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Heart failure: Role of metabolic biomarkers, ejection fraction, and sex

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To my beloved ones

Be kind, for everyone you meet is fighting a hard battle
Ian MacLaren

From Karolinska Institutet, Department of Medicine
Division of Cardiology, Stockholm, Sweden

Heart failure: Role of metabolic biomarkers, ejection fraction, and sex

by

Ulrika Ljung Faxén

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SAMMANFATTNING

Bakgrund
Hjärtsvikt är en vanlig sjukdom som medför sänkt livskvalitet och stor risk för död. Idag delas hjärtsvikt in i tre typer baserat på andelen blod som pumpas ur vänster kammare vid varje hjärtslag: Hjärtsvikt med bevarad (HFpEF), måttligt sänkt (HFmrEF) och sänkt ejektionsfraktion (HFrEF). Kunskap om hur vi ska behandla patienter med HFpEF och i vilken utsträckning HFpEF och HFrEF skiljer sig åt saknas fortfarande i stor utsträckning. På samma sätt är också kunskapen om eventuella könsskillnader vid hjärtsvikt bristfällig, trots att hälften av patienterna är kvinnor.

Syfte
Att undersöka

(1) om den hämning av tillväxthormonaxeln som finns hos patienter med HFrEF även förekommer hos patienter med HFpEF.
(2) nivåer och betydelse av de fetmarelaterade peptiderna leptin och adiponectin hos patienter med HFpEF och HFrEF.
(3) potentiella könsskillnader i livskvalitet hos patienter med HFpEF.
(4) potentiella könsskillnader i nivåer av och prognostisk betydelse av hjärtsviktshormonet N-terminal pro natriuretisk peptid typ B (NT-proBNP) vid kronisk hjärtsvikt

Resultat
Hämning av tillväxtfaktorhormonaxeln
Vid analys av Insulin-like growth factor 1 (IGF-1) och dess bindarprotein (IGFBP-1) fann vi att både HFpEF och HFrEF uppvisade en hämning av tillväxtfaktorhormonaxeln mätt som förhöjda nivåer av IGFBP-1. Förhöjda nivåer av IGFBP-1 var också associerade med förhöjt NT-proBNP eller grad av hjärtsvikt. Emellertid verkade hämningen av tillväxtfaktorhormonaxeln vara mer uttalad i HFrEF och sänkta nivåer av IGF-1 var associerade med sämre prognos enbart hos patienter med HFrEF.

Fetmarelaterade leptin och adiponectin
Vid analys av leptin och adiponectin fann vi förhöjda nivåer av dessa i både HFpEF och HFrEF. Emellertid uppvisade endast HFrEF den så kallade fetmaparadoxen, d.v.s. att förhöjda nivåer av leptin är associerade med bättre prognos. Fynden talar för att HFpEF har en mer konventionell riskprofil avseende leptin och fetma.

Livskvalitet hos kvinnor och män med HFpEF
Vi fann att både kvinnor och män med HFpEF har sänkt livskvalitet. Kvinnorna rapporterade dock sämre generell livskvalitet och associationen mellan livskvalitet och grad av hjärtsvikt var svagare hos kvinnor. En koppling mellan sänkt livskvalitet och sämre prognos fanns endast hos män. Fynden talar för att sänkt livskvalitet hos kvinnor i stor utsträckning förklaras av andra faktorer än hjärtsvikten i sig vilket bör beaktas i eftersträvan att förbättra patienternas livskvalitet.

Betydelse av kön för nivåer av svikthormon vid olika hjärtsviktstyper
Nivåer av svikthormonet NT-proBNP var högre hos kvinnor än hos män i alla de undersökta hjärtsviktstyperna. Faktorer relaterade till nivåer av NT-proBNP och associationen mellan förhöjda nivåer och prognos var emellertid likartade. Detta stödjer att NT-proBNP kan användas för prognostisering av hjärtsvikt oaktat kön, men betydelsen av de relativt stora könsskillnaderna i nivåer hos patienter med HFpEF och låga NT-proBNP nivåer behöver utvärderas.

Slutsats
HFpEF och HFrEF uppvisar väsentliga likheter och skillnader relaterade till metabolism, hormonnivåer och kön. Betydelsen av detta avseende sjukdomsutveckling och eventuell behandling återstår att undersöka.

ABSTRACT

Background

Heart failure (HF) is common and associated with impaired quality of life (QoL) and poor prognosis. There is a ternary classification of HF based on ejection fraction (EF): HF with preserved(HFpEF), mid-range EF(HFmrEF), and reduced EF(HFrEF). How to treat the syndrome of HFpEF, and the extent to which HFpEF and HFrEF are similar, still remain elusive. Likewise, despite the fact that half of the patients with HF are women, the role of sex in HF is often overlooked.

Aims

(1) To investigate whether HFpEF and HFrEF share features of anabolic impairment regarding insulin-like growth factor 1 (IGF-1) and IGF binding protein-1 (IGFBP-1).
(2) To assess levels of the obesity related peptides, leptin and adiponectin, and whether the obesity paradox exists in HFpEF.
(3) To investigate potential sex-specific differences in QoL in HFpEF.
(4) To assess the impact of sex on N-terminal B-type natriuretic peptide (NT-proBNP) in chronic HF across the EF spectrum.

Results

The IGF-1 axis in HFpEF and HFrEF

Serum IGF-1and IGFBP-1 concentrations and their associations with other biomarkers and outcomes were analysed in patients with HFpEF and HFrEF. IGF-1 concentrations were lower and associated with poor prognosis in HFrEF only. However, IGFBP-1 was increased and associated with NT-proBNP in both HF phenotypes. This suggests inhibition of the IGF-1-axis in both syndromes and a possible mechanistic link between IGFBP-1 and natriuretic peptides in HF.

Leptin and adiponectin in HFpEF and HFrEF

Serum leptin and adiponectin concentrations and their associations with other biomarkers and outcomes in patients with HFpEF and HFrEF were analysed. Our findings indicate that the two HF phenotypes share elevated levels of leptin and adiponectin. The obesity paradox regarding leptin, with higher levels being associated with better outcome was nevertheless only demonstrated in HFrEF, pointing towards a more conventional metabolic profile in HFpEF.

Sex and quality of life in HFpEF

We assessed QoL in HFpEF through generic and HF specific QoL instruments. Women with HFpEF express worse global QoL than men. Overall, QoL was only weakly associated with measures of HF severity and the associations were weaker in women. In men only, poor QoL was associated with worse outcome. Overall, this suggests, that in order to improve QoL in HFpEF patients, in particular in women, other factors than HF must be addressed.

Impact of sex on NT-proBNP across HF phenotypes

We analysed concentrations of NT-proBNP, and associations with clinical characteristics and outcomes in the three HF phenotypes, by sex. Women with chronic HF across the entire EF spectrum have higher NT-proBNP concentrations than men. However, associations between NT-proBNP concentrations and clinical characteristics as well as outcomes are largely similar. This supports the current use of NT-proBNP for prognostic purposes across HF phenotypes but the impact of sex-differences in the lower NT-proBNP range warrants further investigation.

Conclusion

HFpEF and HFrEF display important similarities and differences related to metabolic biomarkers, natriuretic peptides, and sex. The impact of these factors on the pathogenesis of and in manifest HF, and as potential therapeutic targets warrants further investigation.

LIST OF ORIGINAL PAPERS

I. HFpEF and HFrEF display different phenotypes as assessed by IGF-1 and IGFBP-1

Faxén UL, Hage C, Benson L, Zabarovskaja S, Andreasson A, Donal E, Daubert JC, Linde C, Brismar K, Lund LH, Journal of Cardiac Failure, 2017, Apr, 23(4):293-303

II. HFpEF and HFrEF exhibit different phenotypes as assessed by leptin and adiponectin

*Faxén UL, Hage C, Andreasson A, Donal E, Daubert JC, Linde C, Brismar K, Lund LH,*International Journal of Cardiology, 2017 Feb 1; 228 709-716

III. Patient reported outcome in HFpEF: Sex-specific differences in quality of life and association with outcome

Faxén UL, Hage C, Donal E, Daubert JC, Linde C, Lund LH, International Journal of Cardiology, 2018, 267 128-232

IV. N-terminal pro-B-type natriuretic peptide in chronic heart failure: The impact of sex across the ejection fraction spectrum

Faxén UL, Strömberg A, Dahlström U, Andersson DC, Lund LH, Savarese G, Manuscript

ABBREVIATIONS

BMI	body mass index
BNP	B-type natriuretic peptide
CAD	coronary artery disease
CI	confidence interval
cGMP	cyclic guanosine mono phosphate
EF	left ventricular ejection fraction
eGFR	estimated glomerular filtration rate
ESC	European Society of Cardiology
EQ-5D	EuroQoL 5 dimensions
EQ-VAS	EuroQoL visual analogue scale
HF	heart failure
GH	growth hormone
HFmrEF	heart failure with reduced ejection fraction
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with mid-range ejection fraction
HOMA-IR	homeostatic model assessment-insulin resistance
HR	hazard ratio
IGF-1	insulin-like growth factor 1
IGFBP-1	insulin-like growth factor binding protein 1
IHD	ischemic heart disease
KaRen	Karolinska-Rennes
KCCQ	Kansas City Cardiomyopathy Questionnaire
ln	natural logarithm
LV	left ventricular
NT-proBNP	N-terminal pro B-type natriuretic peptide
NO	nitric oxide
NP	natriuretic peptide
MLHFQ	Minnesota Living With Heart Failure Questionnaire
NYHA	New York Heart Association
OR	odds ratio
PRO	patient reported outcome
RAAS	renin-angiotensin-aldosterone system
ROS	reactive oxygen species
SBP	systolic blood pressure
SD	standard deviation
SGLT-2	sodium-glucose co-transporter-2
SNS	sympathetic nervous system
T2DM	diabetes type 2
QoL	quality of life

INTRODUCTION

Heart failure (HF) is a common syndrome and affecting 2% of the Western population, 10% above the age of 65 and up to 20% above 75 years[1]. World-wide, more than 26 million people are living with HF and it is associated with poor quality (QoL) of life as well as high morbidity and mortality[2]. The global, overall, annual cost of HF has been estimated to $108 billon[3], but is expected to triple between 2010 and 2030 due to increased prevalence of sedentary life style and aging of the population[4].

