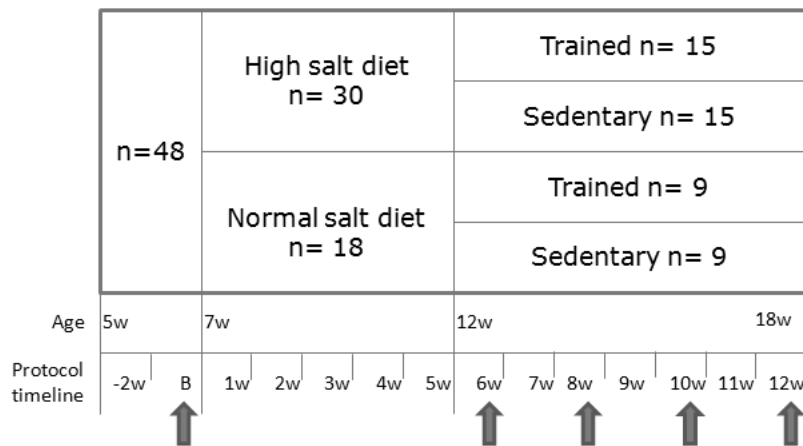
Accordingly, the general purpose of the present study was to investigate whether exercise training could improve overall cardiac performance, attenuate progression towards HF and reverse glucose intolerance in a rat model of hypertensive HF.

## Materials and methods

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publications No. 85-23, revised 1996). The research protocol was approved by the Ethical Committee for Animal Experiments of Hasselt University, Belgium.

### Experimental protocol

A schematic overview of the study protocol is presented in Figure 4.1. Forty eight male Dahl salt-sensitive rats (seven weeks old) were fed with either high (8%, HS, n=30) or normal (0.3%, NS, n=18) NaCl diet (Ssniff Spezialdiäten GMBH, Soest, Germany). Following 5 weeks of diet, animals were assigned either to exercise training (EX) or kept sedentary (SED) for 6 additional weeks.



**Figure 4.1.** Study protocol

Arrows indicate assessment of cardiac function and insulin resistance.

Exercise training consisted in running on a motor-driven treadmill 60min/day at 18m/min and 5% inclination, 5 days/week. Throughout the study, animals received food and water ad libitum, food intake was registered daily. At the end of the experimental protocol, animals were sacrificed with an overdose of Na-pentobarbital (60mg/kg) and further analyses of cardiac tissue were processed.

### Echocardiography

*Conventional echocardiography.* Echocardiography was performed using a Vivid *i* ultrasound machine (GE VingMed, Horten, Norway) with a 10S-RS array transducer. A standard parasternal long-axis image and short-axis views at mid-ventricular level were acquired at a temporal resolution of  $\approx$  200 frames per 64



second. Conventional echocardiographic parameters (e.g. LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), posterior and anterior wall thicknesses) were obtained from the B-mode images at midpapillary level in the parasternal short-axis view. LV fractional shortening (FS)  $([LVEDD - LVESD]/LVEDD*100)$  was also calculated and expressed in %. LV end-systolic (ESV) and end-diastolic volumes (EDV) were calculated by  $\pi*D_M^2*B/6$ , where  $D_M$  indicates the systolic/diastolic diameter of the ventricle in mid-ventricular short-axis view, and B is LV length on parasternal long-axis image. Subsequently, LV ejection fraction (EF) was measured as  $(EDV-ESV)/EDV$ , and expressed in %.

*Strain and strain rate by speckle tracking imaging.* STE data analysis was performed on an EchoPAC workstation (GE Vingmed Ultrasound, version 7.0.1, Horten, Norway), as described previously.<sup>13</sup> Briefly, measurements of radial and circumferential strain and strain rate at midventricular level were performed on selected best-quality two-dimensional images. The endocardium was manually traced in an optimal frame, from which a speckle tracking region of interest was automatically selected. The region of interest width was adjusted as needed to fit the wall thickness from endocardium to epicardium. The software detected and tracked the speckle pattern subsistent to the standard two-dimensional echocardiography after segmenting the ventricular silhouette into 6 segments. The tracking quality was then visually inspected, and, if satisfactorily for at least five segments, the tracing was accepted.

As registration of the electrocardiogram was not always feasible in these animals, end systole and end diastole were therefore defined as the minimum and maximum LV short-axis area, respectively.

### **Assessment of glucose tolerance, insulin resistance and BNP levels**

Glucose tolerance was assessed at baseline, 5 weeks after the start of the diet, and at the time of sacrifice with an 1h oral glucose tolerance test (OGTT). Briefly, after 16h of fasting, glucose (2g/kg body weight as a 50% solution; Merck KGaA, Darmstadt, Germany) was administered via gastric gavage. Before glucose administration and after 15, 30 and 60min, glucose concentration was determined from capillary tail blood collection (Analox GM7, Analis SA, Namur, Belgium). Glucose response was expressed as total area under the curve (AUC), calculated according to the trapezoidal method.<sup>14</sup> At baseline and 60min after glucose administration, a venous blood sample was taken from the tail for determination of blood insulin concentrations. Venous blood samples were centrifuged (4800 g, 6min) and serum was preserved (-80°C) until later analysis. Insulin was measured using an electrochemiluminescence assay (Meso Scale, Gaithersburg, MD). The homeostasis model assessment of insulin resistance (HOMA-IR) was used to assess insulin resistance.<sup>15</sup> HOMA-IR was calculated from fasting glucose and insulin values using the following formula:  $HOMA-IR = (\text{fasting insulin [mU/L]} * \text{fasting glucose [mmol/L]}) / 22.5$ .

Just before sacrifice, an additional venous sample was taken to assess serum B-type Natriuretic Peptide (BNP), measured using a BNP 45 Rat sandwich Elisa kit (Abcam, Cambridge, UK).

### **Fibrosis Measurement**

At the end of the study protocol, animals were sacrificed, and the LV was blotted, weighed and frozen. Sections of 10  $\mu\text{m}$  thick were obtained at midventricular level and stained using collagen-specific picrosirius red. The stained sections were automatically scanned with a digital (microscopic) Mirax slide Scanner system (Carl Zeiss, Göttingen, Germany). Myocardial fibrosis was assessed in four animals per experimental group, and quantified in 4 randomly chosen fields per section, as previously described.<sup>16</sup> 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.

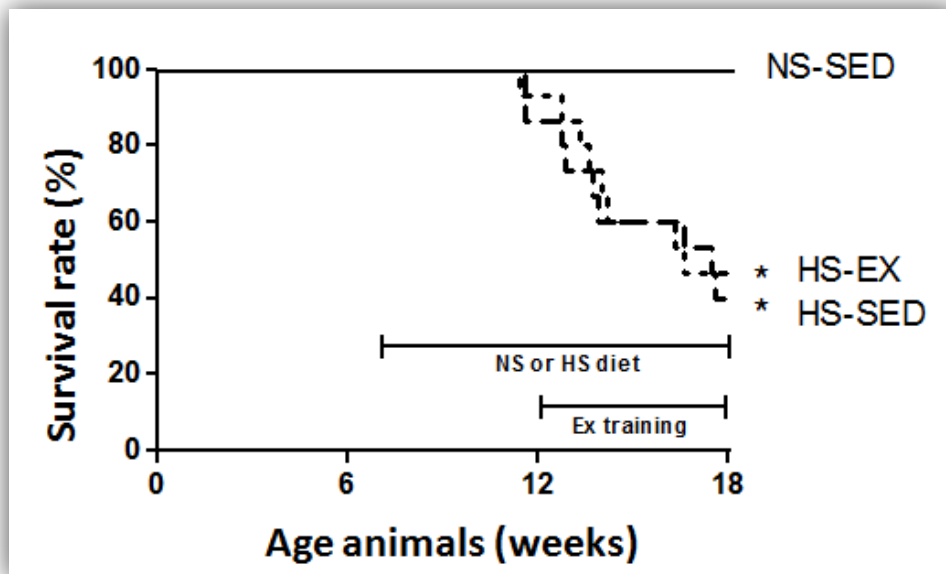
### **Statistical analysis**

Statistical analyses were performed using SAS Enterprise Guide 4.3 and SAS 9.2 (SAS Institute Inc., Cary, NC). Values are expressed as mean  $\pm$  SEM. Survival rate was analyzed using the Kaplan-Meier method and log-rank test. Only rats who survived at least until week 8 of the study protocol (n=9 in each group) were included in analyses regarding exercise effects. Because of missing values for some animals regarding insulin, glucose, BNP and heart weight, sample sizes are reported separately for these measurements. Fibrosis was assessed in a subsample of animals (n=4) who survived until the end of the experiment. Comparisons were performed using Student's two-tailed paired or unpaired test or one-way ANOVA with Tukey post hoc analysis when appropriate. A value of  $p < 0.05$  was considered statistically significant.

## Results

### Early effects of high-salt diet on cardiac function and glucose tolerance in the Dahl salt-sensitive rats

As shown in Figure 4.2, 5 weeks of HS diet did not lead to a substantial mortality. Echocardiographic characteristics of the animals are summarized in Table 4.1. After 5 weeks of HS diet, significant ventricular hypertrophy was present in these animals as evidenced by increased anterior and posterior wall thickness. In addition, LV diameters increased along with a prominent increase in diastolic volumes. These structural changes were accompanied by a significant reduction of circumferential strain, while EF and FS were comparable in HS and NS animals.



**Figure 4.2** Increased mortality induced by 11 weeks of high salt diet is not prevented by addition of exercise training during the last 6 weeks

Kaplan-Meier plot of survival rate in normal salt sedentary group (NS-SED), high salt group (HS-SED) and high salt group undergoing exercise training (HS-EX).  $n = 9, 15$  and  $15$  initially for NS-SED, HS-SED and HS-EX respectively. \* denotes  $p < 0.05$  vs NS-SED.

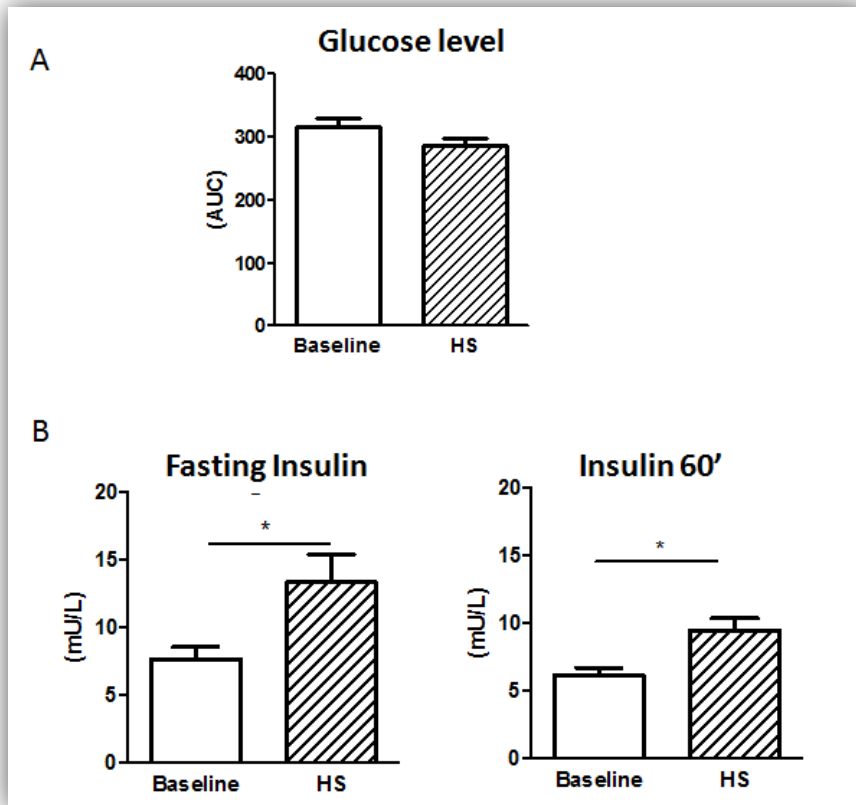
Glucose levels after 5 weeks HS diet were comparable (Figure 4.3A). However, fasting insulin levels significantly increased, suggesting the development of the first stage of insulin resistance in these animals (Figure 4.3B). As a result, calculated fasting HOMA-IR was significantly increased

compared to baseline ( $1.73 \pm 0.34$  vs  $1.05 \pm 0.18$ ,  $p < 0.05$ ). In addition, insulin levels 60' post-glucose were significantly higher after 5 weeks HS diet, suggesting a reduced clearance of insulin (Figure 4.3B).

**Table 4.1 Effect of 5 weeks of high salt diet on conventional echocardiographic parameters**

	Normal salt diet (n=18)		High salt diet (n=18)	
	Baseline	5w	Baseline	5w
<b>HR (beats/min)</b>	396±6	388±7	390±9	380±9
<b>AWT (mm)</b>	1.54±0.03	1.59±0.02	1.54±0.03	2.06±0.04*
<b>PWT (mm)</b>	1.70±0.05	1.71±0.05	1.66±0.06	2.06±0.05*
<b>EDV (μL)</b>	254±9	361±16	294±11	443±13*
<b>ESV (μL)</b>	69±4	111±7	72±5	128±5
<b>CO (ml/min)</b>	73±3	97±4	86±3	120±6*
<b>EF (%)</b>	73 ±1	69 ±1	76 ±1	72 ±1
<b>FS (%)</b>	41±1	38±1	44±1	40±1
<b>Srad (%)</b>	41.9 ± 2.5	42.5 ± 3.2	45.6 ± 2.2	36.2 ± 2.5
<b>SRrad (1/s)</b>	9.7 ± 0.6	8.8 ± 0.5	9.8 ± 0.7	7.7 ± 0.6
<b>Scirc (%)</b>	-19.4 ± 1.0	-18.2 ± 0.9	-19.5 ± 0.5	-14.7 ± 1.1*
<b>SRcirc (1/s)</b>	-5.0 ± 0.2	-4.5 ± 0.4	-5.3 ± 0.2	-4.2 ± 0.3

HR, heart rate; AWT, anterior wall thickness; PWT, posterior wall thickness; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; EDV, end-diastolic volume; ESV, end-systolic volume; CO, cardiac output; EF, left ventricular ejection fraction; FS, fractional shortening;  $S_{rad}$ , radial strain;  $SR_{rad}$ , radial strain rate;  $S_{circ}$ , circumferential strain;  $SR_{circ}$ , circumferential strain rate. Data are presented as mean ± SEM. Nrats= 17 in normal salt diet, and 18 in high salt diet. \* denotes  $p < .05$  vs normal salt at 5w.



**Figure 4.3 Dahl salt-sensitive rats develop insulin resistance after 5 weeks of high salt diet**

(A) Serum glucose levels, measured as area under the curve (AUC), (B) fasting insulin and insulin 60' post-glucose at baseline and after 5 weeks of high salt diet (HS). Data are shown as mean  $\pm$  SEM in Nrats=11. \* denotes  $p < 0.05$ .

**Effect of exercise training on global cardiac performance in the Dahl salt-sensitive rats**

As shown in the Kaplan-Meier plot (Figure 4.2), 6 additional weeks of HS diet led to a progressive increase in animal mortality (up to 60%) as compared to the NS-SED group. Exercise training did not affect mortality rates that remained comparable to those of the HS-SED animals.

Echocardiographic characteristics of animals undergoing exercise training for 6 weeks (HS-EX) or kept sedentary (HS-SED and NS-SED) are summarized in Table 4.2. After 11 weeks of HS diet, the HS-SED group exhibited further worsening of overall cardiac performance, as compared to NS-SED animals. Exercise training was associated with better LV morphology and function in the HS-EX animals. The increased wall thicknesses and LV internal diameters were significantly reduced after exercise towards more physiological values, along with normalized volumes. Although not reaching statistical significance, the reduced hypertrophy was further confirmed in a subset of animals where heart weight/body weight ratios tended to be lower (Figure 4.4A).

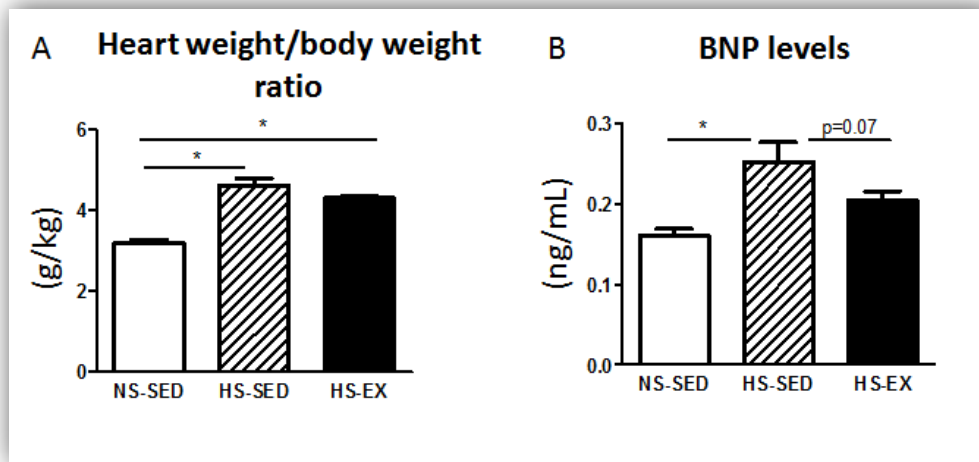
Similarly, STE-derived deformation parameters, *i.e.* strain and strain rate, were significantly improved and normalized after exercise when compared to the HS-SED group (Table 4.2). As a measure of global cardiac impairment, serum levels of BNP were significantly increased after 11 weeks HS diet, but tended to be lower in the exercise group ( $p=0.07$ , Figure 4.4B).

Representative images of the myocardial fibrosis from NS-SED, HS-SED and HS-EX hearts are shown in Figure 4.5A. At 11 weeks of HS diet, myocardial collagen deposition was increased in the sedentary rats when compared to sedentary rats kept on NS diet ( $22.5\pm 3\%$  vs  $8.7\pm 3\%$ ,  $p<0.05$ ). Exercise was associated with significantly reduced myocardial fibrosis when compared to HS-SED animals ( $13.6\pm 1.7\%$  vs  $22.5\pm 0.9\%$ ,  $p<0.05$ , Figure 4.5B). This reduction in collagen content was in line with the normalization of the deformation parameters and displayed a significant correlation between the extent of interstitial fibrosis and circumferential strain ( $p<0.05$ ,  $R^2=0.524$ ; Figure 4.5C).

**Table 4.2** Effect of exercise training on cardiac function in high-salt diet animals

	NS-SED	HS-SED	HS-EX
<b>HR (/min)</b>	403±11	351±14 <sup>†</sup>	348±11 <sup>†</sup>
<b>AWT (mm)</b>	1.58±0.02	2.12±0.06 <sup>†</sup>	1.88±0.04*
<b>PWT (mm)</b>	1.72±0.05	2.23±0.05 <sup>†</sup>	1.93±0.09*
<b>EDV (μL)</b>	421±18	510±29 <sup>†</sup>	464±26
<b>ESV (μL)</b>	132±8	198±15 <sup>†</sup>	166±10
<b>CO (ml/min)</b>	116±4	110±13	104±7
<b>EF (%)</b>	69±1	60±4 <sup>†</sup>	64±1
<b>FS (%)</b>	37±1	31±3	34±1
<b>S<sub>rad</sub> (%)</b>	48.7±4.7	26.6±3.4 <sup>†</sup>	45.6±2.8*
<b>SR<sub>rad</sub> (1/s)</b>	10.7±0.8	5.8±0.6 <sup>†</sup>	8.3±0.8
<b>S<sub>circ</sub> (%)</b>	-16.6±0.7	-11.3±0.8 <sup>†</sup>	-16.9±0.7*
<b>SR<sub>circ</sub> (1/s)</b>	-4.5±0.3	-3.0±0.2 <sup>†</sup>	-4.4±0.3*

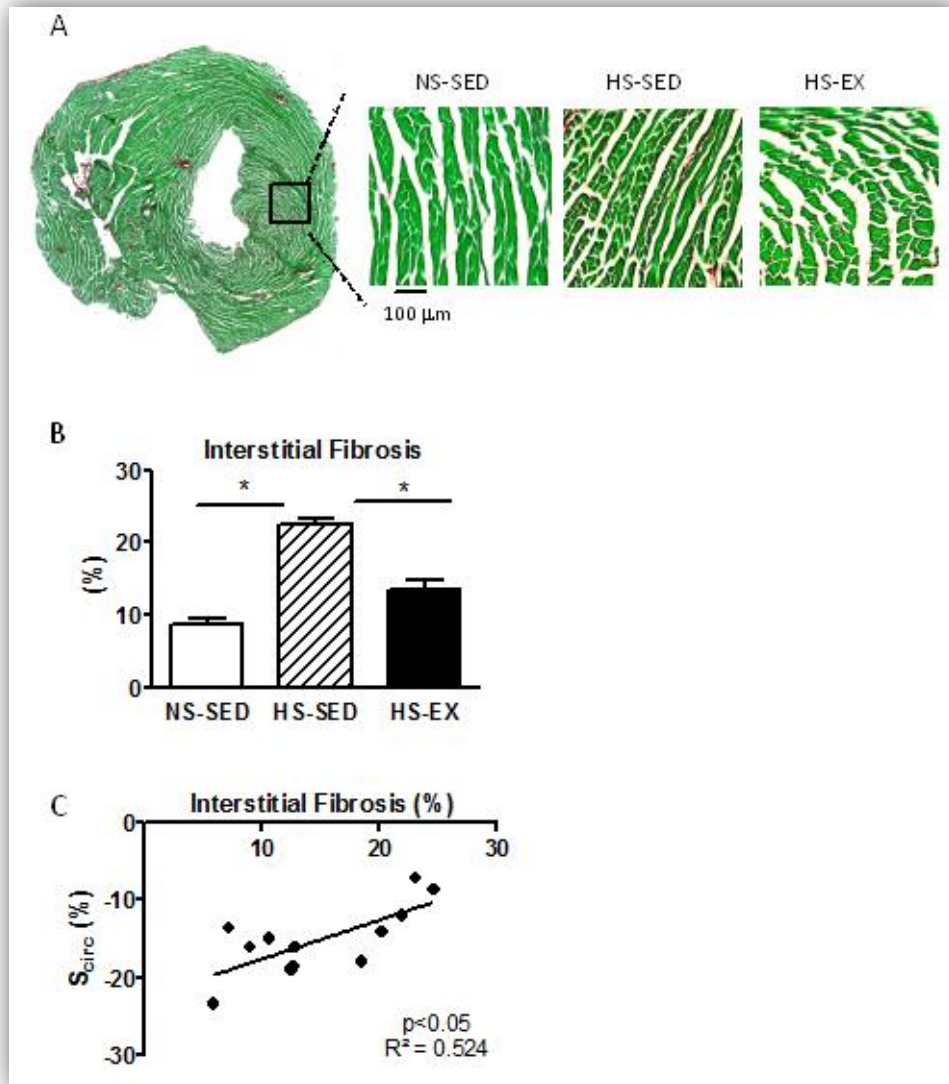
HR, heart rate; AWT, anterior wall thickness; PWT, posterior wall thickness; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; EDV, end diastolic volume; ESV, end systolic volume; CO, cardiac output; EF, left ventricular ejection fraction; FS, fractional shortening; S<sub>rad</sub>, radial strain; SR<sub>rad</sub>, radial strain rate; S<sub>circ</sub>, circumferential strain; SR<sub>circ</sub>, circumferential strain rate. NS-SED, sedentary animals on a normal salt diet; HS-SED, sedentary animals on high salt diet; HS-EX, exercised animals on a high salt diet. N<sub>rats</sub>= 9 in all groups. Data are presented as mean ± SEM. <sup>†</sup>denotes p<0.05 vs NS-SED. \* denotes p<0.05 vs HS-SED.



**Figure 4.4** Effect of exercise training on heart weight/body weight ratio and BNP levels

(A) Heart weight/body weight ratio measured in normal salt (NS-SED,  $n=8$ ), high salt (HS-SED,  $n=6$ ) and high salt undergoing exercise training (HS-EX,  $n=7$ ) groups. (B) Serum BNP levels measured in normal salt (NS-SED,  $n=8$ ), high salt (HS-SED,  $n=9$ ) and high salt undergoing exercise training (HS-EX,  $n=9$ ) groups. Data are shown as mean  $\pm$  SEM. \* denotes  $p < 0.05$ .



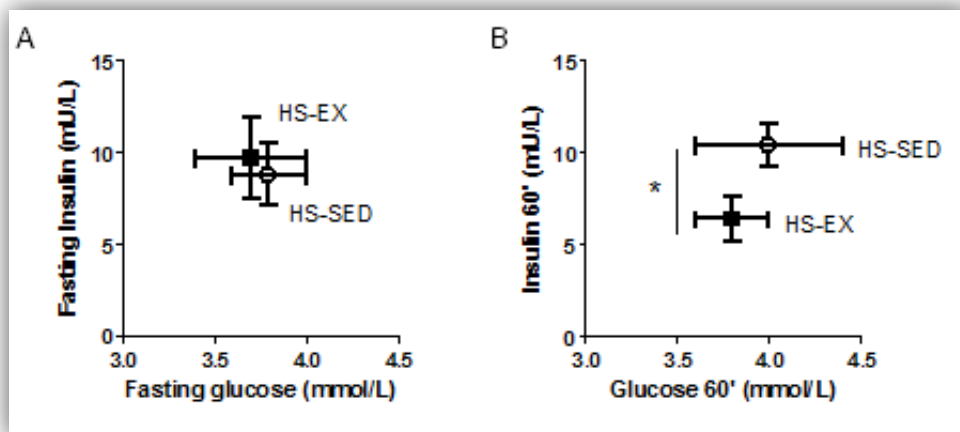


**Figure 4.5 Exercise training is associated with decreased interstitial fibrosis**

(A) Left panel, representative LV section from normal salt intake animal stained with picrosirius red and visualized using Mirax Scan. Right panel, enlargement of an area from a normal salt (NS-SED), high salt (HS-SED) and high salt undergoing exercise training (HS-EX). (B) Interstitial fibrosis content normalized to surface area in NS-SED, HS-SED and HS-EX. (C) Relationship between extent of fibrosis and circumferential strain ( $S_{circ}$ ) in the 3 groups of animals.  $n=4$ /group. Data are shown as mean  $\pm$ SEM. \* denotes  $p < 0.05$ .

### Effect of exercise training on insulin resistance in the Dahl salt-sensitive rats

Potential beneficial effect of exercise training on glucose tolerance and insulin resistance were also investigated in HS animals. As shown in Figure 4.6A, exercise training in rats undergoing HS diet did not change either fasting glucose, nor fasting insulin, resulting in a fasting HOMA-IR comparable between groups ( $1.47 \pm 0.28$  in HS-SED vs  $1.64 \pm 0.42$  in HS-EX). However, despite a comparable glucose level, animals undergoing exercise training displayed a significantly lower insulin level 60' post-glucose administration (Figure 4.6B).



**Figure 4.6** Effect of exercise training on glucose tolerance

Mean insulin expressed as a function of glucose in high salt (HS-SED) and high salt undergoing exercise training (HS-EX), at fasting (A) or 60' post-glucose administration (B). Data are shown as mean  $\pm$  SEM from high salt diet rats (HS-SED,  $n = 6$ ) and high salt diet rats undergoing exercise training (HS-EX,  $n=5$ ). \* denotes  $p < 0.05$ .

### Exercise training has only a minor impact in cardiac function and insulin resistance.

As expected, exercise training in a subpopulation of rats fed with NS diet led to a reduced heart rate ( $348 \pm 8$  vs  $403 \pm 13$  bpm in NS-SED,  $p < 0.05$ ). Exercise training in NS rats did not lead to measurable physiological cardiac hypertrophy, nor changes in cardiac function. In addition, fasting glucose and insulin values were comparable between groups. However, similar to training effects in HS animals, insulin levels 60' post-glucose administration were significantly lower in animals undergoing exercise training.

## Discussion

It is known that co-existence of hypertension and glucose intolerance are independent risk factors for the development of cardiovascular dysfunction in patients.(33) Insulin-resistance has been linked to cardiac hypertrophy, fibrosis and endothelial dysfunction and leads to type 2 diabetes, coronary artery disease and hypertension.(13) Exercise training is known to improve cardiovascular function and prevent glucose intolerance when started before or in the very early phase of the disease.(8, 22, 35) However, when hypertension and glucose intolerance are already established, the effect of exercise training as a therapy to reverse the adverse phenotype is thought to be less efficient. The current study demonstrates that exercise training prevents adverse cardiac remodeling and impaired glucose tolerance induced by high salt diet in a rat model of hypertrophic HF.