Despite decades of success-stories in HF-therapy with neurohormonal antagonists, use of devices, and lately enhancement of adaptive hormonal pathways[5], there is still no evidence based therapy for almost half of the patients suffering from HF. Considering the global burden of the disease, there is an urgent need to expand the understanding of the syndrome, to find new treatment targets, and to develop therapies improving not only survival, but also patient reported outcome (PRO)[6].

About half of the patients living with HF are women. The risk of developing HF, the phenotypic expression of the HF syndrome, outcome and response to therapy are different in men and women[7]. Furthermore, diseases associated with HF, such as obesity, diabetes, and hypertension are known to affect women and men differently[8]. Women are still underrepresented in clinical trials and both preclinical and clinical research are still mainly performed in males or without sex-specific analyses[9].

Against this background, with the overall aim of improving the understanding of heterogeneous syndrome of HF, this thesis addresses biomarkers related to HF, PRO, and the role of sex across the ejection fraction (EF) spectrum.

BACKGROUND

Definition of heart failure and role of ejection fraction

According to the definition by the European Society of Cardiology (ESC), HF is "*a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral edema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress"*[10]. Physiologically this means that the heart is unable to meet the metabolic demands at rest or during exercise, or that the metabolic requirements are met only through increased filling pressures.

Normal filling of the heart in diastole and adequate ejection of blood in systole are essential for cardiac performance. Thus both diastolic and systolic function as well as vascular compliance must be maintained for a normal cardiac function. However, HF research and therapy have for long focused on systolic dysfunction, partly due to the widespread use of low left ventricular (LV) EF for diagnosing HF in clinical practice. When studies in the early 2000 demonstrated a bimodal distribution of EF among HF patients[11,12], the paradigm of using EF to categorize HF evolved. In 2016, the ESC guidelines on HF proposed a ternary classification of chronic HF: HF with preserved (HFpEF), mid-range (HFmrEF), and reduced EF (HFrEF), characterized by EF≥50%, 40-49%, and <40% respectively. Apart from signs and symptoms of HF and EF, the diagnosis of HFmrEF and HFpEF also requires elevated natriuretic peptides (NPs) and relevant structural or functional heart disease such as LV hypertrophy, left atrial enlargement, and/or diastolic dysfunction. Since diastolic dysfunction can exist throughout the EF spectrum, and since systolic function is not necessarily normal in HFpEF, the old nomenclature of systolic and diastolic HF is no longer used[10].

Depending on cohort analyzed, about half of the HF patients have HFrEF, and the remaining HFmrEF or HFpEF[11,13,14], **Figure 1**.

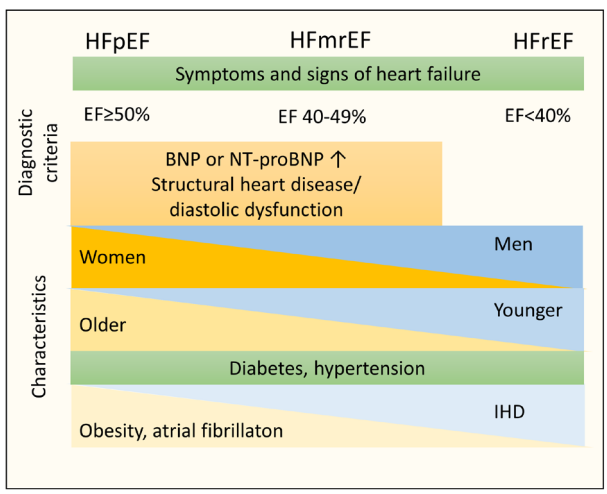

Figure 1. Schematic overview of diagnostic criteria and characteristics of the three EF phenotypes.

HFpEF and HFrEF- one disease or two?

It has been debated whether HFpEF is indeed the same disease as HFrEF with different EF, or whether the two, despite similar symptoms and signs, are actually pathophysiologically different. The prevailing view is that in HFrEF an initial injury (index event), e.g. myocardial infarction, leads to loss of myocardial function. This in turn triggers maladaptive neurohormonal activation, myocardial remodeling including LV dilatation, and eccentric hypertrophy leading to manifest HF. In contrast, in HFpEF a comorbidity driven inflammatory state leads to endothelial damage and microvascular dysfunction through decreased nitric oxide (NO) and cyclic guanosine monophosphate (cGMP), **Figure 2**. Ultimately this results in concentric LV remodeling and reduced myocardial compliance. The bimodal distribution of EF, the lack of benefit in HFpEF of the neurohormonal antagonists, and different macroscopic and myocellular patterns of LV remodeling support this paradigm. Furthermore, the disease progression, comorbidity profile, and the sex-distribution are different in HFpEF compared with HFrEF[11,15].

The introduction of HFmrEF to the European HF guidelines in 2016 was made to dichotomize between "true" HFrEF or HFpEF and the "grey or mixed area" between these syndromes. This is considered important in terms of etiology, demographics, co-morbidities, response to therapies, and design of interventional trials[10].

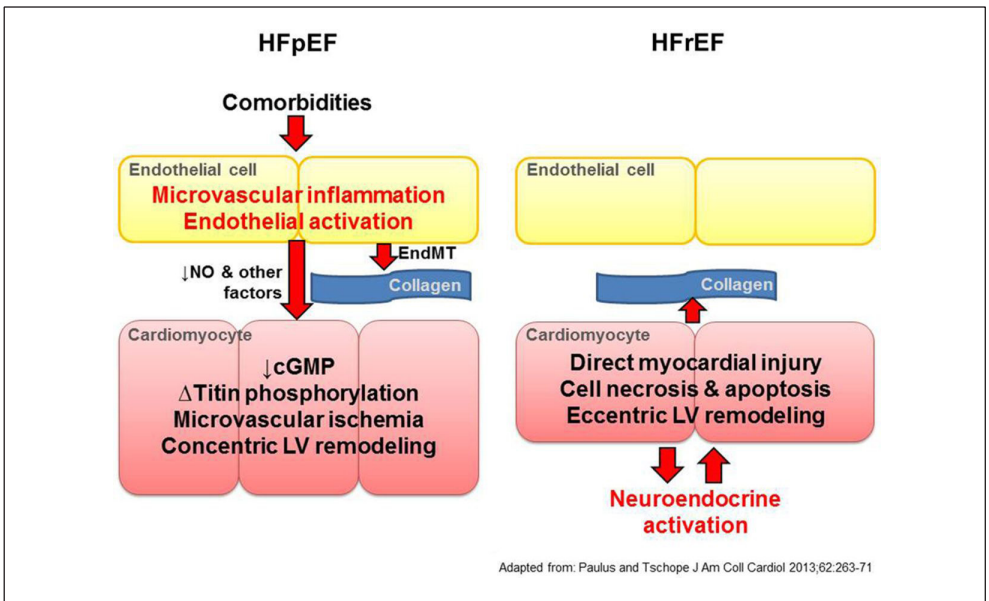

Figure 2. Overview of the pathogenesis in HFpEF and HFrEF. EndMT, endothelial mesenchymal transition. Heart, Lam CSP, Lund LH, 2016, reproduced with permission from the publisher.

HFrEF- pathophysiology, risk factors, clinical characteristics, and treatment

As described, HFrEF is caused by a direct injury or disease state affecting the myocardium, leading to reduced LV contractility. About 2/3 of HFrEF cases are caused by ischemic heart disease (IHD). Other primarily cardiac aetiologies include cardiomyopathies, myocarditis,

and valvular diseases. Extra-cardiac causes of HFrEF are abundant, including endocrine disorders, systemic inflammatory diseases, alcohol- or drug-abuse, or toxic reactions. Important risk factors are IHD, diabetes, smoking, and hypertension[16].

The initial myocardial injury reduces cardiac output, which leads to compensatory neurohormonal activation to preserve oxygen delivery. These mechanisms include activation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS). While these mechanisms initially are adaptive, they become maladaptive in long term, leading to cardiac remodeling with eccentric hypertrophy, LV dilatation, and cardiomyocyte dysfunction. This will further reduce cardiac output, and creates the vicious circle of worsening HF. The basis of HF therapy involves inhibiting these neurohormonal pathways with β-blockers and RAAS-inhibitors. Moreover, adaptive responses in HF can also be targeted, such as with the inhibition of the enzyme neprilysin, that increases the bioavailability of presumably cardioprotective NPs[10,17].

HFpEF- pathophysiology

HFpEF is characterized by multiple impairments in ventricular diastolic and systolic function, vascular function, and reserve capacity[18]. The pathophysiological hypothesis is that common non-cardiac diseases, such as hypertension, obesity, diabetes type 2 (T2DM), anemia, pulmonary disease, or chronic kidney disease induce a pro-inflammatory state. This leads to endothelial dysfunction, microvascular inflammation, recruitment of immune cells, reactive oxygen species (ROS), and lower bioavailability of NO resulting in microvascular dysfunction and cardiac remodeling[15,19,20]. The pluricellularity of the heart and the important role of other cell-types than cardiomyocytes, such as endothelial cells, immune cells, cardiac stem cells, and fibroblasts are recognized. In particular, the role of the microvascular endothelial cells is stressed, both as a sensor of the local environment in the heart and in the bloodstream and as an effector in endothelium derived signaling, affecting adjacent cells. Apart from NO, a plethora of small molecules, peptides, and proteins such as prostacyclin, angiotensin-II, endothelin, growth-factors, and inflammatory cytokines are involved in this complex cellular crosstalk[21].