### **Exercise training attenuates cardiac hypertrophy and improves the overall cardiac performance.**

The characteristics of the high salt-induced cardiac hypertension and the transition from eccentric hypertrophy to decompensated HF in the Dahl salt-sensitive rat model have extensively been described in the literature.(6, 14, 26) In the current study, animals fed with HS diet displayed features of cardiac hypertrophy that are comparable to previously reported findings.(11, 14, 26) In addition to wall hypertrophy, cardiac dysfunction caused by high salt diet was accompanied by a modest increase in LV volumes, and an overall decrease in global function. Furthermore, 11 weeks of high salt diet resulted in significantly decreased radial and circumferential deformation parameters in concordance with previous echocardiographic studies in the Dahl salt-sensitive rats.(12, 16) These changes were associated with an increase in myocardial collagen content (Figure 4.5). Although the loss of myocardial contractility in hypertensive HF has been attributed mainly to changes in LV geometry (25), several studies have demonstrated that alterations in the extracellular matrix components impair the overall contractile capacity of the ventricle despite the presence of normal cardiomyocyte contractility.(39) These findings suggest that fibrosis itself may directly cause systolic myocardial dysfunction independently of the contractility status of the cardiomyocytes. However, in the current study, we did not evaluate contractility of single cardiomyocytes and this aspect requires further research.

It is well known that exercise, as a physiological stimulus, leads to beneficial hypertrophy and as such, one could anticipate that in addition with the hypertrophic stimuli induced by high salt diet, heart wall thicknesses might further increase after exercise training. However, the effect of exercise training on hypertrophy in the hypertensive setting is still under debate. Comparable heart weight to body weight ratios after exercise training have been reported by some groups (1, 3), while others show that salt-sensitive rats exhibit further

hypertrophy compared to salt resistant rats.(32) Our results demonstrate no further hypertrophy after exercise in animals with high salt diet (Table 2) and are also in agreement with the group of Miyachi *et al.* (22) who reported an unchanged LV mass and a comparable heart weight/tibia length ratio after exercise training. These observations could be related to the moderate intensity exercise used in the current study, suggesting that this might be a safe strategy for exercise therapy in hypertensive HF patients.

In this study, exercise training improved the overall cardiac performance, as demonstrated by lower LV volumes and BNP levels. Furthermore, 6 weeks of exercise training reversed the high salt diet-related impairment in myocardial contractility as evidenced by a complete recovery of systolic strain and strain rate deformation parameters. This improvement in contractility was associated with reduced interstitial fibrosis. Our observations are in agreement with previous experimental and clinical investigations on the role of exercise training focusing on different conditions of HF.(5, 23, 27)

#### **Exercise training partially improves glucose tolerance.**

Before the manifestation of hypertension, one early hallmark of Dahl salt-sensitive rats is elevated insulin levels.(36) Using the hyperinsulinemic euglycemic clamp technique and measuring glucose uptake in peripheral muscles (such as the soleus), high salt diet has been reported to further increase insulin resistance.(24) In our study, using a non-invasive oral glucose tolerance test, we also demonstrate that Dahl sensitive rats with high salt diet develop insulin resistance, characterized by elevated fasting and 60' post-glucose administration insulin levels necessary to maintain normal glucose levels. Interestingly, exercise training did not reduce fasting insulin. However, levels of insulin 60' post-glucose administration were significantly reduced after exercise training, suggesting an increased clearance of insulin and an increased insulin sensitivity in skeletal muscles, as they are known to be major players in insulin regulation. Our data suggest that exercise training plays a role in this process and could reverse the adverse glucose intolerance induced by high salt diet. This hypothesis is supported by data from other groups that suggest that development of insulin resistance induced by high salt diet could be prevented by early exercise training, in part through enhancement of impaired GLUT- 4 translocation and mRNA expression in skeletal muscle.(31) However, in our study, we did not examine changes in skeletal muscles, nor examined the specific pathways involved (such as GLUT-4, IRS-1 and PI-3-kinase pathway) and further studies are required to elucidate the underlying molecular mechanisms involved.

#### **Increased mortality due to high salt diet is not prevented by exercise training in the Dahl salt-sensitive rats.**

In this study, we show that Dahl salt-sensitive rats fed with high salt from the age of 7 weeks display a 60% mortality after 11 weeks of high salt diet. This

rather high mortality rate was not prevented nor improved by the exercise regime given to the animals. In the same model, mortality rates have been shown to be variable between research groups. Indeed, some display a 20-25% after 10-12 weeks of high salt diet (6, 14) while others report a more dramatic effects displaying up to 100% mortality after 7 weeks of high salt diet.(19) Although cause of death was not investigated in our study, the known reasons for high mortality in this model are hypertension-induced stroke and overt HF.(32) It has been previously reported that exercise training is able to improve survival in Dahl salt-sensitive rats, when started before or simultaneously with the high salt diet (18, 34, 35), however the extent of this improvement might not only depend on the moment when the training is started but also on the modalities (e.g. duration, intensity) of the exercise training (18, 22). To our knowledge, the impact of exercise training as a secondary prevention strategy in hypertrophic HF related to hypertension has only been described by one group.(22) In their study, they report an improved survival rate in Dahl-sensitive rats undergoing swimming training possibly attributed to attenuation of LV concentricity. An additional possible mechanism contributing to the improved survival could be the reduced blood pressure via activation of nitric oxide synthase in response to exercise training, mechanism previously described in other rat models of hypertension.(9, 37, 40)

### **Limitations**

There are several limitations that should be recognized. First, no hemodynamic studies have been performed in these animals in order to measure ventricular pressures. However, the Dahl salt-sensitive rat is a well-established animal model of hypertensive HF and it has been extensively used to study the characteristics of myocardial hypertrophy and its transition to HF.(6, 12, 14, 22, 24) Second, STE-derived strain and strain rate parameters were measured only in the radial and circumferential direction since the anatomical position of the rodent heart impedes acquiring data from an apical view. As a result, longitudinal function could not be assessed. Although recent advances in the field of cardiac ultrasound imaging systems have facilitated the measurement of longitudinal function in small animals using speckle-tracking based strain analyses (2), the additional value of measuring longitudinal function in rats may be questioned as it has been previously demonstrated that the long-axis contribution to heart function is relatively small in these animals.(30)

### **Conclusion**

Our study demonstrates that exercise training in Dahl salt-sensitive rats fed a high salt diet is able to improve pathological cardiac remodeling and insulin resistance: cardiac deformation parameters and volumes were fully restored to normal values, whereas hypertrophy, LV diameters and glucose intolerance improved significantly. The specific exercise regimen might be an important factor to determine the extent of the reverse remodeling and adjusted modalities of exercise training (e.g. duration, intensity, forced or voluntary,

running, swimming or high-intensity interval training) might offer new insights not only as a preventive strategy, but also as a treatment for insulin resistance and HF.

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## Chapter 6

### Study 4

**Exercise training improves insulin release during glucose tolerance testing in stable chronic heart failure patients.**



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## Abstract

**Purpose.** Chronic heart failure (CHF) patients often present with (pre)diabetes, which negatively influences prognosis. Unlike the proven effect of exercise on glucose regulation in the general population, its effect in CHF is unclear. Therefore, the present study aimed to investigate the effect of exercise training on glucose regulation in stable CHF patients.

**Methods.** Twenty-two CHF patients were randomized into a training (EX, n=15) and a control group (CON, n=7). Before and after a 12w training intervention involving endurance and resistance training, glucose tolerance (2h oral glucose tolerance test), exercise tolerance (cardiopulmonary exercise test), muscle strength (isokinetic dynamometer), heart function (echocardiography), HbA1c, body composition (DEXA) and quality of life (Eq5d) were assessed.

**Results.** At baseline, glucose levels 2h after glucose intake were elevated in both groups. Whereas area under the glucose curve did not change, area under the insulin curve decreased following training (EX  $-13\pm 23\%$  vs CON  $+22\pm 33\%$ ;  $p<0.05$ ). Changes in the ratio of mitral peak velocity of early filling / early diastolic mitral annular velocity and waist-to-hip fat mass ratio were related to changes in insulin curve. Exercise training improved oxygen uptake at the 2<sup>nd</sup> ventilatory threshold (EX  $+10\pm 5\%$  vs CON  $-8\pm 5\%$ ;  $p<0.05$ ) and isokinetic strength endurance of the upper leg (EX  $+25\pm 9\%$  vs CON  $-6\pm 5\%$ ;  $p<0.05$ ). Lean body tissue was increased by  $2.2\pm 0.5\%$  in EX (vs CON  $+0.2\pm 0.6\%$ ;  $p<0.05$ ). No changes were observed regarding heart function and quality of life.

**Conclusions.** Our data suggest that exercise training attenuates worsening of glucose regulation typically seen in a stable CHF population.

## Background

(Pre)diabetes is a frequently occurring characteristic of chronic heart failure (CHF) patients, with detrimental consequences on morbidity and mortality.<sup>1-3</sup> Regular exercise is an essential strategy in the prevention and treatment of (pre)diabetes in non-CHF populations because it improves insulin sensitivity as well as insulin independent glucose uptake.<sup>4-6</sup> In CHF, exercise therapy is also an essential part of the overall treatment. It improves, amongst others, exercise tolerance, skeletal muscle abnormalities and health-related quality of life.<sup>7,8</sup> However, although the impact of exercise therapy on insulin resistance and fasting blood concentrations of glucose and insulin have been studied before in CHF, the results of these studies do not provide a clear picture.<sup>9,10</sup>

Exercise intervention studies describing the effect on glucose metabolism in CHF are difficult to compare because of heterogeneous exercise modalities and methods for assessing glucose and insulin abnormalities.<sup>10</sup> The greatest training effects were found in 2 studies by Kemppainen et al. using the euglycemic hyperinsulinemic clamp technique to assess insulin resistance, but these studies were not randomized and their results could not be confirmed by Sabelis et al.<sup>11-13</sup> Furthermore, training studies reporting fasting venous blood samples are inconsistent, with predominantly non-significant effects.<sup>11-20</sup> To our knowledge, there are no studies investigating the effect of exercise training on glucose tolerance in CHF, using a 2h oral glucose tolerance test in combination with hemoglobin A1c, which are the recommended screening tools for (pre)diabetes in a clinical setting.<sup>4</sup> In addition, it is not known whether exercise-induced improvements in glucose regulation are necessarily related to changes in peak oxygen uptake, muscular fitness, fat mass and/or lean tissue mass in this population.

In keeping with the above line of reasoning, the first aim of the present prospective randomized study was to test the hypothesis that exercise training improves glucose regulation in stable CHF patients. The second aim was to explore whether changes in glucose regulation are related to changes in exercise tolerance, skeletal muscle strength, body composition and cardiac function.

## **Patients and methods**

### **Patients**

Patients diagnosed with CHF were recruited from the heart failure clinic. Inclusion criteria were (1) history of heart failure >6 months, (2) clinically stable (not hospitalized) >3 months and (3) optimal and stable pharmaceutical therapy (maximal tolerated dose of beta blockers and angiotensin converting enzyme inhibitor or angiotensin receptor antagonist, and spironolactone in heart failure NYHA III). Exclusion criteria were (1) glucose lowering therapy, (2) contraindication to exercise testing or training, (3) other chronic diseases (pulmonary disease, end-stage renal disease, cancer) and (4) engagement in a training program. A power analysis was not performed because of lacking data regarding impact of exercise on glucose tolerance assessed with oral glucose tolerance testing in a heart failure population. Therefore, based on previous studies, sample size was estimated on 30 patients.<sup>11,12</sup> Subjects were asked to avoid changes in daily level of physical activity and diet during the study period. Furthermore, pharmacologic treatment remained constant throughout the study period. After receiving detailed information, patients gave their written informed consent. Ethical approval of the study was obtained from the committees of the Jessa hospital and Hasselt University. The study conforms with the principles outlined in the Declaration of Helsinki.

### **Study design**

Following randomization, a prospective controlled trial was performed in which structured combined exercise training was tested against usual care. Baseline measurements were performed over a time period of 3 days and consisted of assessment of glucose tolerance, skeletal muscle strength, body composition and health-related quality of life (day 1), exercise tolerance and echocardiography (day 3). Randomization was performed in a 2-1 ratio (training intervention: EX - control group: CON), based on sex, age, etiology of CHF and glucose values. The protocol was repeated following 12 weeks of training or usual care. Glucose tolerance was the primary outcome measure; skeletal muscle strength, body composition, exercise tolerance, heart function and health-related quality of life were secondary outcome measures.

### **Cardiopulmonary exercise testing protocol**

Symptom-limited exercise testing was performed on an electronically braked cycle ergometer (eBike 1.8, GE Healthcare) in a non-fasting condition and under usual medication. Exercise tests took place at a standardized time for each patient, within the time range of the training sessions. After 1min of rest followed by 1min of unloaded cycling, the initial load was set at 20W for 1min, and was increased by 10 or 20W every 2min until exhaustion. Cycle load increments were based on previous exercise testing at the heart failure clinic, aiming to yield a test duration of approximately 10min. A 12-lead

electrocardiogram was monitored continuously (Cardiosoft 6.6). Minute ventilation (VE), oxygen uptake ( $\text{VO}_2$ ), and carbon dioxide output ( $\text{VCO}_2$ ) were acquired breath-by-breath, and averaged over 10s intervals (Jaeger Masterscreen CPX).<sup>21</sup> Peak  $\text{VO}_2$  and peak respiratory exchange ratio (RER) were expressed using the highest 10s average obtained during the last minute of the test. The first ventilatory threshold was set at the nadir of the  $\text{VE}/\text{VO}_2$  curve, the second ventilatory threshold was set at the nadir of  $\text{VE}/\text{VCO}_2$  curve.<sup>22</sup> Percent-predicted peak  $\text{VO}_2$  was calculated according to normative values proposed by Wasserman et al.<sup>23</sup> The  $\text{VE}/\text{VCO}_2$  slope was calculated via least squares linear regression ( $y = mx + b$ ,  $m = \text{slope}$ ) using data obtained from the unloaded cycling until the second ventilatory threshold. The oxygen uptake efficiency slope was calculated using  $[\text{VO}_2 = m(\log_{10}\text{VE}) + b]$ , where  $m = \text{oxygen uptake efficiency slope}$  using data obtained during the entire exercise period.

### **Muscle strength**

Maximal voluntary unilateral strength of the upper leg and arm were evaluated in a seated position on an isokinetic dynamometer (System 3; Biodex Medical Systems), which provides a reliable measurement with comparable learning effects in both groups.<sup>24</sup> The rotational axis of the dynamometer was aligned with the transverse knee (elbow) joint axis and connected to the distal end of tibia (handgrip). Two maximal isometric contractions were performed by extensors and flexors at a knee and elbow angle of 45° and 105°, respectively. Maximal contractions (4s) were interspersed by 30s rest intervals. Hereafter, and after 3 submaximal trial contractions, subjects performed 20 maximal isokinetic knee extensions at a velocity of 180°/s to assess strength endurance. Knee extensions were initiated at a joint angle of 90° towards 160°. Total work (J) and average power (W) were calculated and used as strength endurance descriptors.

### **Body composition**

A Dual Energy X-ray Absorptiometry scan (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer) was performed. Fat tissue mass and lean tissue mass were obtained for the whole body and for the following separate regions: legs, trunk, gynoid and android region. Waist-to-hip fat ratio (android fat (g)/gynoid fat (g)) and trunk fat/ limb fat limbs ratio were calculated.

### **Echocardiography**

Standard echocardiographic and myocardial velocity imaging (MVI) data were acquired using a 2.5 MHz probe on a Vivid 7 ultrasound machine (GE Vingmed). The modified Simpson method was used for determination of left ventricular volumes and global left ventricular ejection fraction. Conventional Doppler measurements of early and late diastolic transmitral flow, their ratio (E/A), the early filling deceleration time, isovolumetric relaxation time, and duration of late diastolic flow, and pulmonary venous curves were made to assess diastolic function. In addition, the ratio of mitral peak velocity of early filling (E) to early

diastolic mitral annular velocity ( $E'$ ; assessed from the medial and lateral mitral annulus from the apical four-chamber view) was calculated for the estimation of left ventricular end-diastolic filling pressures.

### **Health-related quality of life**

Health-related quality of life was assessed using the EQ5D.<sup>25</sup>

### **Glucose regulation, HbA1c and BNP**

Following an overnight fasting period but with usual medication intake, patients arrived at the research center at 9 am. After a 30min resting period, the 2h oral glucose tolerance test (OGTT) was started. Baseline blood glucose and insulin concentrations as well as Hemoglobin A1c (HbA1c) and B-type Natriuretic Peptide (BNP) were determined via a venous blood sample taken from an antecubital vein. Hereafter, 75g glucose (Merck KGaA, Darmstadt, Germany) dissolved in 250mL water was ingested and 1- and 2 hours blood samples were taken for glucose and insulin analysis. Blood samples for glucose and insulin (collected in serum separation tubes) and BNP (collected in EDTA tubes) were maintained at room temperature for 30min, centrifuged, and serum and plasma were frozen at  $-80^{\circ}\text{C}$  until later analysis. Blood samples for HbA1c (collected in EDTA tubes) were processed on the test day. Glucose was determined with an Olympus AU analyzer (Beckman Coulter, Switzerland), insulin and BNP with ADVIA Centaur (Siemens Medical Solutions Diagnostics) and HbA1c with Hi-Auto A1C Analyzer (Menarini Diagnostics). Prediabetes was defined as fasting plasma glucose 5.6-6.9mmol/L or 2h plasma glucose 7.8-11.0mmol/L or HbA1c 5.7-6.4%, and diabetes mellitus as fasting glucose  $\geq 7.0$ mmol/L or 2h glucose  $\geq 11.1$ mmol/L or HbA1c  $\geq 6.5\%$ .<sup>4</sup> Glucose and insulin responses were expressed as total areas under the curve, calculated according to the trapezoidal rule.

### **Exercise intervention**

The exercise program was consistent with current recommendations.<sup>26</sup> Briefly, patients trained 5 times every 2 weeks for 12 weeks. The program consisted of a 5min warming-up at 0-25W on a cycle ergometer (Gymna, Ergo-fit Ergo Cycle 157) followed by progressive endurance and resistance training.

Endurance training involved first 2 cycling bouts and thereafter 2 bouts of walking on a treadmill (Gymna, trac 3000 S Alpin). The exercise intensity in the first training session was set at the power output and heart rate just before the second ventilatory threshold, determined from the incremental cardiopulmonary exercise test. Thereafter, heart rate and patient reactions (dyspnea and perceived exertion) were monitored and power output was adapted accordingly. During the following training sessions, power output was adapted according to heart rate and ratings of perceived exertion (Borg 14-16) in such a way that critical power was achieved (highest training intensity that could be maintained with constant heart rate during the exercise bout). Heart rate was monitored continuously (Polar, Oy, Finland) and manually checked. Exercise bout duration



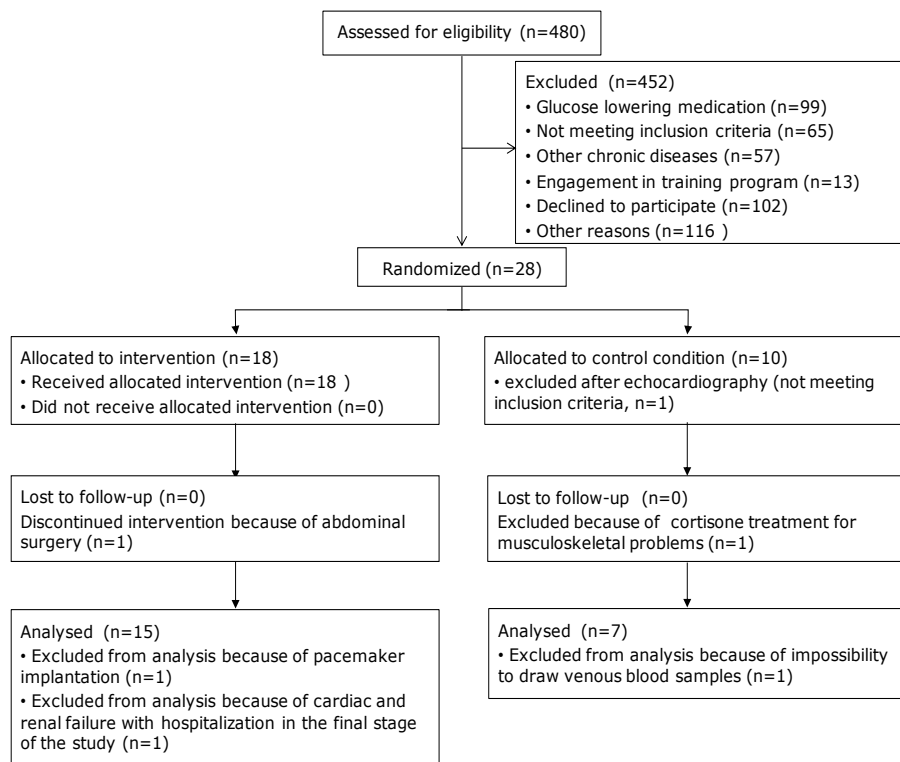
was progressively increased from 6-8min (0-6 weeks) to 8-10min (6-12 weeks). The four exercise bouts were alternated with rest periods of 2 minutes. Resistance training consisted of leg press (Technogym, Belgium), leg extension (Gymna) for the lower limbs, chest press and vertical traction for the upper body (Fysioplus). For the purpose of exercise prescription, 3- 5 repetition maximum (RM) was assessed after 2 familiarization sessions, and converted into 1RM using the following formula:  $1RM = \text{weight lifted} / [1,0278 - 1993(\text{repetitions to fatigue} * 0,0278)]$ .<sup>27</sup> Then, training loads were set at the maximum attainable load between 50-70% 1RM. This was repeated after 6 weeks. Repetitions were gradually increased starting from two sets of 10 repetition to three sets of 15 repetitions, aiming at increasing repetitions (with 2-3) after every 5 training sessions.

### **Statistical analysis**

Statistical analyses were performed using SAS Enterprise Guide 4.3, SAS 9.2 (SAS Institute Inc.) and R2.10.1 software. All measures are presented as mean  $\pm$  standard error. Changes in continuous data were compared using nonparametric Wilcoxon 2-sample test. Paired comparisons were performed to study changes within groups using nonparametric Wilcoxon signed rank test. Categorical data were compared using Fisher's exact test. Because the glucose- and insulin curves consist of longitudinal data (3 time points for each patient), relations between changes in the curves and changes in other patient characteristics (explanatory variables) were investigated with mixed model analysis. All tests were two-sided with a P-value of 0.05 as the threshold for statistical significance.

## Results

A patient flow diagram is presented in Figure 6.1 and baseline patient characteristics are presented in Table 6.1. Patients in EX and CON did not differ at baseline regarding general characteristics, exercise tolerance, muscle strength, body composition, heart function and health-related quality of life, with the exception of body mass index. Patients in EX attended on average 93% of the sessions (range 73-100%). Sixty percent of all registered heart rates during cycling were within the prescribed intensity range or higher, while 90% were in range or higher during walking.



**Figure 6.1 Patient flow diagram**

**Table 6.1 Baseline patient characteristics**

Variable	Training (n=15)	Control (n=7)	Difference (p value)
Age (years)	66.6 ± 3.1	64.4 ± 6.3	1.0
Sex (% male)	10 (67%)	6 (86%)	.62
Smoker (%)	2 (13%)	4 (57%)	.05
Aetiology (% IHD)	6 (40%)	4 (57%)	.65
BMI (kg/m <sup>2</sup> )	28.8 ± 2.1	24.0 ± 1.6	<b>&lt;.04</b>
NYHA class I (%)	5 (33%)	1 (14%)	
NYHA class II (%)	7 (47%)	3 (43%)	.62
NYHA class III (%)	3 (20%)	3 (43%)	
LVEF (%)	39 ± 3	35 ± 2	.65
ACE-inhibitor/AIIA (%)	13 (87%)	7 (100%)	1.0
% of optimal daily dose	79 ± 13	79 ± 14	.77
Selective β-blocker (%)	11 (73%)	6 (86%)	1.0
% of optimal daily dose	41 ± 4	42 ± 12	.46
Non-selective β-blocker (%)	3 (20%)	1 (14%)	1.0
% of optimal daily dose	83 ± 17	200 (/)	.16
Aldosterone antagonist (%)	9 (60%)	3 (43%)	.65
% of optimal daily dose	83 ± 8	100 (/)	.27
diuretic (%)	12 (80%)	6 (86%)	1.0
% of usual daily dose	106 ± 29	100 ± 22	.65

*IHD, ischaemic heart disease; BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction, AIIA, angiotensin II antagonist. Data are presented as mean ± SE or as frequency and percentage.*

### Exercise tolerance

Following 12 weeks of training, exercise duration and VO<sub>2</sub> (mL/min and % predicted) at the second ventilatory threshold increased significantly (p<0.05, see Table 6.2) compared to CON. Exercise tolerance (VO<sub>2</sub>, exercise duration and cycling power output) at the first ventilatory threshold and at peak exercise did not differ between groups.

### Muscle strength

Average power and total work of isokinetic strength endurance as well as isometric extension strength of the elbow were increased in EX compared to

CON (Table 6.2). Isometric strength measures of the upper leg and elbow flexion were not altered in either group.

### **Body composition**

Lean tissue mass increased by  $2.2\pm 0.5\%$  in EX vs  $+0.2\pm 0.6\%$  in CON ( $p=0.04$ ), which seemed to be concentrated at the legs ( $p=0.06$ ). Changes in fat mass and fat distribution parameters were comparable (see appendix 6.1). However, fat percentage of the trunk and ratio of fat mass of the trunk/ limbs were decreased in EX (within group analysis:  $p<0.05$ ).

### **Heart function**

Left ventricular ejection fraction, E/E' and E/A ratio did not change significantly in either group throughout the study (see appendix 6.2). Likewise, changes in BNP concentrations were not significant (EX:  $281\pm 95$  to  $240\pm 98$ pg/mL vs CON:  $285\pm 117$  to  $347\pm 155$ pg/mL).

### **Health-related quality of life**

Health-related quality of life in terms of total EQ5D score and self-rated health on visual analog scale were comparable in both groups before and after intervention. Also shifts in different subdomains of the EQ5d did not reach significance (data not shown).

### **Glucose regulation**

Glucose values were comparable at baseline, but fasting insulin was higher in EX ( $p<0.05$ ). Prediabetes or newly diagnosed diabetes was present in 12/15 subjects in EX and 5/7 in CON at baseline (Figure 6.2). Glucose and insulin profiles during the 2h OGTT are presented in Figure 6.3. After training, mean fasting insulin remained stable in EX ( $14\pm 2$  to  $15\pm 3$  mU/L) and increased in CON ( $6\pm 2$  to  $24\pm 9$  mU/L), leading to a significant relative change between groups. Compared to CON, mean insulin values at 1h decreased in EX (EX:  $147\pm 43$  to  $115\pm 17$  mU/L vs CON  $93\pm 21$  to  $116\pm 24$  mU/L, relative change between groups:  $p<0.05$ ). This was also reflected in area under the insulin curve ( $-12.8\pm 6.0\%$  in EX versus  $+22.5\pm 12.8\%$  in CON,  $p<0.05$ ). Changes in glucose profiles did not differ between groups. HbA1c remained stable in EX ( $5.7\pm 0.1\%$ ) and increased slightly in CON ( $5.6\pm 0.1$  to  $5.7\pm 0.1\%$ , not significant).

### **Contributors to changes in glucose regulation**

Mixed model analysis revealed 9 possible explanatory variables for changes in insulin values during OGTT: changes in E, E/E' ratio, knee flexion strength, elbow flexion strength, total fat mass, fat percentage, waist-to-hip fat mass ratio, fat mass of the trunk and fat mass of the legs. To avoid interference between variables in the model, only changes in E/E' ratio, knee flexion strength, total fat mass and waist-to-hip fat mass ratio were combined in one model with random intercept. After manual backward selection procedure,

changes in E/E' and waist-to-hip fat mass ratio showed significant interaction effects with changes in insulin profile during OGTT (see Table 6.3). Changes in E/E' also showed a main effect for changes in overall insulin values.