The diastolic dysfunction in HFpEF is linked to both myocyte hypertrophy and passive stiffness due to fibrosis and phosphorylation of titin, as well as impaired active relaxation[15,22-24].

HFpEF- risk factors, clinical characteristics, and treatment

Patients with HFpEF tend to be older and to have a higher prevalence of obesity, hypertension, and atrial fibrillation compared with patients with HFrEF[14,16,25]. The aging population and the increase in prevalence of comorbidities are leading to a growth in prevalence of HFpEF by 10% per decade[18].

In contrast to HFrEF, there is a female predominance in HFpEF[26] and there seem to be sex specific traits in cardiac structure and function making women more prone to develop HFpEF with aging. Arterial stiffening is greater in women and women appear more disposed to develop concentric LV remodeling with pressure overload[8,27]. In HFpEF, diastolic impairment is also more pronounced in women[28].

There is currently no evidenced based therapy for patients with HFpEF. Contrary to expectations, conventional neuro-hormonal antagonists, like β-blockers and RAAS-blockade have not been convincingly efficient[29-32]. Numerous novel interventions, such as sildenafil, organic or inorganic nitrates, and soluble guanylate cyclase stimulators have been studied but have generally failed or not yet been convincingly proven efficient[33]. Reasons for the lack of success might be the heterogeneity of HFpEF and the failure to match treatment with phenotype, disease stage, and severity[6,34-37]. Furthermore, only a minority of patients presenting to hospitals and clinics are actual candidates for interventional trials with strict selection criteria, there is also a concern about the generalizability of trial results[36]. As such, HFpEF is considered one of the major challenges in contemporary cardiology[38].

HFmrEF

The middle HF phenotype, HFmrEF was introduced not because of a suspicion of it being a pathophysiologically unique HF phenotype, but rather due to the heterogeneity of the group, the possible transition of patients from one EF category to another, the imprecise EF measure, and to stimulate and refine research[10,39,40]. Between 13 and 24% percent of patients in population based studies of HF have HFmrEF. On group level, HFmrEF seem to be in between HFpEF and HFrEF regarding age-, sex- and comorbidity-profile, with the important exception of IHD, regarding which HFmrEF is more similar to HFrEF[14,41-44]. In post-hoc analyses, patients with HFmrEF also seem to respond to conventional HF therapy in a similar way as HFrEF[45,46].

Prognosis in heart failure

HF is associated with high mortality. The prognosis in HFpEF is appears to be slightly better than in HFrEF[25]. Similar prognosis across the EF spectrum has been reported from the American *Get With the Guidelines* registry with a one-year mortality of 37,5 vs, 35.1 vs 35.6% after acute HF hospitalization in HFrEF, HFmrEF, and HFpEF respectively. Hospital readmission rates were nevertheless higher in HFrEF and HFmrEF vs. HFpEF; 30.9 and 28.4 vs. 24.3 % [47]. In chronic HF, 1-year mortality in Europe was higher in HFrEF (8.8%) vs. HFpEF (6.3%), with HFmrEF intermediate (7.6%)[44]. In a Swedish cohort of a mix of in- and outpatients, crude 1-year mortality was 15% in HFrEF, 14% in HFmrEF, and 17% in HFpEF[14].

Patient reported outcomes

While focus in interventions in HF, in particular clinical trials, for long has been on reducing "hard endpoints", like mortality or rehospitalization, a holistic approach to HF care, including patient satisfaction and PRO is now emphasized[48]. The Food and Drug Administration even stresses the use of PRO as a clinical trial endpoint[49].

It is well established that patients with HFrEF experience impaired QoL, and women with HFrEF report lower QoL than men[7,50]. As in HFrEF, QoL is impaired in HFpEF and has been associated with poor prognosis[50-53]. There is a substantial variability in QoL, independent of HF severity, and impaired QoL in patients with HF appears largely explained by other factors

than HF itself[52,54]. Furthermore, while poor QoL is associated with poor outcomes, improved QoL in the trial setting is often linked to better outcome[55].

There are several instruments for assessment of QoL, both HF-specific focusing on disease specific impairments, and generic instruments. Disease specific instruments may be preferred to generic when assessing specific treatment effects. Generic instruments provide a broader assessment of QoL, rather than the impact of a particular disease[56]. The most highly ranked and commonly used HF specific instruments are Kansas City Cardiomyopathy Questionnaire (KCCQ), Minnesota Living with Heart Failure Questionnaire (MLHFQ), and Chronic Heart Failure Questionnaire[57]. All three have a good reliability (internal consistency, test-retest reliability, and interrater reliability) and validity. The choice of instrument depends rather on the setting in which they are used, since for example their qualities regarding forms of administration (KCCQ and MLHFQ can be self-administered) and sensitivity to change differ (KCCQ and Chronic Heart Failure Questionnaire are superior to MLHFQ)[57].

Commonly used generic QoL instruments are Short-Form 36 Health Survey, the Sickness Impact Profile, and the EuroQoL 5D (EQ-5D)[56].

Role of sex

Half of the patients living with HF are women and the prevalence of HF in adults in the United States between 2011-2014 was 2.4% in men vs. 2.6% in women[1]. While women are diagnosed with HF later in life, the overall lifetime risk is about 20% in both sexes[7]. Both in HFrEF and HFpEF/HFmrEF, prognosis is better for women, despite lower QoL and greater functional impairment in women[7,25,58,59].

While men are overrepresented in HFrEF, women are more likely to develop HFpEF[26]. Sex-specific differences in cardiac structure and function and the loss of protective estrogen after menopause are possible explanations[27]. As mentioned, ventricular arterial stiffening is greater in women and women more likely develop concentric LV remodeling with pressure overload[8,27]. The diastolic dysfunction is also more pronounced in women with HFpEF[28].

Furthermore, comorbidities related to HF seem to affect women and men differently and women with diabetes or hypertension have a higher risk of developing HF than men[8,60]. Autoimmune diseases and iron-deficiency, that are related to HF and inflammation, are also more common among women. In addition, pregnancy related disorders like preeclampsia are evidently unique for women[8], **Figure 3.**

The evidence-based treatment for HFrEF is largely based on trials where women have been underrepresented with a fraction of women below 30%[7,61]. In HFpEF, evidence based therapy is lacking, but despite the high prevalence of HFpEF among women, women are often excluded from trials, likely due to higher age or comorbidity burden[61,62]. When hospitalized for HF, women are equally likely to receive diuretics, but less likely to be treated with vasoactive therapy and evidence-based oral therapy[63].

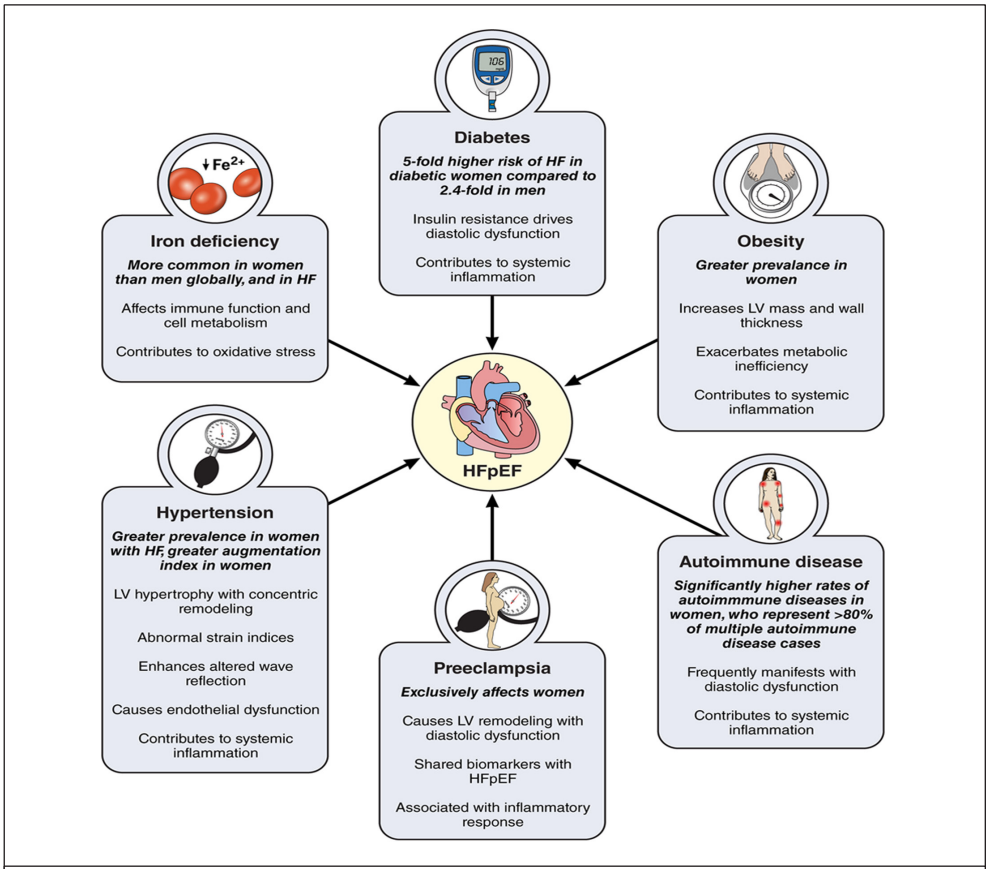

Figure 3. The influence of comorbidities in the pathogenesis of HFpEF in women. Beale et al., Circulation, 2018. Reproduced with permission from the journal.

Natriuretic peptides

The NP family includes a large number of peptides and peptide fragments, e.g. A-type NP, B-type natriuretic peptide (BNP), and C-type NP[64]. While they all seem to contribute to the adaptive neurohormonal system in HF, the most well-known and clinically used is BNP, or the cleaving fragment of the pro-hormone, N-terminal-proBNP(NT-proBNP)[65,66].

In the healthy state, BNP is mainly secreted in the atria, whereas with increased ventricular wall stress in HF, secretion is shifted to the ventricle. BNP acts through the NP-receptor A, a guanylate-cyclase receptor, and activation leads to increased formation of cyclic guanosine mono-phosphate (cGMP). The actions of BNP counteracts the activation of the RAAS and SNS by causing vasodilation, natriuresis, and opposing adverse remodeling[64,67]. NT-proBNP is produced in equimolar amounts as BNP, but exists in higher plasma concentrations due to longer half-life. The biologic role of NT-proBNP, if any, is not known. However, due to a longer half-life and stability in vitro, NT-proBNP is widely used instead of BNP to measure BNP activity[67].

The use of NPs is well established for diagnostic[10,68-70] and prognostic[13,71-73] purposes in HF. NPs are also used for trial selection purposes and as surrogate outcomes in both acute and chronic HF trials, although the latter has not convincingly been shown to translate into better outcomes[74]. Considering their adaptive effects in HF, they are used and investigated as treatment targets[17,64] and for guidance of therapy[43,75-77].

Low NPs are considered to have a strong negative predictive value in excluding HF[10], although normal BNP is indeed found in patients with HFpEF despite increased filling pressures[78]. Levels of BNP/NT-proBNP are higher in HFrEF compared to HFmrEF and HFpEF[79]. High levels are associated with severity of HF and poor prognosis in both the acute and chronic setting across all EF phenotypes[79-81].

Females, both healthy and with acute decompensated HF, have higher NP concentrations than men, which may at least partially be explained by sex-hormones, i.e. higher oestrogen levels [82-85]. Nevertheless, despite higher concentrations in females with acute HF, the short term prognostic ability of BNP is similar in both sexes across the EF spectrum[13]. In contrast, in chronic HF higher[86], similar[28], and lower [59] NT-proBNP concentrations are reported in females vs. males. Likewise regarding long term prognosis, data is diverging and a mix of EF phenotypes limits the interpretation[87-89]. In chronic HF, population based studies are lacking but in the trial setting of mixed HFpEF/HFmrEF, data supports similar prognostic power in females and males[59].

Role of obesity and diabetes

Obesity and T2DM are not only risk factors for IHD and HFrEF, but may also participate in the pathogenesis of HFpEF through low grade inflammation and microvascular disease[15,90-92], **Figure 4**. The adipose tissue is highly metabolically active through the excretion of both pro-

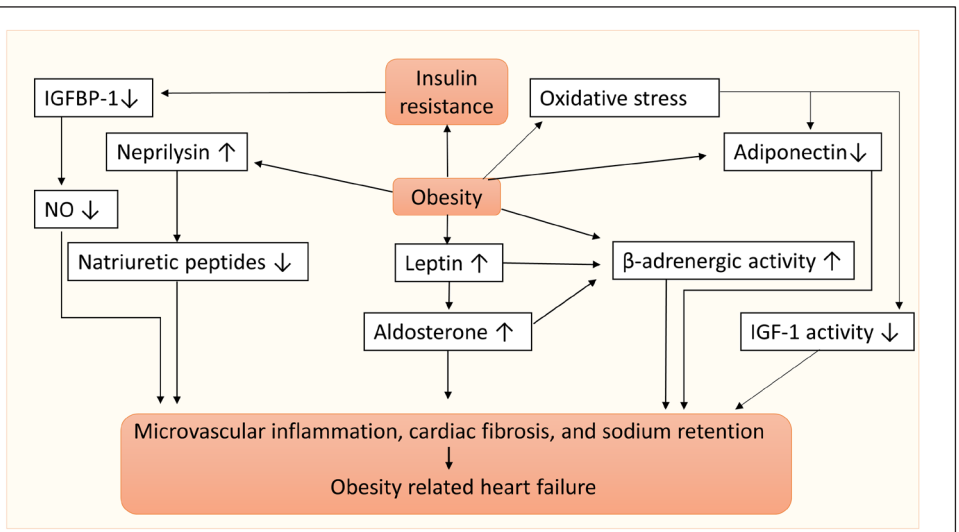

Figure 4. Overview of potential pathogenic mechanisms caused by obesity and insulin resistance contributing to the evolution of obesity related HF or HFpEF.

and anti-inflammatory mediators or *adipokines,* such as leptin or adiponectin. In addition, the adipose tissue is involved in the NP clearance via secretion of neprilysin and expression of the degradation receptor, NP receptor C[93]. Obesity also leads to increased aldosterone levels, both through RAAS activation, through adipokine induced adrenal stimulation, and through direct production of aldosterone from adipose tissue[92,94]. Moreover, obesity and insulin-resistance lead to oxidative stress and an imbalance in the somatotropic axis, thereby further amplifying the cardio-metabolic risk-profile[95].

Despite being a risk factor for incident HF, obesity is associated with better prognosis in manifest HFrEF, referred to as the obesity paradox[96,97]. Whether the obesity paradox manifests a subgroup of patients with better reserve capacity or less severe disease, or if there is a mechanistic link between better prognosis and obesity is not clear[98].

Metabolic biomarkers

Insulin-like growth factor 1

Insulin-like growth factor 1 (IGF-1) is a peptide hormone, structurally similar to insulin, produced in most cells. Circulating IGF-1 is mainly produced in the liver. IGF-1 is the effector peptide of GH acting through the IGF-1 receptor which resembles the insulin-receptor. In addition to metabolic and anabolic effects, IGF-1 stimulates myocardial contractility and has anti-inflammatory effects [99,100]. IGF-1 levels are mainly reduced in HFrEF[100], but normal[101] or even increased concentrations have been reported in less severe HF[102]. These discrepancies are not fully understood, but are possibly explained by IGF-1 concentrations being dependent on age, severity of HF, and assay variability[100].

In HFrEF, lower IGF-1 concentrations are associated with a catabolic state with cytokine activation, endothelial dysfunction, adverse remodeling, impaired skeletal muscle function, and worse outcomes[103-105]. IGF-1 is believed to exert inotropic actions through increased intracellular Ca^{2+} transients and sensitivity, and through a shift in myosin isoforms. Thus, suppression of IGF-1 production or inhibition of IGF-1 could contribute to HF severity in HFrEF[100,106]. Moreover, administration of GH in HFrEF increases IGF-1 and improves myocardial function and cardiac output[100].

Insulin-like growth factor binding protein 1

In general, decreased IGF-1 activity may be secondary to impaired GH-secretion, GH resistance, increased inhibition of IGF-1, malnutrition, or insulin-deficiency[100]. The activity of IGF-1 is tightly regulated by insulin-like growth factor binding proteins (IGFBPs) where IGFBP-1 is considered particularly important for IGF-1 activity regulation. Although present in much lower concentrations than IGFBP-3, IGFBP-1 is usually unsaturated and has a high diurnal variability and thereby accounts for the greatest changes in IGF-1 activity. IGFBP-1 has numerous IGF-1 inhibitory actions such as peripheral binding of IGF-1, potent inhibition of IGF-1 at receptor level[107], and inhibition of IGF-1 production in the liver, independently of insulin[108,109].

The role of IGFBP-1 besides regulation of IGF-1 is still largely unexplored. IGFBP-1 may even potentiate the effects of IGF-1[107] and it may also have IGF-1 independent actions[110].

While obesity and peripheral insulin resistance are associated with lower IGFBP-1, high IGFBP-1 is associated with female sex, older age, lower body mass index (BMI), and lower levels of insulin[111]. Oxidative stress, hypoxia, inflammation, stress hormones, malnutrition, and insulin deficiency, all of which may be present in, and have a role in the evolution of both HFrEF and HFpEF, increase IGFBP-1[112-114]. Increased levels of IGFBP-1 have been described in hypertrophic cardiomyopathy and during congestion IGF-1 decreased and IGFBP-1 increased[115]. Although higher levels of IGFBP-1 are associated with a favorable lipid-profile, absence of insulin resistance, and female sex; high levels of IGFBP-1 have been associated with risk for incident HF[111]. Furthermore, high levels of IGFBP-1 have been associated with hospitalization for HF after myocardial infarction[116].

Adipokines- leptin and adiponectin

Leptin and adiponectin are cytokines, commonly referred to as *adipokines*, secreted mainly by the adipose tissue. In obesity, there is hyperleptinemia and levels of adiponectin are reduced. Leptin regulates satiety and is considered proinflammatory. In contrast, adiponectin is regarded as cardioprotective in reducing oxidative stress and inflammation, both in the heart and the vasculature. Hence, higher adiponectin levels in healthy individuals are associated with a favourable cardiovascular risk profile[117,118].

The role of leptin and adiponectin in HF is complex. As obesity, leptin and adiponectin can behave paradoxically in HF. Despite a catabolic state, leptin may be elevated[119] and higher concentrations of leptin are associated with better prognosis in HFrEF[120]. Correspondingly, higher levels of adiponectin are, despite the presumed beneficial effects of adiponectin, associated with worse outcomes in HF. This is sometimes referred to as the adiponectin paradox[121].

Leptin

Although mainly produced by adipocytes, leptin is also found in various cell types such as cardiomyocytes and smooth muscle cells. High levels of leptin are associated with obesity, hypertension and insulin resistance[117]. Besides metabolic effects, leptin has various cardiovascular effects. Leptin mediates positive inotropic and chronotropic effects through central SNS stimulation and RAAS activation[122]. Leptin is also believed modulate vascular function via stimulation of endothelial NO-synthesis. However, in hyperleptinemia, there is a reduced response to leptin through interaction with inflammatory biomarkers, resulting in inhibition of the NO generating effect of leptin[123]. This suggests a link between leptin resistance, inflammation and endothelial dysfunction[122,123].

Elevated plasma leptin has been reported in established HFrEF[119,124]. High concentrations of leptin have also been associated with arterial stiffness[125]. Additionally, an association between leptin and diastolic dysfunction in the general population and in patients with CAD has been described[126,127]. Interestingly, this association was more prominent in women. Hence, leptin is believed to play a role in the pathogenesis of HFpEF[128,129].