**Table 6.2 Changes in exercise tolerance and skeletal muscle strength after 12 weeks of exercise training versus control condition.**

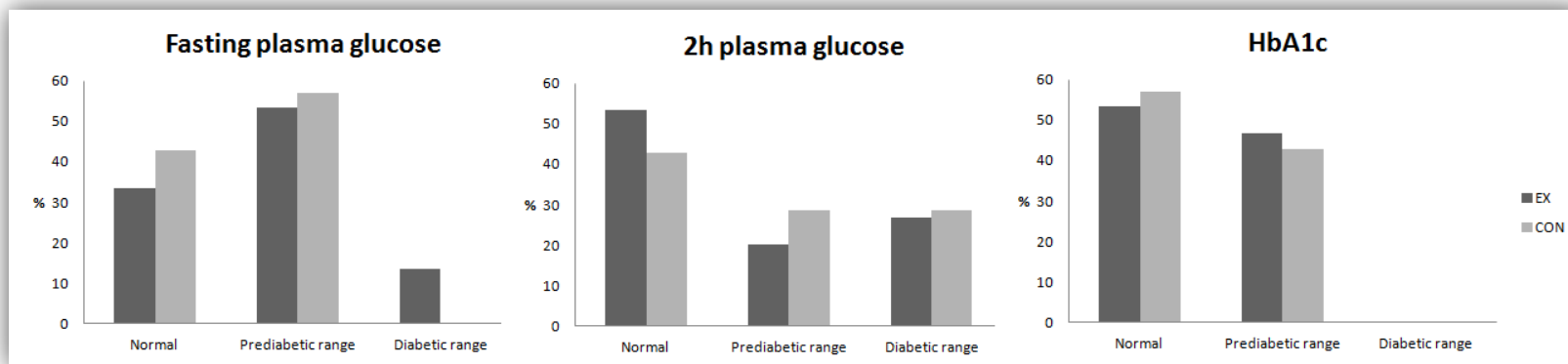
	Training (n=15)			Control (n=7)			Difference
	Pre	Post	% change	Pre	Post	% change	p
<b>Peak exercise</b>							
Exercise duration (min)	8.3 ± 0.7	9.4 ± 0.8	14.4 ± 6.2	9.0 ± 1.4	9.0 ± 1.1	3.0 ± 4.5	.15
VO <sub>2</sub> (mL/min/kg)	17.8 ± 1.0	18.1 ± 1.1	2.7 ± 3.9	18.0 ± 3.1	16.9 ± 3.1	-6.1 ± 4.5	.11
VO <sub>2</sub> (% predicted)	83 ± 5	85 ± 6	3.5 ± 4.1	67 ± 7	63 ± 6	-6.0 ± 4.6	.11
Load (Watt)	87 ± 8	93 ± 8	9.2 ± 4.2	80 ± 15	80 ± 15	1.8 ± 4.2	.20
Oxygen pulse (mL/ beat/min)	13.5 ± 1.0	13.6 ± 1.0	1.3 ± 2.9	11.8 ± 1.0	11.3 ± 1.0	-4.0 ± 4.4	.24
<b>VT1</b>							
Exercise duration (min)	1.7 ± 0.3	1.9 ± 0.3	60.8 ± 42.2	1.6 ± 0.5	1.7 ± 0.4	32.2 ± 23.3	.75
VO <sub>2</sub> (mL/min/kg)	9.9 ± 0.5	10.3 ± 0.7	4.2 ± 6.9	10.0 ± 1.3	9.1 ± 1.4	-9.3 ± 10.4	.31
VO <sub>2</sub> (% predicted)	47 ± 3	48 ± 3	5.1 ± 7.1	39 ± 5	35 ± 6	-9.3 ± 10.3	.31
<b>VT2</b>							
Exercise duration (min)	5.2 ± 0.5	6.4 ± 0.6	25.0 ± 8.2	4.8 ± 0.9	4.6 ± 1.0	-6.1 ± 11.7	<b>&lt;.04</b>
VO <sub>2</sub> (mL/min/kg)	13.8 ± 0.9	14.8 ± 0.9	9.3 ± 4.8	13.2 ± 1.7	12.0 ± 1.4	-8.2 ± 5.5	<b>&lt;.04</b>
VO <sub>2</sub> (% predicted)	64 ± 4	70 ± 5	10.1 ± 5.0	51 ± 5	47 ± 5	-8.1 ± 5.4	<b>&lt;.03</b>

VO<sub>2</sub>, oxygen uptake; VT1, first ventilator threshold; VT2, second ventilator threshold. Data are presented as mean ± SE. P-values reflect differences in relative changes between groups.

**Table 6.2 (continued)**

	Training (n=15)			Control (n=7)			Difference
	Pre	Post	% change	Pre	Post	% change	p
<b>Slopes</b>							
VE/VCO <sub>2</sub>	31.6 ± 1.2	32.5 ± 1.4	3.1 ± 3.5	34.2 ± 3.3	34.6 ± 3.3	1.6 ± 3.5	1.0
OUES	1533 ± 95	1643 ± 129	7.1 ± 4.3	1243 ± 198	1280 ± 237	0.2 ± 5.1	.31
<b>Maximal isometric strength</b>							
Knee extension 45° (Nm/kg)	124 ± 9	136 ± 8	11.1 ± 3.8	138 ± 18	139 ± 17	2.2 ± 4.8	.22
Knee flexion 45° (Nm/kg)	73 ± 6	78 ± 6	10.0 ± 5.4	78 ± 9	81 ± 6	7.1 ± 5.6	.92
Elbow extension 105° (Nm/kg)	40 ± 2	47 ± 3	17.6 ± 7.8	46 ± 4	44 ± 5	-4.4 ± 7.4	<b>&lt;.04</b>
Elbow flexion 105° (Nm/kg)	39 ± 2	43 ± 3	11.5 ± 5.3	41 ± 3	43 ± 4	4.9 ± 9.1	.38
<b>Strength endurance</b>							
Total work (J)	1087 ± 109	1202 ± 391	14.3 ± 4.7	1157 ± 183	1054 ± 147	-6.0 ± 5.2	<b>&lt;.03</b>
Average power (W)	90 ± 9	106 ± 35	25.3 ± 9.1	107 ± 20	97 ± 15	-6.3 ± 5.1	<b>&lt;.01</b>

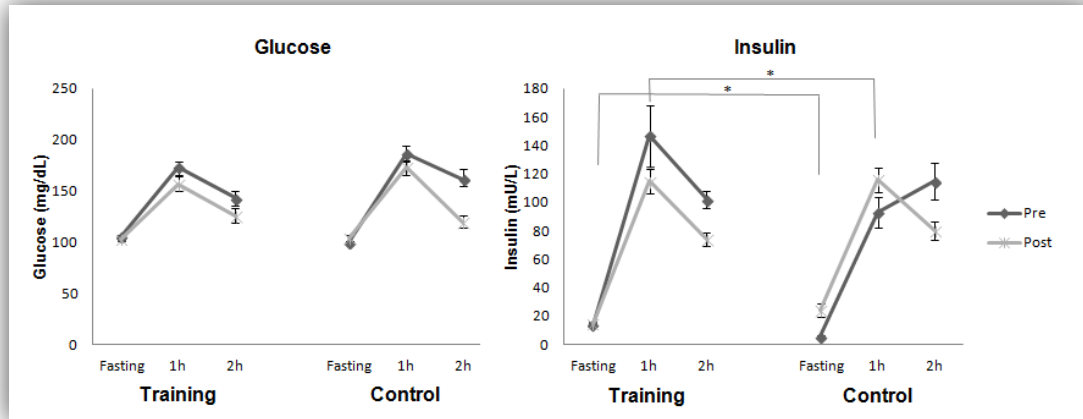
*OUES, oxygen uptake efficiency slope. Data are presented as mean ± SE. P-values reflect differences in relative changes between groups.*



**Figure 6.2 Proportion of patients meeting criteria for normal glucose tolerance, prediabetes and diabetes at baseline.**

*There were no significant differences in proportions between the exercise intervention group and the control group.*





**Figure 6.3** Changes in glucose and insulin concentrations during a 2h glucose tolerance test after 12 weeks of exercise training versus control condition.

Data are presented as mean  $\pm$  SE. \*  $p < 0.05$ .

**Table 6.3** Results of the final mixed model regression analysis for changes in insulin response during the oral glucose tolerance testing.

Parameter	Time	Estimate	SE	Overall p-value
<b>Time</b>	Fasting	3.33	10.81	
	1h	-0.22	9.94	0.0715
	2h	-26.96	9.78	
<b>E/E'</b>	Fasting	-1.14	1.52	
	1h	7.43	1.60	0.0006
	2h	0.06	0.42	
<b>Waist-to-hip fat mass ratio</b>	Fasting	-12.98	102.73	
	1h	450.13	112.70	0.0035
	2h	-79.35	104.17	

## Discussion

Twelve weeks of exercise training decreased insulin release during OGTT in CHF patients. These results confirm that structured exercise training is a good strategy for improving glucose regulation in stable CHF patients. Moreover, changes in insulin concentrations during OGTT were related to changes in left ventricular end-diastolic pressure (E/E') and body composition (waist-to-hip fat mass ratio).

Most patients in the present study were prediabetic or (undiagnosed) diabetic at the start of the study, which is well known in current literature.<sup>10</sup> Some mechanisms have been proposed to explain the development of insulin resistance in CHF patients: advanced age, sympathetic nervous system overactivity, inflammation, obesity, heart failure medication, hormonal changes, reduced muscle mass, altered muscle metabolism, and physical inactivity.<sup>10</sup> While glucose area under the curve was not altered following exercise, insulin area under the curve was decreased by 13%.

The changes in insulin curves during OGTT were significantly related to changes in E/E' and waist-to-hip fat mass ratio. We have previously reported an association between glucose regulation and E/E' in an observational study, in which the baseline data of the present study population were combined with data from another patient cohort.<sup>28</sup> Our results now show that this association is still present when studying evolutions induced by exercise training. Although average changes in E/E' were comparable between groups because of high individual variation, we observed a positive correlation between changes of both factors in analysis of the total study population, indicating that a decrease in left ventricular end-diastolic pressure is accompanied by a decrease in insulin response during OGTT. Possible mechanisms linking both factors proposed by Stahrenberg et al. include increased myocardial mass through the growth stimulating effect of insulin and activation of the sympathetic nervous system by insulin.<sup>29</sup> In this respect, both attenuation of pathological hypertrophy (animal models) and sympatho-inhibitory effects (human) of exercise training have been described.<sup>30-32</sup> The association between changes in insulin release and waist-to-hip fat mass ratio suggests that training programs in CHF should focus on decreasing abdominal fat, and therefore aim at high caloric expenditure, in order to improve glucose regulation.

There is abundant evidence for the effect of exercise training in the prevention and management of type 2 diabetes mellitus in non-CHF populations.<sup>5,33,34</sup> Moreover, improved insulin sensitivity in response to exercise therapy has been extensively reported.<sup>35</sup> Specifically, a comparable decrease in blood insulin levels during an oral glucose tolerance test has been described in overweight young adults.<sup>36</sup> However, it is difficult to compare these results with studies describing the effect of exercise training in CHF patients, because of the heterogeneity in methods.<sup>11-18,20</sup> From five randomized controlled trials, 3 studies described decreased fasting glucose after high intensity endurance training, and one after moderate intensity endurance training.<sup>13,15,16,18,20</sup> One

study also reported a decrease in fasting insulin values.<sup>16</sup> The most promising training effects were presented by Kemppainen et al. Although these studies were not randomized, they showed that combined exercise training improved whole-body insulin-stimulated glucose uptake by 23-25%.<sup>11,12</sup> These results were not confirmed in the randomized trial of Sabelis et al., possibly because of lower prescribed exercise intensity compared to the former studies.<sup>13</sup> All studies mentioned above recruited CHF patients with reduced ejection fractions.

Changes in exercise tolerance were present by an increased second ventilatory threshold, but peak exercise tolerance was not affected. Two reasons can possibly explain why peak  $\text{VO}_2$  did not show comparable improvements as in other CHF studies using a similar training intervention (12-31%).<sup>37</sup> Firstly, the study was performed in a heterogeneous stable CHF patient population with 50% of participants older than 70 years, while most training studies in CHF examined a younger population. Secondly, the intervention could be defined as hospital-based phase III rehabilitation, which means that the highest improvement rates during spontaneous recovery (after a hospitalization period) have already passed.

This study has some limitations. (1) The exclusion of several patients during the study disturbed the 2:1 randomization balance, and was therefore responsible for baseline differences in the final study population, resulting in the comparison of an overweight exercise group to a normal weight control group. This may have contributed to the relations noted between changes in area under the insulin curve and waist-to-hip fat mass ratio. Furthermore, these exclusions also resulted in a reduction of power (post hoc power calculation 65%). Therefore, larger studies are necessary to confirm these results. (2) Subjects were asked to avoid changing daily physical activity and diet over the course of the 12 week study but these were not measured. (3) There was no objective measure to show that the subjects were in fact sedentary before training. Furthermore, we suggest that future studies should also collect muscle biopsies in order to describe glucose-related molecular adaptations in response to exercise training in CHF patients.

Our findings have clinical implications concerning exercise therapy in CHF patients. The presented data suggest that it is essential to assess the insulin response during a glucose tolerance test in order to be able to observe relevant changes in glucose regulation. In addition, (pre)diabetic CHF patients could have additional benefit from exercise therapy aiming on reducing abdominal fat. In this regard, exercise sessions emphasizing caloric expenditure should be prescribed.

In conclusion, exercise training attenuates worsening of glucose regulation typically seen in a heterogeneous stable CHF population. Therefore, exercise training should be recommended, not only to improve exercise tolerance, but also to prevent diabetes in order to improve prognosis.

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**Appendix 6.1 Changes in body composition after 12 weeks of exercise training versus control condition.**

	Training (n=15)			Control (n=7)			Difference
	Pre	Post	% change	Pre	Post	% change	p
Fat mass (kg)	29.7 ± 4.1	29.6 ± 4.2	-1.03 ± 1.2	20.6 ± 3.3	20.6 ± 3.3	0.7 ± 2.9	.86
Fat mass (%)	34.9 ± 2.1	34.2 ± 2.2	-2.2 ± 1.0	28.9 ± 3.4	29.0 ± 3.3	0.5 ± 2.5	.34
Fat mass/heighth <sup>2</sup> (kg/m <sup>2</sup> )	10.5 ± 1.5	10.5 ± 1.6	-1.0 ± 1.2	7.2 ± 1.3	7.3 ± 1.3	0.7 ± 2.8	.86
Lean mass (kg)	52.4 ± 2.4	53.7 ± 2.5	2.2 ± 0.5	48.6 ± 2.2	48.8 ± 2.4	0.2 ± 0.6	<b>.0376</b>
Lean mass legs (kg)	16.3 ± 0.8	16.9 ± 0.8	3.7 ± 0.7	15.3 ± 0.8	15.5 ± 0.9	0.9 ± 1.1	.06
Lean mass/heighth <sup>2</sup> (kg/m <sup>2</sup> )	18.3 ± 0.7	18.8 ± 0.7	2.1 ± 0.4	16.8 ± 0.6	16.9 ± 0.6	0.2 ± 0.6	<b>.0483</b>
Android/gynoid ratio	1.19 ± 0.05	1.77 ± 0.05	-0.6 ± 2.2	1.08 ± 0.06	1.06 ± 0.08	-2.6 ± 2.8	.86
Fat trunk/fat limb ratio	1.54 ± 0.09	1.46 ± 0.08	-4.3 ± 1.6	1.40 ± 0.11	1.35 ± 0.10	-3.7 ± 2.4	.44

*Data are presented as mean ± SE. P-values reflect differences in relative changes between groups.*

**Appendix 6.2 Changes in cardiac function after 12 weeks of exercise training versus control condition.**

	Training (n=15)		Control (n=7)		Difference p
	Pre	Post	Pre	Post	
LVEF (%)	39 ± 3	38 ± 3	35 ± 2	38 ± 5	.34
E (m/s)	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	.59
A (m/s)	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	.97
E/E' ratio	19.8 ± 3.2	17.2 ± 2.2	20.2 ± 6.0	16.1 ± 4.0	.76
E/A ratio	1.2 ± 0.1	1.4 ± 0.2	1.5 ± 0.4	1.5 ± 0.3	.91
DT (ms)	184 ± 16	192 ± 14	181 ± 14	181 ± 18	.39
IVRT (ms)	97 ± 4	116 ± 11	90 ± 10	109 ± 15	.36
MV Adur (msec)	135 ± 5	130 ± 11	137 ± 13	126 ± 8	.37
PG Tricuspid (mmHg)	26 ± 2	27 ± 2	35 ± 6	36 ± 9	.18
LVEDD (mm)	58 ± 3	57 ± 3	58 ± 3	59 ± 1	.84
LAEDD (mm)	61 ± 3	58 ± 3	59 ± 3	58 ± 4	.68

*LVEF, left ventricular ejection fraction; E, peak early mitral inflow filling velocity; A, peak mitral filling velocity at atrial contraction; DT, deceleration time of mitral E wave; IVRT, isovolumic relaxation time; MV Adur, duration of mitral A wave; PG Tricuspid, peak gradient over tricuspid valve; LVEDD, left ventricular end-diastolic diameter; LAEDD, left atrial end-diastolic diameter; Data are presented as mean ± SE. P-values reflect differences in relative changes between groups.*



## Chapter 7

### General discussion



The final chapter of this dissertation starts with a short recapitulation of the research goals. Hereafter, some methodological issues are addressed and the findings described in the previous chapters are integrated and discussed. Next, clinical implications and possible directions for future research are formulated.

## Recapitulation of the research goals

Despite advances in medical therapy for cardiac patients in general, the prognosis for HF patients is still poor.<sup>1</sup> Especially the group of HF patients with diabetes are at high risk for a worse outcome and therefore management of the 'co-morbidity' diabetes is acknowledged as a component of the holistic care of patients with HF.<sup>2,3</sup> In this context, and following a thorough literature search, the following research goals were identified.

The **first aim** of this thesis was to describe the prevalence of impaired glucose regulation in patients with acute (Chapter 3) and chronic (Chapter 4) HF using the oral glucose tolerance test.

**The second aim** was to gain more insight in the relation between impaired glucose tolerance and (1) severity of HF, (2) the intake and dosage of typical medical therapies for HF and (3) body composition and muscle strength in stable HF patients (Chapter 4).

The **third aim** was to investigate the impact of exercise therapy on glucose tolerance in HF and to explore the influencing parameters (Chapter 5, Chapter 6).

## Methodological issues

### Oral glucose tolerance testing

Throughout this project, glucose regulation was investigated using oral glucose tolerance testing. In **Chapter 3** and **Chapter 4**, the prevalence of impaired glucose regulation was described in prospective observational studies using the 2h glucose tolerance test in combination with HbA1c. The glucose tolerance test is proposed by the World Health Organisation (WHO) and the American Diabetes Association as one of the tools to detect (pre)diabetes, but these authoritative organizations do not agree regarding the combination of different diagnostic methods, nor in diagnostic criteria.<sup>4,5</sup> While the WHO proposes the combination of fasting plasma glucose (FPG) and 2h plasma glucose during OGTT (2hPG) with a supporting role for HbA1c, the ADA recommends more stringent criteria for FPG and 2hPG with a more prominent role for HbA1c in the diagnostic process.

In both observational studies, many patients presented with normal FPG combined with elevated 2hPG, and therefore could not be classified in any category using the WHO criteria (IGT= elevated FPG + elevated 2hPG). This finding is in concordance with results from major studies reporting the value of FPG and 2hPG in the diagnosis of diabetes: the DECODE study in 1517 previously undiagnosed patients demonstrated that 59% of patients with elevated FPG had a 2hPG that did not indicate diabetes, and conversely, 52% of patients with elevated 2hPG did not meet the FPG criterion for diabetes.<sup>6</sup> Similarly, in the US-NHANES study, only 44% of previously undiagnosed diabetics met both the fasting and the 2h glucose criteria.<sup>7</sup> For that reason, we chose to adopt the ADA criteria for diagnosis of diabetes and impaired glucose tolerance (the presence of elevated fasting glucose, or elevated 2h glucose in OGTT, or elevated HbA1c), which were more suitable to classify the glucose regulation profiles of our patient population. Undoubtedly, this decision has led to more dramatic figures regarding prevalence of (pre)diabetes in this project, compared to previous observational studies.

In both observational studies, glucose values after glucose loading brought forth more new diagnoses of diabetes compared to FPG and HbA1c: the majority (72% in acute HF and 79% in chronic HF) of newly diagnosed diabetics displayed a 2hPG in the diabetic range, while fasting glucose and HbA1c were in the normal or prediabetic range. In acute HF patients, the limited sensitivity of HbA1c could indicate that the disturbed glucose regulation is an acute reaction on illness and stress from hospitalization which is not yet reflected in this marker of prolonged hyperglycaemia.

The glucose tolerance test was also used to assess the impact of exercise training in animals (**Chapter 5**, 1h OGTT) and in patients with chronic HF (**Chapter 6**, 2h OGTT). Our results showed that exercise training induced minimal changes in glucose curves during OGTT, but clear reductions in insulin levels necessary to maintain these glucose curves. Although it seems likely that

exercise training is able to reduce glucose levels, training studies in non-HF study populations have also reported unchanged glucose values alongside reduced insulin release after exercise training.<sup>8,9</sup> On the one side, this could be an argument to state that a diagnostic glucose tolerance test without the assessment of insulin values is not sensitive enough to detect changes induced by a short period of exercise training. Another consequence is the fact that a training intervention or exercise therapy of 12 weeks is not able to cause shifts diagnosis fo (pre)diabetes, based on glucose values during OGTT and HbA1c. As a consequence, our data support the use of insulin sensitivity indices based on a combination of glucose and insulin values during OGTT, as described by Hays and colleagues.<sup>10</sup> Specifically when focusing on the effects of exercise training on the role of the skeletal muscle in glucose uptake, the skeletal muscle insulin sensitivity index (=rate of decline in plasma glucose concentration from peak to nadir/mean plasma insulin concentration, as introduced by Abdul-Ghani et al in 2007) is promising, although it has not been adopted in exercise training studies, to my knowledge.<sup>11</sup>

*In conclusion, while adopting the ADA criteria for glucometabolic classification, we found that fasting plasma glucose and HbA1c are not adequate to identify patients with diabetes in the HF population. On the other hand, oral glucose tolerance testing is more efficient to detect significant impaired glucose tolerance. Oral glucose tolerance testing with insulin assessment proved to be even more sensitive, and was able to detect exercise-induced changes.*

### **Patient population**

In all three clinical studies of this project (**Chapter 3, 4 and 6**), a general HF patient population was included. All patients with diagnosis of HF, according to the definition provided by the European Society of Cardiology <sup>2</sup>, were eligible and no further selection criteria regarding systolic function, sex nor age were imposed. While being aware that this decision can lead to a heterogeneous study population, this was a deliberate choice. Especially in the observational studies, the purpose was to describe glucose tolerance in a general HF population, with clinical relevance to the everyday practice in inpatient as well as outpatient HF clinics.

## Integration of the findings of the separate studies

The results and conclusions of the previous chapters are integrated in the following sections: first, the prevalence of impaired glucose regulation in HF patients is discussed, followed by the effect on prognosis and by the description of determinants of glucose tolerance. Last, the impact of exercise therapy on impaired glucose regulation in HF is summarized.

### 1) *Prevalence of impaired glucose regulation in HF patients*

The prevalence of diabetes reported in literature varies widely from 16% to 46% in acute HF during hospital admission and from 8% to 41% in chronic HF.<sup>12,13</sup> The large variation in prevalence of diabetes in different studies can at least partly be explained by differences in study design and inclusion criteria (particularly considering age, systolic function and aetiology of HF), partly because of geographic/ethnic characteristics of study subjects but also by different diagnostic criteria for diabetes (see supra). Furthermore, it is not only probable that a large proportion of diabetic HF patients are undiagnosed<sup>14</sup>, these figures would be even higher should they include patients with prediabetes. The glycaemic state in acute HF patients was investigated in **Chapter 3**. In this observational study, 32% of the patient population was previously diagnosed with diabetes. In addition, it was demonstrated that from 98 patients with no previous diagnosis of diabetes, only a nonsignificant proportion (1%) presented with a normal glucose profile on the day of hospital discharge. In **Chapter 4**, 80% of the study population of 56 patients with stable chronic HF (not previously known with diabetes) presented with an abnormal glucose regulation. Glucose values were in the prediabetic range in 42% of acute and in 55% of chronic HF patients. Results regarding newly diagnosed diabetes were more divergent: more than half of the hospitalized patients could be classified as diabetics, versus 25% of chronic stable patients from the HF clinic.

Possible explanations for the more severely impaired glucose regulation at the end of a hospital admission for HF could be the following: (1) hyperglycaemia and other abnormal metabolic factors are commonly found in seriously ill patients, caused by a highly complex interplay of counter-regulatory hormones such as catecholamines, growth hormone, cortisol, and cytokines.<sup>15</sup> As only patients who were clinically stable for more than three months were included in the study in **Chapter 4**, these mechanisms which are exacerbated during critical illness have, at least partly, attenuated. (2) The more intensive diuretic therapy to relieve congestion during a hospital stay for HF is likely to induce hyperglycaemia to a higher extent compared to the optimized diuretic therapy in chronic patients. (3) The glucose tolerance of patients in **Chapter 3** was assessed at the end of their hospital stay, which is a period of severely reduced physical activity or even immobilization. The devastating effects of bed rest on insulin resistance was illustrated by Hamburg and colleagues, who

showed that even a short period of 5 days bed rest induced a 67% increase in the insulin response to glucose loading as well as increased glucose curves during OGTT in healthy persons.<sup>16</sup> The insulin resistance resulting from bed rest during HF admissions is more than likely even worse, as the median length of hospital stay in our study was 7-9 days, which is comparable to the findings of the EuroHeart Failure Study II.<sup>17</sup> Even though the physical activity level (assessed by questionnaires) in the study group of chronic HF patients in **Chapter 4** was relatively low, we can assume that this is still considerably higher compared to hospitalized patients. However, these possible explanations for the differing results in **Chapter 3 and 4** are speculative, because these studies lack supporting data.

The prevalence of impaired glucose regulation reported in **Chapter 3 and 4** is higher compared to the general population. In the 34 OECD (The Organization for Economic Co-Operation and Development) countries, 6.9% of people between 20 and 79 years were estimated to have diabetes in 2011.<sup>18</sup> In Belgium, it was reported that less than 5% of adults suffer from diabetes.<sup>18,19</sup> The estimated prevalence of impaired glucose tolerance is estimated 10% in Europe and 8% in Belgium.<sup>19</sup> Regarding the cardiovascular patient population, the glycaemic state of 4196 patients was described using WHO criteria in the Euro Heart Survey on diabetes and the heart in 2004.<sup>20</sup> In this multicentre European prospective observational study, 31% had previously diagnosed diabetes. Furthermore, from the group of patients with an acute coronary event, 36% presented with impaired glucose regulation and 22% with newly diagnosed diabetes. From the group of cardiovascular patients with scheduled hospital admissions, these proportions were 37% and 14%, respectively.

Two other HF studies had a similar set up compared to the presented work. The study of Matsue and colleagues was similar to the study in acute HF patients in **Chapter 3** in respect to patient population (inclusion of patients with reduced as well as preserved systolic function in the same age range) and assessment of glucose tolerance at the day of discharge.<sup>21</sup> They reported that OGTT revealed 9% newly diagnosed diabetics and 34% prediabetic patients. In stable chronic HF on the other hand, the proportion of (pre)diabetes described in **Chapter 4** is much higher than the 23% prediabetes and 18% newly diagnosed diabetes in a selected population of chronic HF patients with reduced LVEF reported by Egstrup and colleagues.<sup>22</sup> This is likely due not only to the more stringent ADA criteria, but also to differing patient populations.