Adiponectin

Adiponectin, existing as polymers, is abundantly present in plasma and the highest levels are found in lean subjects. Similarly to leptin, women have higher adiponectin levels than men[118].

Low levels of adiponectin are associated with comorbidities related to HF like obesity, insulin resistance, and hypertension. Adiponectin is considered a marker of, or potentially even a factor effectuating, cardio-protection[130]. Adiponectin deficiency leads to hypertension, LV hypertrophy, diastolic dysfunction, and, in the presence of pressure overload, cardiovascular alterations resembling HFpEF in experimental models[131]. Correspondingly, overexpression of adiponectin attenuates cardiac remodeling[130].

In HFpEF adiponectin is still poorly studied, although experimental data support adiponectin deficiency in the pathogenesis of HFpEF[130,131]. An association between diastolic dysfunction and lower adiponectin concentrations was found in a small study of patients with EF >50% and mild HFpEF[132], similar to findings in patients with CAD[133,134].

Despite low adiponectin levels being associated with the evolution of HF, adiponectin levels are increased and associated with poor prognosis in manifest HFrEF[135-137]. The reason for the adiponectin paradox in HFrEF is not well known but adiponectin concentrations are positively associated with severity of HF and NP concentrations in congestive HF[135,137]. Furthermore NPs increase the production of adiponectin in adipocytes from HF patients [138] and adiponectin resistance has been described in HFrEF[139].

AIMS

The overall aim was to investigate metabolic biomarkers and the impact of sex regarding PRO and NPs across the ejection fraction spectrum in patients with HF with particular focus on HFpEF.
Specific aims were:

To investigate whether the impairment of the IGF-1 axis shown in HFrEF exists also in HFpEF through assessment of IGF-1 and IGFBP-1 **(Study I)**.

To investigate leptin and adiponectin concentrations and their prognostic associations in HFpEF and HFrEF, and whether the obesity paradox is present in HFpEF **(Study II)**.

To assess potential sex differences in PROs in HFpEF, including the associations with HF severity and outcomes **(Study III)**.

To assess the impact of sex on NT-proBNP concentrations and prognosis in HF across the ejection fraction spectrum **(Study IV)**.

THESIS AT A GLANCE

Study	I	II	III	IV
Aim	To assess if HFpEF and HFrEF share features of anabolic impairment regarding IGF-1 and IGFBP-1	To assess levels of leptin and adiponectin and whether the obesity paradox exists in HFpEF	To investigate potential sex-specific differences in PROs in HFpEF	To assess the impact of sex on concentrations of NT-proBNP, and associations with clinical characteristics and outcomes of high NT-proBNP across the HF phenotypes
Design	Prospective observational cohort studies			Registry based cohort study
Time of data collection	HFpEF: 2007-2011 HFrEF: 2009-2014		2007-2011	2000-2012
Data source	KaRen, MetAnEnd, Hälsa Ohälsa		KaRen	SwedeHF
Study population	Chronic HFpEF, HFrEF and controls from the normal population		Chronic HFpEF	Chronic HF across the EF spectrum
Patients	300 85 HFpEF 79 HFrEF 136 Controls	234 84 HFpEF 79 HFrEF 71 Controls	378 HFpEF	15,849 1811 HFpEF 2122 HFmrEF 5914 HFrEF
Outcomes	HFpEF: HF hospitalization or death HFrEF: Transplantation, LV assist device, or death		HF hospitalization or death	1. HF hospitalization or death 2. All-cause death
Main statistical analyses	ANCOVA, Pearson's correlation, Kaplan Meyer, Cox regression		Spearman's correlation, Cox Regression	Logistic regression, Kaplan Meyer, Cox Regression
Results/ Conclusion	Both HF phenotypes share impairment in the IGF-1 axis through increased IGFBP-1. IGF-1 was lower and associated with outcomes in HFrEF only.	HFpEF and HFrEF share elevated leptin and adiponectin, but the obesity paradox regarding leptin could only be confirmed in HFrEF.	Females express worse general QoL. Poor QoL seems less explained by HF in females and was associated with worse outcome in males only.	Despite higher concentrations in females, determinants of concentrations and association with prognosis were similar in females and males.

METHODS

Data sources

Study I-III the Karolinska Rennes

The Karolinska Rennes study (KaRen) was an observational, prospective, multicentre study conducted in France and Sweden during 2007-2011. The primary aim was to investigate the prevalence and prognostic role of electrical dys-synchrony in HFpEF. Predefined sub-studies were echocardiography, cardiopulmonary exercise testing, serological biomarkers, and PRO.

Patients were included at hospitalization for acute decompensated HFpEF and inclusion criteria were: (1) Acute presentation with clinical signs and symptoms of HF according to the Framingham criteria; (2) LVEF ≥45 % by echocardiography during the first 72 h; and (3) BNP >100pg/mL or NT-proBNP >300pg/mL. The aim was to study a real-life cohort of HFpEF and the exclusion criteria were mainly factors which prevented the patients from completing the study. Key exclusion criteria were: evidence of primary hypertrophic or restrictive cardiomyopathy or infiltrative heart disease, isolated right HF, pericardial constriction, chronic pulmonary disease requiring oxygen, end-stage renal disease requiring dialysis, and anticipated or indication for cardiac surgery or percutaneous intervention. Patients were scheduled for a follow up visit in stable state 4-8 weeks after the acute presentation and were then followed for at least 18 months. In total 539 patients were included and 438 patients attended the follow up visit. The primary outcome was hospitalization for HF or all-cause death[140].

In **Study I** and **II** only patients from the pre-specified KaRen biomarker study were investigated and for **Study III** all patients with complete PRO assessment at the follow up visit were included.

Study I-II MetAnEnd

Patients with HFrEF, were obtained from the Metabolic Anabolic Endothelial Function Heart Failure study cohort (MetAnEnd-HF) at Karolinska University Hospital. Between January 2009 and September 2014, patients with advanced HF and EF <40% referred to the hospital were included in MetAnEnd-HF. Exclusion criteria were only inability to participate or participation in a pharmacological intervention study. The patients were followed prospectively and the composite endpoint was all-cause death, implantation of LV assist device, or heart transplantation. Information regarding vital status, implantation of LV assist device or heart transplantation was obtained from patient charts and the Population Register in December 2014.

Study I-II Hälsa Ohälsa

The control population for the biomarker studies was obtained from the Hälsa Ohälsa study conducted in 1995- 1998. In the study, individuals aged 18 and above from the general population were randomly selected through their personal identification number for a questionnaire study concerning health. Of these, 488 again randomly selected, individuals across age-groups were invited to a medical examination including biomarker analysis. Individuals free from self-reported cardiovascular disease or known hypertension were included as controls in **Study I** and **II**[118,141].

Study IV- the Swedish Heart Failure Registry

The Swedish Heart Failure Registry (SwedeHF, www.swedehf.se) is a national quality registry founded in 2000. It covers almost 90% of the hospitals and about 10% of primary care

centres in Sweden. The inclusion criterion is *"Clinician–judged HF"*. About 80 variables are recorded at discharge from hospital or after outpatient clinic visit in a web-based case report form, managed by Uppsala Clinical Research Center, Uppsala, Sweden (www.ucr.uu.se). In about 90% of the registrations, EF is reported categorized as <30, 30-39, 40-49, and ≥50 %, enabling differentiation between the three different HF phenotypes[142]. To obtain outcome data the registry is linked to The Population and the Patient Registries administered by The Swedish Board of Health and Welfare (www.socialstyrelsen.se), through the Swedish personal identification number.

Instruments for patient reported outcome

The EQ-5D-3L is a generic QoL instrument. Part one is descriptive with five dimensions; mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. There are three response options for each dimension (no problems, some problems, and extreme problems). Part two is the EuroQoL Visual Analogue Scale (EQ-VAS), which records the patient's self-rated global health on a VAS-scale. The endpoints are labelled "Worst imaginable health state" (0) and "Best Imaginable Health State" (100). Hence higher values of EQ-VAS denotes better QoL[143-145].

The MLHFQ is a HF specific instrument. Totally 21 items cover the effects on QoL of functional limitations, symptoms, and psychological reactions associated with HF or HF treatment. The response options range from no limitation (0) to very much limited (5). A total score of 0-105 is given and a higher score will indicate worse QoL[146,147].

Statistics

Data are displayed as counts (%) and median and interquartile range (IQR), except for **Study III** where mean and standard deviation (SD) were used for normally distributed, continuous baseline characteristics. Continuous variables were analysed with non-parametric Mann-Whitney U test or Kruskal-Wallis, or t-test as appropriate. Proportions were compared with Fisher's exact test or Chi^2-test depending on frequency distribution.

Biomarker data in **Study I** and **II** and QoL data in **Study III** were analysed by the analysis of covariance (ANCOVA) with adjustment for clinically relevant variables. Associations were assessed by Pearson's (**Study I-II**) and Spearman's correlations (**Study III**), and with multivariable logistic regression **(Study IV)**. Unadjusted survivor functions were estimated through the Kaplan-Meier method (**Study IV**), and associations with outcomes were analysed with Cox Proportional Hazards models (**Study I-IV**). Results from regression models are presented as odds ratio (OR) or hazard ratio (HR) as appropriate and 95% confidence interval (CI). To test significant differences between sexes in **Study IV**, interaction terms between sex and the other variables considered was included in the multivariable models regression models. The presence of missing data in **Study IV** was addressed through multiple imputation with chained equations (n=10), run in blocks defined according to HF type and sex. The statistical significance level was set to 0.05 in all analyses except for correlations in **Study I** and **II** where the α-level was set to 0.003 and 0.002 respectively due to multiple analyses (Bonferroni adjustment). All- p-values were 2-sided.
Statistical analyses were performed in IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY) (**Study I and II**) and Stata 14.1 (StataCorp, College Station, Texas) (**Study III-IV**).

Ethical considerations

All the studies were performed in accordance with good clinical practice guidelines and the Declaration of Helsinki and all patients provided written informed consent[148]. For registration in SwedeHF, individual consent is not required but the patients are informed and able to opt out. The establishment of SwedeHF and all studies in this thesis were approved by ethical committees in Sweden.

Description of studies

Study I

Aim

To investigate concentrations of IGF-1 and IGFBP-1, associations of these with HF severity and outcomes, and whether impairment of the IGF-1 axis in HFrEF exists also in HFpEF.

Patients

Patients with HFpEF from the KaRen study (n=85), with HFrEF from MetAnEnd-HF (n=79), and individuals without self-reported cardiovascular disease aged 40 years and above from the Hälsa Ohälsa study (n=136) were included in the analysis.

Methods

The patients were examined and underwent echocardiography in stable state. Fasting blood samples were collected in and IGF-1 and IGFBP-1 analyses performed by in-house radioimmuno-assays. Of note, due to the age-dependency of IGF-1 (decreasing concentrations with increasing age), age adjusted IGF-1 SD-score was calculated based on the regression of IGF-1 concentrations in healthy[149]. Concentrations of IGF-1 and IGFBP-1, as well as their associations with other relevant biomarkers and outcomes were assessed.

Endpoints

For HFpEF time to HF hospitalization or all-cause death, for HFrEF time to implantation of LV assist device, heart transplantation, or all-cause death.

Study II

Aim

To investigate concentrations of leptin and adiponectin, associations with HF severity and outcomes and whether the reverse metabolic profile is present in HFpEF as in HFrEF.

Patients

Patients with HFpEF from the KaRen study (n=84), patients with HFrEF from MetAnEnd-HF (n=79), and individuals aged 60 years or above without self-reported cardiovascular from the Hälsa Ohälsa study (n=71) were included in the analysis.

Methods

The patients were examined and underwent echocardiography in stable state. Fasting blood samples were collected and leptin and adiponectin analyses were performed by radio-immunoassays Merck Millipore® (HL-81 K and HADP-61K). Concentrations of leptin and adiponectin and their associations with other relevant biomarkers and outcomes were assessed.

Endpoints
For HFpEF time to HF hospitalization or all-cause death, for HFrEF time to implantation of LV assist device, heart transplantation, or all-cause death.

Study III

Aim
To assess PRO in HFpEF and potential sex differences.

Patients
Of the 539 patients included in KaRen, 438 patients attended the follow up visit. Of these 387 patients had complete assessments of PRO. Since the KaRen study was designed prior to the new definition of HFpEF, 9 patients with EF <50% were excluded, and the remaining 378 patients were analysed.

Methods
The patients underwent clinical examination, ECG, and echocardiography at the 4-8 weeks visit in stable state. Two validated PRO instruments were used, the generic EQ-5D-3L and the HF-specific MLHFQ. Self-reported QoL was assessed and the associations of QoL with HF severity and outcomes.

Endpoint
Time to first HF hospitalization or all-cause death.

Study IV

Aim
To assess the impact of sex on NT-proBNP concentrations, associations between clinical characteristics and high NT-proBNP, and the associations with outcomes in HFpEF, HFmrEF, and HFrEF.

Patients
Between May 11th 2000 and December 31st 2012, 36,255 outpatient registrations were recorded in SwedeHF. Excluding patients with missing EF, follow up <1 day, missing NT-proBNP, and repeated registrations left 9847 patients for analysis. In the case of more than one registration, the first assessment was considered.

Methods
Concentrations of NT-proBNP were assessed in females and males in all EF phenotypes respectively. Associations between NT-proBNP above the median in females and males in each HF-type, and clinical characteristics and outcomes were investigated. The majority of health facilities in Sweden use the NT-proBNP analysis by Roche Diagnostics, Bromma, Sweden (www.equalis.se).

Endpoints
The primary endpoint was time to HF hospitalization or all-cause death and the secondary endpoint time to all-cause death. End of follow-up was December 31st 2012.

RESULTS

Study I

Baseline characteristics

Compared with HFrEF, patients with HFpEF were older, more commonly female, with lower NYHA class, better renal function, higher BMI, and lower NT-proBNP. There were no statistically significant differences in comorbidities although numerically IHD, defined as previous coronary artery by-pass grafting and percutaneous coronary intervention, was more common in HFrEF. Use of neurohormonal antagonists and diuretics were more common in HFrEF, whereas calcium channel blockers were more common in HFpEF. Selected baseline characteristics are shown in **Table 1**. The controls were younger and had lower BMI compared with the HF patients. Blood pressure and sex ratio in controls were similar to in HFpEF.

Table 1. Baseline characteristics of the patients in **Study I** expressed as median and lower and upper quartiles and numbers and percentage.

				p-value		
	HFpEF n=85	**HFrEF n=79**	**Control n=136**	**HFpEF: HFrEF**	**HFpEF: control**	**HFrEF: control**
Age years	73 (67;79)	64 (52;69)	58 (49;66)	<0.001	<0.001	0.018
Female	44 (52)	13 (16)	68 (50)	<0.001	0.890	<0.001
NYHA I II III IV	19(22) 46(54) 20 (24) 0	1 (1) 4 (5) 65 (82) 9(11)		<0.001		
BMI kg/m^2	28 (25;33)	27 (23;30)	25 (23;27)	0.036	<0.001	0.017
Systolic blood pressure mmHg	140 (128;153)	108 (96;122)	137 (126;149)	<0.001	0.100	<0.001
Pulse pressure mmHg	65 (50;75)	39 (30;50)	50 (43;60)	<0.001	<0.001	<0.001
EF %	64 (55;68)	22 (15;28)		<0.001		
NT-proBNP pg/mL	983 (463;2303)	3425 (1333;5988)		<0.001		
HOMA-IR	3.5 (2.0;5.9)	2.6 (1.4;5.3)	2.0 (1.3;2.8)	0.250	<0.001	0.004
eGFR mL/min/1.73m^2	66 (51;80)	54 (39;66)	76 (68;85)	<0.001	<0.001	0.001
Hemoglobin g/L	13.1 (12.2;14.1)	13.3 (12.2;14.4)	14.3 (13.5;15.2)	0.497	<0.001	<0.001

Concentrations of IGF-1

Median (IQR) concentrations of IGF-1 were: 173 (137-207) in HFpEF vs. 149 (105-219) in HFrEF vs. 163 (133-205) µg/L in controls (p overall= 0.002). Age adjusted IGF-1 SD-scores were 1.21 (0.57-1.96) vs. 0.09 (-1.40–1.62) vs. 0.22 (-0.47-0.96) arbitrary units, respectively (p overall <0.001). Concentrations and pair-wise comparisons are shown in **Figure 5**, and in men only in **Figure 6.** The difference between IGF-1 concentrations in HFpEF and HFrEF was significant also after adjustment for sex, BMI, insulin, and NYHA class (p=0.032).

In patients with functional class NYHA II-III age adjusted IGF-1 SD-scores were 1,31 (0.70-2.28) in HFpEF (n=66) vs. 0. 24 (-1.32-1.65) in HFrEF (n=69) (p<0.001 crude and p=0.012 adjusted for sex, BMI, and insulin).

Concentrations of IGFBP-1

In HFpEF and HFrEF, IGFBP-1 was increased compared to controls. Median (IQR) IGFBP-1 was 48 (28-79) µg/L in HFpEF vs. 65 (29-101) in HFrEF vs. 27 (14-35) in controls (p overall <0.001). When adjusted for age, sex, BMI, and insulin; IGFBP-1 remained significantly higher in HFrEF and HFpEF compared to controls (p <0.001 for both), but lower in HFpEF compared to HFrEF (p=0.021). When adding NYHA class to the model as a marker of HF severity, the difference between HFpEF and HFrEF was no longer significant (p=0.451). Coherently, in patients with NYHA class II-III, levels of IGFBP-1 were similar in HFpEF 48 (28-78) and HFrEF 60 (29-91) (unadjusted p=0.369 and adjusted for sex, age, BMI, and insulin, p=0.262), **Figure 5**.

The analyses of IGF-1 and IGFBP-1 were repeated in men only. The findings were largely similar, except that no measure of IGF-1 significantly differed between patients with HFpEF and controls, **Figure 6**.

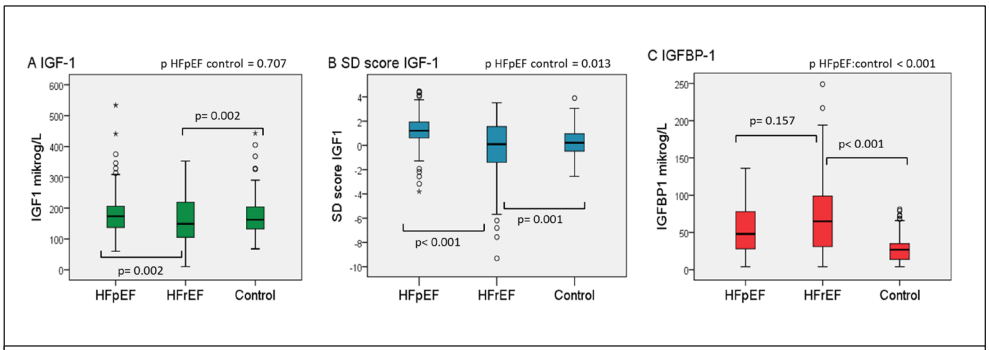

Figure 5. Concentrations of IGF-1 (A), IGFBP-1 (C), and age-adjusted IGF-1 SD score (B) in HFpEF, HFrEF, and controls. P denotes crude comparisons between groups.