*It can be concluded that the prevalence of impaired glucose regulation is severely increased in acute as well as chronic HF patients compared to the general population and to patients with coronary events, even when taking differences in diagnostic criteria into account.*

## **2) Effect of disturbed glucose regulation on mortality and morbidity**

Studies investigating the prognostic impact of (*non-fasting*) admission glucose on outcome in HF patients provide heterogeneous results. A powerful association between admission glucose and short term (30 day) mortality was reported in a large multinational cohort study from Mebazaa et al, including data from 6212 patients.<sup>23</sup> For 60 day mortality, Barsheshet and colleagues reported similar results in an Israeli study population.<sup>24</sup> Regarding long-term prognosis in acute HF, two British studies concluded that the admission glucose was also an important predictor.<sup>25,26</sup> However, contradictory to these findings, Kosiborod and colleagues found no association between admission glucose levels and mortality after 30 days and 1 year of follow-up, in a major multicentre trial throughout the United States including more than 50,000 elderly patients.<sup>27</sup> These results were confirmed in a more recent study of Barsheshet et al.<sup>28</sup>

The relation of *FPG* and *2hPG* to mortality has been described in a male cohort followed in the Baltimore Longitudinal Study of Aging by Sorkin et al.<sup>29</sup> They concluded that (1) risk of all-cause mortality is not increased in men with increased FPG and a normal 2hPG, and (2) risk increases progressively in men with increased FPG as the 2hPG becomes progressively worse. In order to investigate the impact of glucose tolerance, assessed by OGTT and HbA1c, on mortality and morbidity in acute HF, 192 patients were followed up for a median time period of 470 days in **Chapter 3**. Our findings showed that elevated 2h glucose during OGTT, but not fasting glucose nor HbA1c, demonstrated a higher risk for worse outcome. The predictive effect of 2hPG was more convincing on a continuous scale, as opposed to comparison of patients with normal-prediabetic-diabetic 2hPG values on a categorical scale: risk of mortality combined with hospitalization for cardio-renal causes (primary endpoint) increased with 8% and risk for mortality alone (secondary endpoint) with 19% with every increase of 1 mmol/L in 2hPG. However, our data could not confirm the results of the study of Matsue et al (see supra), who reported a 3-4 fold higher risk in HF patients classified as glucose intolerant.<sup>21</sup>

Looking into the prognostic impact of glucose regulation in patients with chronic HF, Egstrup and colleagues followed a cohort of more than 300 patients with reduced systolic function for a median time of 591 days.<sup>22</sup> After demonstrating the importance of 2hPG in the diagnosis of diabetes in chronic HF, they also showed that previously as well as newly diagnosed diabetic patients are at higher risk for mortality compared to patients with prediabetes and normal glucose tolerance. Furthermore, they found a stepwise increasing mortality rate with increasing glucose intolerance. In addition, in a study of Doehner et al, impaired insulin sensitivity assessed by intravenous glucose tolerance test was independently associated with worse long-term prognosis in patients with reduced systolic function.<sup>30</sup> Although this project did not provide data regarding mortality in relation to glucose regulation in a chronic HF population, it is safe to deduce that 53% of the study population in **Chapter 4** (30/56 patients had elevated 2hPG) is at higher risk for mortality compared to other chronic HF patients with normal glucose values during OGTT.

In the hypertensive and hypertrophic animal model for HF described in **Chapter 5**, we aimed to describe mortality in relation to glucose regulation. We found that Dahl salt-sensitive rats fed with high salt from the age of 7 weeks display a 60% mortality after 11 weeks of high salt diet. In the same model, mortality rates have been shown to be variable between research groups. Indeed, some describe a 20-25% mortality after 10-12 weeks of high salt diet<sup>31,32</sup> while others report a more dramatic effects displaying up to 100% mortality after 7 weeks of high salt diet.<sup>33</sup> Although cause of death was not investigated in our study, reasons for high mortality in this model are described as hypertension-induced stroke and overt HF.<sup>34</sup> Unfortunately, in our study the impaired glucose regulation could not be related to morbidity (weight loss and echocardiographic parameters), nor with mortality.

*Taken together, our findings confirm and extend evidence for the importance of glucose tolerance for morbidity and mortality in patients after a hospital admission for acute HF.*

### **3) Determinants of glucose tolerance in HF patients**

The prospective observational study in **Chapter 4** aimed to investigate the determinants for impaired glucose tolerance in chronic HF patients. The findings of this study can be complemented by some additional comments based on findings from acute HF (age, body mass index, LVEF; **Chapter 3**) and from the animal model (cardiac hypertrophy, BNP; **Chapter 5**).

#### ***Glucose tolerance in relation to severity of HF, heart function and cardiac hypertrophy***

In chronic HF due to systolic dysfunction, LVEF has been described to be associated with impaired glucose regulation assessed by fasting insulin and glucose values<sup>35</sup>, intravenous glucose tolerance testing<sup>36</sup> and an euglycaemic hyperinsulinaemic clamp procedure.<sup>37</sup> Conversely, an association between diastolic dysfunction and impaired glucose tolerance assessed by OGTT has also been reported in patients with type 2 diabetes<sup>38</sup>, in patients with coronary artery disease<sup>39</sup> and in patients with risk factors for HF<sup>40</sup>. Because chronic HF patients with reduced as well as preserved LVEF were included in the chronic HF patients of **Chapter 4**, we expected to find a relation between glucose tolerance and LVEF. However, LVEF was not independently associated with overall glucose values or shape of the glucose curve. Likewise, patients with acute HF in **Chapter 3** had comparable LVEF in all glucometabolic classification groups.

Another unexpected finding was that levels of BNP, a marker of increased myocardial wall stress, were not associated with glucose response during OGTT in **Chapter 4**. On the other side, E/E' ratio, another marker reflecting left ventricular end-diastolic pressure, appeared to be related to glucose response during OGTT. Although the association in the analyses between E/E' ratio and glucose response during OGTT was strong, this result cannot easily be interpreted. Therefore, it is desirable to confirm these findings in a larger patient population before drawing further conclusions.



In the hypertensive and hypertrophic Dahl salt sensitive rat model, it is known that plasma insulin levels are elevated from weaning age, in contrast to the Dahl salt-resistant rat.<sup>41</sup> Using the euglycaemic hyperinsulinaemic clamp technique and measuring glucose uptake into isolated soleus muscle, Ogihara and colleagues showed that a high salt diet could further increase insulin resistance in Dahl S rats.<sup>42</sup> According to Shehata, this is possibly the result of salt-induced oxidative stress and inflammation.<sup>43</sup> It was hypothesized in **Chapter 5** that glucose tolerance would develop alongside early diastolic dysfunction and worsen with exacerbating HF. However, under the conditions of the experiment, worse glucose regulation could not be related to cardiac hypertrophy, nor cardiac function and BNP production. Again, according to Shehata, the overnight fasting before OGTT causes increased insulin signaling in a greater extent in Dahl S rats on high salt diet because of decreasing body weight.<sup>44</sup> This in analogy with literature reporting that fasting significantly enhanced the insulin signaling pathway in lean compared to obese subjects.<sup>45</sup> Possibly, the fasting and 1h insulin levels did not increase to the expected levels in our study because of decreasing body weight in animals increasingly suffering from HF.

*In conclusion, glucose tolerance was not associated with LVEF, nor cardiac dimensions. In addition, although we previously showed that glucose tolerance was more prevalent in acute versus chronic HF, it was not related to BNP levels. Echocardiographic measures of ventricular filling pressures show an association with glucose tolerance which was not the case with measures of systolic function.*

#### **Other determinants of impaired glucose regulation in HF**

Standard medications in the treatment of HF are known to influence glucose regulation (see introduction), and were therefore studied in relation to glucose tolerance in **Chapter 4**. The findings from the 56 patients with chronic HF did not provide an answer to the research question whether patients with different glucometabolic classification could be characterized by differing HF therapy. As the great majority of the study population was optimally treated (according to the guidelines of the ESC<sup>2</sup>), pharmacological treatment was similar between patients with different glucose regulation. However, even though we did not find any differences in our patient population recruited in our HF clinic, the importance to understand the pathogenesis of the hyperglycaemia, also in relation to HF therapy, has to be stressed, as recently stated in a report from the ADA.<sup>4</sup>

The ideal body weight for CHF patients has been the subject of debate. On the one hand, a low body mass index is a risk factor for mortality in CHF while the presence of obesity (BMI 30-35 kg/m<sup>2</sup>) is associated with lower mortality.<sup>46</sup> On the other hand, more detailed body composition parameters may give another view on the beneficial effects of obesity in CHF. In this respect, Oreopoulos and colleagues suggested that higher lean mass is protective in CHF, while fat mass is associated with detrimental effects such as higher fasting

glucose.<sup>47</sup> The BMI, which was the only measure of body composition in the acute HF patients in **Chapter 3**, was comparable in patients with different glucometabolic profile derived from OGTT. Only patients who were diagnosed with diabetes before hospital admission showed a higher BMI. Comparison of BMI in surviving versus deceased patients, showed a comparable BMI in both groups, in contradiction with the obesity paradox in HF. However, the classic link of glucose intolerance with increasing obesity was confirmed in chronic HF patients in **Chapter 4**, in which detailed data regarding body composition were available. As higher fat mass and fat distribution around the trunk were predictors for glucose response during OGTT, our data agree with the logic from Oreopoulos et al. However, although lean mass was quantitatively not different between groups, it is probable that muscle quality and function are also decisive factors for glucose tolerance.

Although this work did not provide data in respect to histological and molecular properties of the skeletal muscle, some arguments can be proposed in favor of the role of changed muscle quality in impaired glucose uptake and usage in the skeletal muscle of CHF patients. First, the fiber type shift from (insulin sensitive) type I fibers to (insulin insensitive) type II fibers in HF has been known for decades.<sup>48</sup> Second, Doehner et al have showed a decreased density of GLUT4 proteins in skeletal muscle of CHF patients, independent of body composition assessed by dual energy x-ray absorptiometry.<sup>49</sup> Furthermore, not only is the mitochondrial density lower in CHF patients, the activity of mitochondrial enzymes involved in oxidative metabolism is also decreased.<sup>50,51</sup>

Muscle function in terms of extension strength of the upper leg was related to overall glucose values in univariate analysis in **Chapter 4**. In addition, extension strength of the upper leg was negatively correlated with fasting insulin values. This confirmed the hypothesis that higher skeletal muscle strength of the upper leg is associated with better glucose tolerance. Although we believe that higher muscle strength is a reflection of increased levels of physical activity, this was not confirmed by the physical activity levels assessed using the International Physical Activity Questionnaire. Possibly, this methodology is not sensitive enough to correctly reflect daily activity in our patient population, and accelerometers may have resulted in more reliable results.

*In summary, glucose tolerance is related to waist-to-hip fat ratio in patients with chronic HF. Muscle function also seems to be associated with glucose regulation, but this finding needs further research in terms of muscle quality and physical activity levels.*

#### **4) Impact of exercise therapy on impaired glucose regulation in HF**

There is abundant evidence for the effect of increased physical activity or exercise training in the prevention and management of type 2 diabetes mellitus in non-CHF populations.<sup>52-54</sup> More specifically, improved insulin action in response to exercise therapy has been extensively reported and was summarized by Hawley and Lessard.<sup>55</sup> Increased insulin action in skeletal muscle from insulin resistant individuals after exercise training has been attributed to a

rapid increase in the expression of both GLUT4 mRNA and protein in skeletal muscle, increased expression and activity of key signaling proteins involved in insulin transduction (e.g. AMPK, AS160) and an increase in the oxidative capacity of the skeletal muscle by up-regulating lipid oxidation and the expression of proteins involved in mitochondrial biogenesis.<sup>56</sup> As proposed in the introduction, and summarized in figure 1.2, there are many reasons to believe that exercise therapy can also be a strategy to improve glucose regulation in HF. The exercise interventions provided in the human study (in patients with chronic HF; **Chapter 6**) and in the animal model (**Chapter 5**) were followed by reduced insulin release during OGTT while glucose curves were not altered in both studies. A comparable decrease in blood insulin levels during OGTT has been described in other exercise intervention trials.<sup>8,9</sup> The changes in insulin curves during OGTT were significantly related to changes in waist-to-hip fat mass ratio in **Chapter 6**. The latter suggests that exercise training programs in CHF should focus on decreasing abdominal fat, and therefore aim at high caloric expenditure, in order to improve glucose regulation. Moreover, this finding corresponds to the concept of Pedersen and colleagues, who postulate that abdominal obesity is linked with the development of insulin resistance (amongst others) through inflammatory pathways associated with physical inactivity.<sup>57</sup> In this regard, exercise is protective against insulin resistance through the production of myokines (humoral factors produced and released by muscle cells) as well as through the reduction of abdominal obesity.<sup>58</sup>

It is difficult to compare the results from **Chapter 6** with studies describing the effect of exercise training in CHF patients with reduced systolic function, because of the heterogeneity in methods (see table 1.2).<sup>59-67</sup> From five randomized controlled trials, three studies described decreased fasting glucose after high intensity endurance training, and one after moderate intensity endurance training.<sup>60,61,65-67</sup> One study also reported a decrease in fasting insulin values.<sup>61</sup> Exercise intervention trials using whole-body insulin-stimulated glucose uptake to investigate insulin resistance and glucose regulation are contradicting, possibly because of differing exercise prescription.<sup>62,63,66</sup> We cannot draw conclusions regarding the impact of the training modalities used in our studies. Both in the studies from **Chapter 5 and 6**, a relatively high exercise intensity was prescribed for continuous endurance training. In the human study, this was complemented with resistance exercises (endurance type with low intensity and high repetitions), based on literature suggesting that insulin sensitivity can be improved by resistance training.<sup>68</sup> Frequency of exercise trainings varied between **Chapter 5 and 6**, with patients training 2-3 times a week, and animals training 5 times a week. Literature is not conclusive regarding the ideal exercise modalities to improve glucose regulation and insulin sensitivity. Exercise duration has been described to be a determining factor for improving insulin sensitivity in the STRRIDE trial in sedentary overweight subjects.<sup>69</sup> More recently, a graded dose (exercise intensity\*duration\*frequency)-response relationship was described for improvements in insulin sensitivity in healthy volunteers, without evidence of a threshold or maximal dose-response effect.<sup>70</sup> Another research group, however, showed that minimal activity of longer duration

is more effective in improving insulin action compared to short bouts of more vigorous activities, and therefore emphasized the importance of non-sitting time.<sup>71</sup> Finally, it is known that effects of exercise interventions are short-lived when the intervention is terminated. Lifelong adherence to an increased physical activity lifestyle should therefore be promoted.