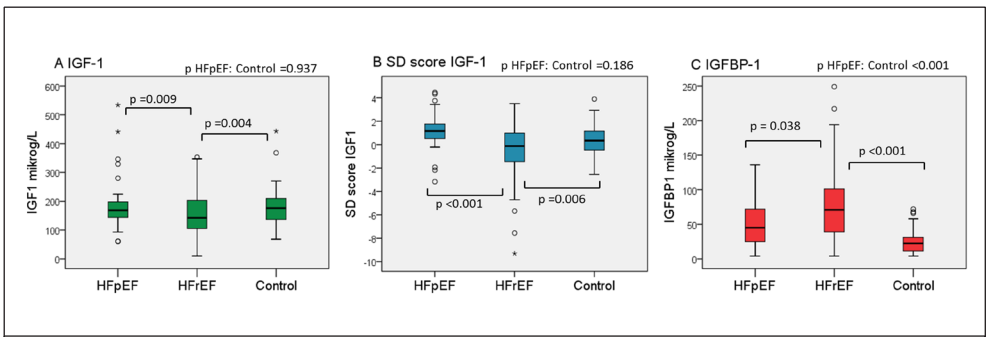

Figure 6. Concentrations of IGF-1 (A), IGFBP-1 (C), and age-adjusted IGF-1 SD score (B) in men with HFpEF, HFrEF, and male controls. P denotes crude comparisons between groups.

Associations with HF-severity

There was a negative association between IGF-1 and IGFBP-1 in HFpEF (r=-0.390, p<0.001); and approaching significance in HFrEF (r= -0.320, p=0.004, α-level 0.003). There was no significant association between IGF-1 and age, NT-proBNP, or estimated glomerular filtration rate (eGFR) in neither HFpEF nor HFrEF. In contrast, NT-proBNP and IGFBP-1 were associated in both HFpEF (r= 0.458, p<0.001) and HFrEF (r=0.533, p<0.001). In both HFpEF and HFrEF; IGFBP-1 was associated with insulin (r= -0.430, p<0.001; and r=-0.383, p=0.001). Results in men only were similar. Selected correlations are shown in **Figure 7**.

Associations with outcomes

Median (IQR) follow-up time was 576 (468-1349) days in HFpEF and 403 (195-992) days in HFrEF. The endpoint occurred in 35 (41%) patients with HFpEF of which 6 patients (17%) died and 29 (83%) were hospitalized for HF. Corresponding data for HFrEF was 50 (63%) events, of which 27 (54%) were deaths and 23 (46%) implantation of LV assist device or heart transplantation.

Hazard ratios per *ln* unit increase in IGF-1, IGF-1 SD-score and IGFBP-1 for the composite endpoints in HFpEF and HFrEF respectively are shown in **Figure 8**. In HFpEF, there was no association between baseline IGF-1 or IGF-1 SD-score and outcomes, in uni- or multivariable analyses. In HFrEF, higher IGF-1, and likewise higher age-adjusted as SD-score, was associated with better outcome.

Regarding IGFBP-1 there were no associations with outcomes in neither HFpEF nor HFrEF. Results in men were similar.

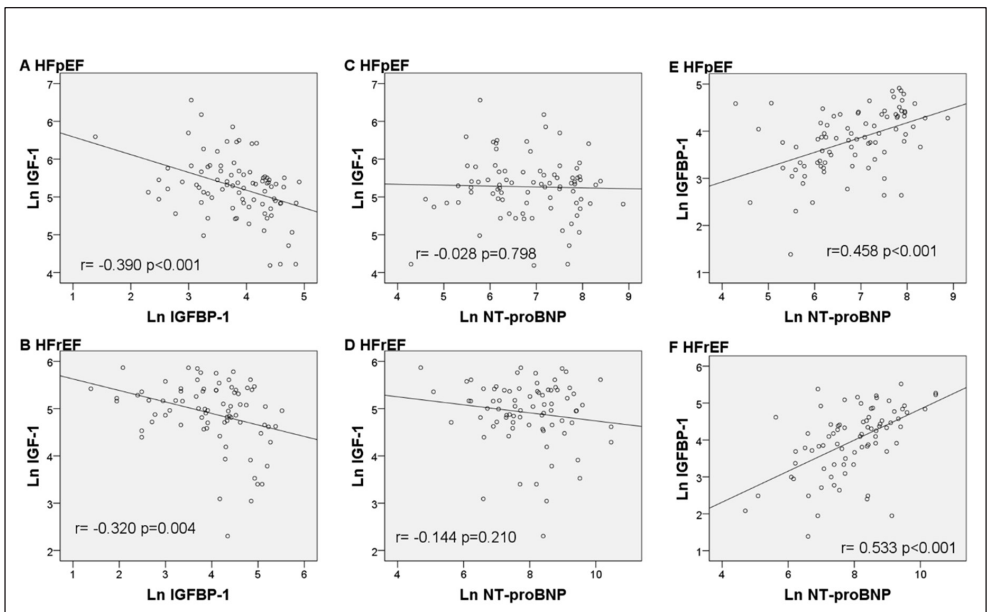

Figure 7. Pearson's correlations between IGF-1, IGBP-1, and NT-proBNP in HFpEF and HFrEF.

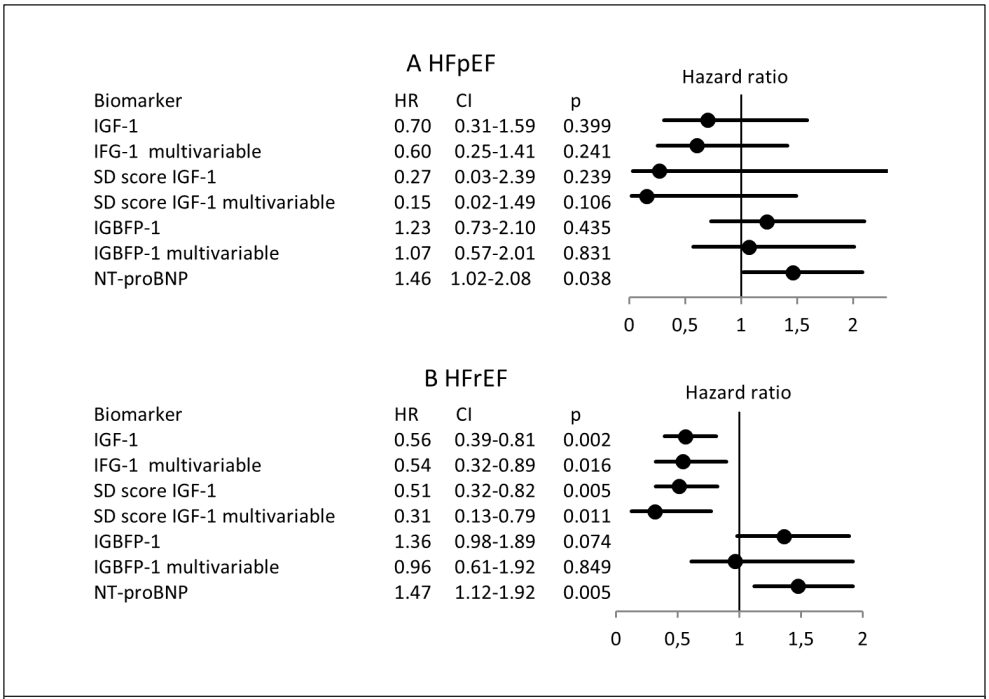

Figure 8. Association between IGF-1 (crude and age adjusted SD-score), IGFBP-1, and NT-proBNP with the composites of HF hospitalization or death in HFpEF and implantation of LV assist device, heart transplantation, or death in HFrEF. Insulin resistance (HOMA-IR) and HF severity, measured as NT-proBNP, were adjusted for in the multivariable models.

Study II

Baseline characteristics

Compared to HFrEF, patients with HFpEF were, as in **Study I**, older (median 73 vs. 64 years, p<0.001), more commonly female (52 vs 16%, p<0.001), and in lower NYHA class. Patients with HFpEF also had better renal function, higher BMI, and lower NT-proBNP (median 966 vs. 3425 pg/mL, p<0.001). Regarding comorbidities there were no statistically significant differences but previous coronary artery by-pass grafting and percutaneous coronary intervention were numerically more common in HFrEF, as in **Study I**. Therapy also differed between HFpEF and HFrEF similar to in **Study I**.

The controls were in between HFpEF and HFrEF regarding age (median 67 years). BMI was lower in controls compared to in HFpEF, whereas similar to in HFrEF. Considering the controls did not have known hypertension, surprisingly they had higher blood pressure compared to both HF phenotypes. Median systolic blood pressure was 140 mmHg in HFpEF vs. 108 in HFrEF vs. 165 in controls (p for all comparisons <0.001).

Concentrations of leptin

Leptin concentrations were median (IQR) 23.1 (10.2-51.0) in HFpEF vs. 15.0 (6.2-33.2) in HFrEF vs. 10.8 (5.4-18.9) ng/mL in controls (p overall <0.001). Concentrations of leptin were

higher in HFpEF than in HFrEF (p=0.007), however there was no difference between the HF groups when adjusted for sex, BMI, and age (p=0.123), nor when NYHA class was added to the model as adjustment for HF severity (p=0.834). Results in men only were similar.

Concentrations of adiponectin

Crude levels of adiponectin did not differ between groups, 11.8 (7.9-20.1) µg/L in HFpEF vs. 13.7 (7.0-21.1) in HFrEF vs. 10.5 (7.4-15.1) in controls (p overall 0.159). There was no significant difference between the HF groups crude or adjusted for age, sex, BMI, and NYHA class. In analyses of men only, adiponectin, adjusted for BMI and age, was higher in both HFpEF (p=0.044) and HFrEF (p=0.001) compared to in controls. Absolute concentrations and adjusted pair-wise comparisons for the entire cohort and men separately, are shown in **Figure 9**.