*To conclude, the exercise interventions used in this project were able to reduce insulin release during OGTT, while glucose responses during OGTT remained unaltered. Exercise-induced changes in insulin release were associated with changes in waist-to-hip fat mass ratio.*

## Clinical implications of our findings

### ***Should the glucometabolic state be assessed in all HF patients?***

Despite the evidence in favor of screening for impaired glucose tolerance in this work, a few words regarding the possibilities and drawbacks for the OGTT as a screening tool are in place. When evaluating the outcome in patients who were offered screening, three biases can be described for screening for diabetes using OGTT.<sup>72,73</sup>

First, the chronic HF patient population of **Chapter 5 and 6** was composed of volunteers, which holds a *self-selection bias*: the patients who choose to participate in these studies are likely to differ from those who don't. Some may even suspect impaired glucose regulation and consider participation in the study as a thorough checkup.

Second, a *lead-time* (time between diagnosis of a disease by screening and the usual time of diagnosis with clinical signs) *bias* may also be present: the use of OGTT during hospitalizations and in HF clinics will lead to earlier diagnoses of (pre)diabetes in many HF patients, and more cases will be discovered before the manifestation of clinical signs. Consequently, there is an over-representation of earlier diagnosed cases, whose survival will be increased by exactly the amount of time their diagnosis was advanced by the screening. Only if an effective early intervention can be offered during the lead-time, prognosis could be improved and a screening would be advantageous.

Third, there is a *length bias*: it is possible that screening with OGTT will pick up patients with a slowly progressing dysfunction in glucose regulation, while fasting glucose values and HbA1c will pick up patients with a rapid progression of the disease, which could be totally different patient populations. A better prognosis in patients with impaired glucose regulation detected with screening could therefore be explained by a difference in disease progression.

In addition to these drawbacks for screening for impaired glucose tolerance and diabetes, the early diagnosis of "another disorder" could possibly increase anxiety and depression in the short term in patients with HF.<sup>74</sup> Personal experience (during patient recruitment phases) confirms that many patients do not want to know their glucometabolic state, because the burden of HF alone

causes enough worries. Many patients and relatives express that they cannot take other bad news.

While an increased risk for worse outcome was described for elevated 2hPG, the clinical relevance of this finding can be debated. Especially because the predictive effect was reduced when age was taken into account, our results do not promote the use of the OGTT when looking for prognostic parameters. However, it is clear that clinical follow-up is necessary in patients with elevated 2hPG in order to prevent worsening of glucose regulation as well as comorbidities associated with diabetes mellitus. In this respect, according to Cochrane, should screening be initiated, we should have conclusive evidence that screening can alter the natural history of the disease in a significant proportion of those screened.<sup>75</sup> In this respect, healthcare professionals might opt to follow the patient more closely and to repeat the testing in 3-6 months. Although it is still an unresolved question, it seems logical that the first therapeutic actions lie in nonpharmacologic lifestyle changes with dietary modifications and exercise training, possibly focusing on reducing the waist-to-hip fat mass ratio. Particularly the achievement of physical activity goals will remain a challenge in this specific patient population and needs more intensive promotion. In addition to lifestyle changes, glucose lowering medication could carefully be considered: although metformin was considered contra-indicated in CHF patients because of negative effects regarding lactic acidosis and fluid retention<sup>15</sup>, it is nowadays commonly used.<sup>27,53</sup>

*With the results of this work in mind, even though some biases for screening studies can be described, we can formulate an advice in favour of glucose tolerance testing in acute and chronic HF patients in order to provide early intervention to prevent worsening of glucose regulation and comorbidities of diabetes.*

### **Physical activity and exercise in HF patients**

For decades, it is known that exercise therapy and exercise training can result in many benefits for HF patients. Our findings add to this knowledge that exercise training can improve glucose regulation in HF. However, although the beneficial effects of exercise therapy for HF patients have extensively been described, it is still not generally implemented in phase II rehabilitation and it suffers from a low referral rate.<sup>76,77</sup> Also after referral, patient compliance is generally low. What's more, phase III rehabilitation in a community setting is almost non-existing in Belgium.

*Taken together, we feel that there is a key role for exercise in the prevention and treatment of (pre)diabetes in HF patients. However, over the whole spectrum of exercise therapy in hospital settings, lifelong exercise in community settings and general increase in physical activity, there is still a long way to go to integrate exercise in the lives of HF patients.*

## Directions for future research

Based on literature review and results obtained during this project, it is evident that many aspects of the pathological process and the impact of exercise therapy in CHF patients with impaired glucose regulation are still to be investigated. Therefore, some suggestions for future research are formulated.

First, all surviving patients who underwent an OGTT during their hospital stay in **Chapter 3** will be invited for a follow-up by their family doctor, 1-2 years after their hospital stay. With these results, we want to investigate the predictive strength of impaired glucose regulation during hospitalization for the evolution of glucose regulation into maintained/overt diabetes or into healthier values after the acute stress of severe illness has passed.

Second, studies investigating the effect of sedentary lifestyles and the effect of minimal intensity activities on glucose tolerance were performed in healthy persons or non-HF patient populations. It would be interesting to investigate the impact of bed rest and sedentarism on glucose regulation in acute and chronic HF settings.

Third, as the skeletal muscle is a key component in the pathological process of impaired glucose regulation, the impact of exercise therapy on cellular and molecular mechanisms in the skeletal muscle needs more investigation.

Fourth, our animal model isolated one HF-related factor, (salt-induced) cardiac hypertrophy, to investigate the association with glucose regulation. Other studies may investigate other isolated determinants (e.g. hormonal imbalance, pressure overload, diastolic dysfunction, ...) in relation to glucose regulation.

## Final conclusions

The novel insights provided by this doctoral work may lead to a more comprehensive evaluation of glucose tolerance in acute and chronic HF settings. Furthermore, they can lead to a better understanding of the prognostic impact and of the factors associated with impaired glucose tolerance in HF patients. In addition, our findings support more intensive promotion of physical activity for HF patients in order to improve glucose regulation.

### **Take-home messages:**

The majority of HF patients seen in clinical practice suffers from undiagnosed impaired glucose regulation.

From all three markers for diagnosis of (pre)diabetes (fasting plasma glucose, 2h plasma glucose during OGTT and HbA1c), elevated 2h plasma glucose during OGTT is the better predictor for mortality combined with rehospitalization for cardio-renal causes.

In chronic HF patients, impaired glucose regulation in chronic HF patients is related to waist-to-hip fat mass ratio.

Exercise training is able to reduce insulin release in reaction to glucose loading in the same chronic HF population.

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**Samenvatting**

**Summary**



Heart failure (HF) is one of the most important causes of morbidity and mortality in the Western world. Despite advances in medical therapy for cardiac patients in general, the prognosis for HF patients is still poor. Moreover, many patients with chronic HF are believed to have unrecognized diabetes, which is associated with a worse prognosis. In this thesis, the prevalence and characteristics of impaired glucose regulation, as well as the impact of exercise therapy on glucose regulation were studied in HF.

We found evidence that the prevalence of impaired glucose regulation is severely increased in acute as well as chronic HF patients. It was demonstrated that from 98 patients hospitalized for acute HF, only a nonsignificant proportion (1%) presented with a normal glucose profile on the day of hospital discharge. Furthermore, our results showed that from all glycaemic markers, increased fasting blood glucose and HbA1c concentrations contain little prognostic value, while impaired glucose tolerance was the better predictor for morbidity (cardio-renal events) and mortality (**chapter 3**). In a second observational study, the majority (80%) of 56 patients with stable chronic HF (without diagnosed diabetes) presented with an abnormal glucose regulation (**chapter 4**). In acute and chronic HF patients, oral glucose tolerance testing was more efficient to detect significantly impaired glucose regulation compared to fasting glucose and HbA1c concentrations (**chapter 3-4**). In the investigation of the relation between impaired glucose regulation and possible explanatory parameters in chronic HF patients, glucose tolerance was associated with echocardiographic measures of ventricular filling pressures, but not with measures of systolic function nor cardiac dimensions. Furthermore, we found that increased waist-to-hip fat ratio was a predictor of impaired glucose regulation. Muscle function also seemed to be associated with glucose regulation, but this finding needs further research (**chapter 4**).

Available literature and official guidelines do not provide effective therapies to improve glucose regulation in HF patients. Therefore, the impact of exercise training on glucose regulation in HF was investigated in an animal model for HF (**chapter 5**) and in stable chronic HF patients (**chapter 6**). The exercise interventions used in both experiments were able to reduce insulin release during oral glucose tolerance testing, while glucose responses remained unaltered. In stable chronic HF patients, exercise-induced changes in insulin release were associated with changes in waist-to-hip fat mass ratio (**chapter 6**).

These findings are clinically relevant because they may lead to a more comprehensive evaluation of glucose tolerance in acute and chronic HF settings. Furthermore, they can lead to a better understanding of the prognostic impact and of the factors associated with impaired glucose regulation in HF patients. In addition, our findings support more intensive promotion of physical activity for HF patients in order to improve glucose regulation.



Hartfalen (HF) is één van de belangrijkste oorzaken van mortaliteit en overlijden in de Westerse wereld. Ondanks de verbeterde therapieën voor hartpatiënten in het algemeen, blijft de prognose slecht voor patiënten met HF. Terwijl het geweten is dat een verstoorde glucoseregeling de prognose verder negatief beïnvloedt, wordt er toch verondersteld dat veel patiënten met HF lijden aan niet-vastgestelde diabetes. In deze thesis werden de prevalentie en karakteristieken van verstoorde glucoseregeling alsook de invloed van fysieke training op de glucoseregeling bestudeerd in HF.

We hebben aangetoond dat de prevalentie van verstoorde glucoseregeling zeer hoog is in acuut (**hoofdstuk 3**) en in chronisch HF (**hoofdstuk 4**). In een studiepopulatie van 98 patiënten, gehospitaliseerd voor acuut HF, vertoonde slechts een kleine minderheid (1%) een normaal glucoseprofiel op de dag van ontslag uit het ziekenhuis. Daarenboven hadden verhoogde nuchtere bloed glucose en HbA1c concentraties weinig prognostische waarde, terwijl een verstoorde glucosetolerantie een betere predictor was voor (cardio-renale) morbiditeit en mortaliteit (**hoofdstuk 3**). Ook bij patiënten met stabiel chronisch HF (zonder gediagnostiseerde diabetes), vertoonde 80% van de studiepopulatie een verstoorde glucoseregeling (**hoofdstuk 4**). Zowel bij patiënten met acuut als chronisch HF was de orale glucose tolerantie test efficiënter in het identificeren van patiënten met een verstoorde glucoseregeling, vergeleken met nuchtere glucose en HbA1c concentraties (**hoofdstuk 3-4**). In de zoektocht naar mogelijke verklarende factoren voor een verstoorde glucoseregeling in chronisch HF werd een verband vastgesteld met echocardiografische parameters die de vullingsdrukken binnen de ventrikels beschrijven. Anderzijds, werd er geen relatie gevonden met metingen van systolische pompfunctie of cardiale dimensies. Verder toonden onze resultaten ook het belang van een verhoogde buik-heup vetverhouding voor een verstoorde glucoseregeling bij patiënten met chronisch HF. Ook spierfunctie kon gerelateerd worden aan glucoseregeling, maar dit gegeven moet zeker verder onderzocht worden (**hoofdstuk 4**).

Reeds verschenen literatuur en officiële richtlijnen geven niet aan welke therapieën de glucoseregeling van patiënten met HF kunnen verbeteren. Daarom werd het effect van fysieke training op de glucoseregeling bestudeerd in een diermodel voor HF (**hoofdstuk 5**) en in patiënten met stabiel chronisch HF (**hoofdstuk 6**). De trainingsinterventies van beide experimenten resulteerden in een verlaging van de insulinerespons tijdens een glucose tolerantie test, terwijl de glucosecurve dezelfde bleef. In de humane studie konden de veranderingen in insulinerespons na training gerelateerd worden aan veranderingen in de buik-heup vetverhouding (**hoofdstuk 6**).

Deze bevindingen zijn klinisch relevant omdat ze kunnen leiden tot een uitgebreidere evaluatie van de glucosetolerantie binnen de HF klinieken. Ze kunnen ook leiden tot een beter begrip van de prognostische impact en van de bepalende factoren van een verstoorde glucoseregeling. Daarenboven moedigen onze resultaten een doorgedreven promotie aan van fysieke activiteit voor patiënten met HF voor het verbeteren van de glucoseregeling.



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## About the author

An Stevens was born on December 24, 1979 in Maaseik (Belgium). She graduated in Latin-Sciences at the Stedelijke Humaniora Dilsen in 1997. Afterwards, she started her studies in Physical Education and obtained a Master degree in 2001, followed by a European Master Degree in Adapted Physical Activity in 2002 at the Catholic University of Leuven.

In the following 4 years, she combined scientific work at the cardiac rehabilitation department of the Catholic University of Leuven with activities as an exercise therapist in cardiac rehabilitation at the University Hospital Gasthuisberg. During these years, she also supervised training sessions in Harpa, a sports club for cardiac patients, in her free time. After moving from Leuven to Hoeselt, she was a teacher at the Stedelijke Humaniora and the Stedelijke Bouwvakschool in Dilsen for 3 years (2007-2010).

During the doctoral project, she also followed several courses. First, she followed the Course on Laboratory Animal Science at Hasselt University. To improve her scientific skills and knowledge in the research field of heart failure, she received international training organized by the European Association for Cardiovascular Prevention and Rehabilitation: "Exercise Rehabilitation and Long Term Management of Heart Failure Patients" in Bern 2011 and "3<sup>rd</sup> Training Course on the use of Cardiopulmonary Exercise Testing in Cardiology" in Veruno 2013. Followed courses regarding transferrable skills (Project Management, Academic Writing and Presenting, amongst others) were organized by the Doctoral School of Medicine and Life Sciences. In addition, she was appointed as a tutor in 'Rehabilitation of Cardio-respiratory and Metabolic Disorders', and in 'Didactics and Physical Education' in the Master programme of Rehabilitation Sciences and Physiotherapy.

An is married to Kris Hansen and is the proud mother of Arne (°2006), Arthur (°2007) and Roos (°2008).

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