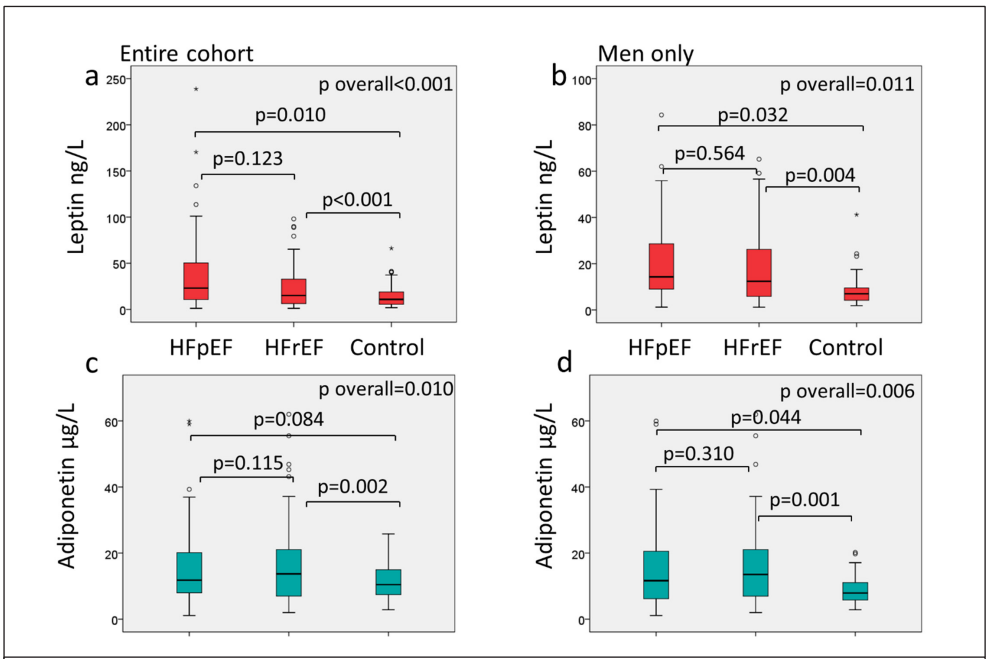

Figure 9. Crude levels of leptin and adiponectin in the entire (a+c) cohort and men only (b+d). P for pairwise comparisons and overall, adjusted for age, sex, and BMI (a+c), and for age and BMI (b+d).

Associations with biomarkers

In all groups, leptin was positively associated with BMI (HFpEF r=0.740, HFrEF r=0.595, and controls r=0.593, p for all <0.001). Similarly, there was a positive association between leptin and insulin (HFpEF r=0.685, HFrEF r=0.487, and controls r=0.358 p <0.001 for all). In HFrEF only, there was a significant association of leptin with NT-proBNP(r=-0.364 p=0.001).

Adiponectin showed an inverse association with BMI in the heart failure groups (r=-0.386 p<0.001 in HFpEF and r=-0.379 p=0.001 in HFrEF), but not in controls (r=-0.154 p=0.202). In HFrEF, adiponectin was associated with with NT-proBNP (r=0.396 p<0.001), however not in HFpEF considering the adjusted α-level of 0.002 (r=0.238, p=0.030), **Figure 10 a-d**.

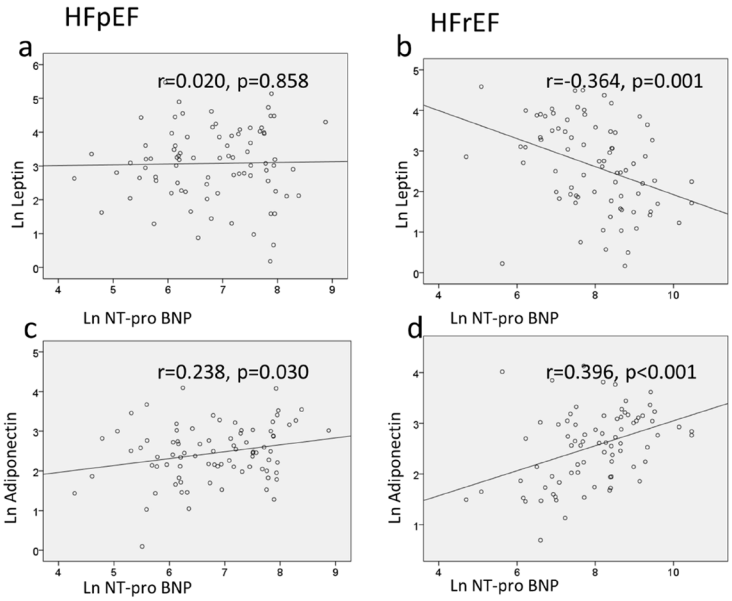

Figure 10. Associations between the adipokines and NT-proBNP in HFpEF and HFrEF.

Associations with outcomes

Median follow-up time (IQR) was 572 (467-1369) days in HFpEF and 402 (196-873) days in HFrEF. The endpoint occurred in 34 (40%) patients with HFpEF of which 6 patients (18%) died and 28 (82%) were hospitalized for HF. Corresponding data in HFrEF were 50 (63%) events, of which 27 (54%) were deaths and 23 (46%) received LV assist device or were transplanted**. Figure 11** shows HRs per unit *ln* increase in leptin and adiponectin in HFpEF and HFrEF for the composite endpoints.

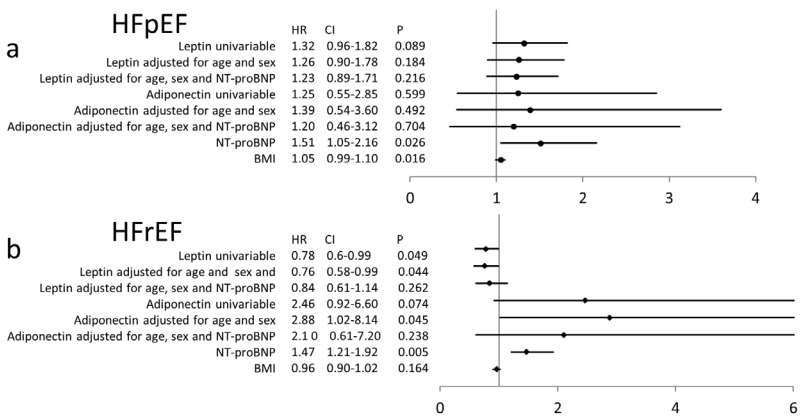

Figure 11. Association between leptin, adiponectin, NT-proBNP, and BMI and the composites of HF hospitalization or death in HFpEF and implantation of LV assist device, heart transplantation, or death in HFrEF.

High leptin levels were associated with reduced the risk of the composite endpoint in HFrEF only, both crude and adjusted for age and sex. When adjusting for HF severity by including NT-proBNP in the model, the association was no longer significant.

While there was no association between adiponectin and the composite outcome in HFpEF, hihger adiponectin was associated increased risk in HFrEF after adjustment for age and sex, HR 2.88 (95% CI 1.02-8.14, p=0.045). The association was however not independent of HF severity.

Study III

Baseline characteristics

A total of 378 patients were included in the analyses, **Figure 12**. Of these, 215 were women (57%). Women were older and had higher EF. There were also signs of higher filling pressures, measured as E/e' in women. Levels of NT-proBNP were similar, median (IQR), 1408 (507-2369) vs. 1480 (611- 2840) ng/L (p=0.17). In contrast, in the cohort of patients where BNP was assessed (n=35), BNP levels were higher in women than in men, median (IQR), 301 (229- 476) vs. 108 (98- 570) ng/L (p=0.041). Kidney function, was similar in both sexes.
Comorbidities were no different across sexes except for women having a lower prevalence of CAD (27 vs. 38%, p=0.016), and anaemia (35 vs. 51%, p=0.004). Therapy did not differ between sexes. Baseline characteristics are shown in **Table 2**.

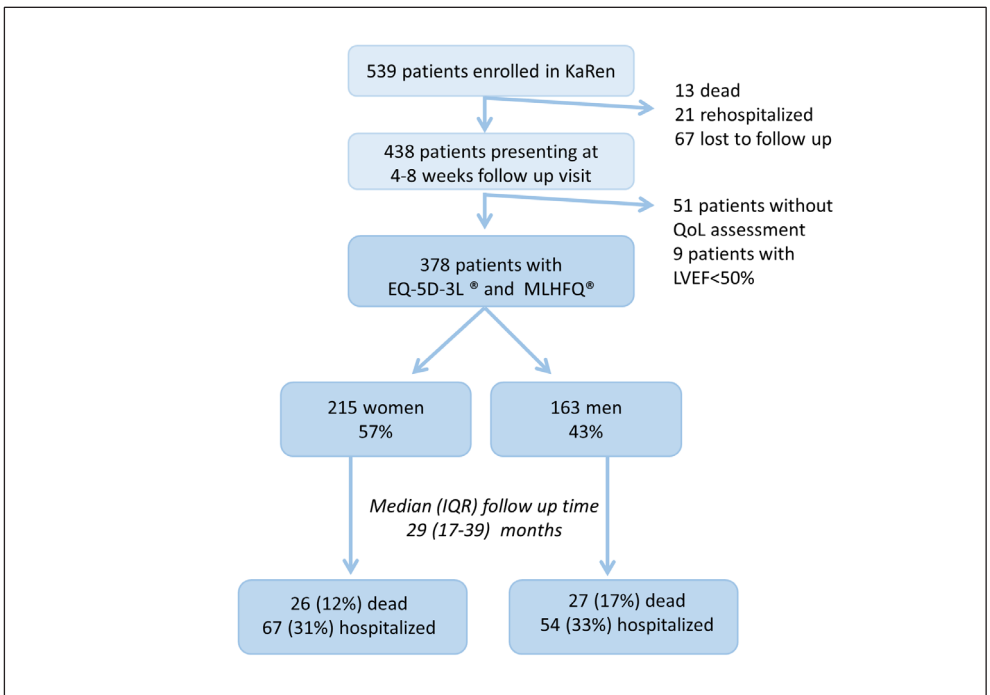

Figure 12. Study outline, patient selection and follow up.

Table 2. Baseline characteristics by sex, **Study 3**. To include both BNP and NT-proBNP in the multivariable analyses, quartiles were calculated based on the entire population. For NT-proBNP: Q1 <532, Q2 532-1438, Q3 1439-2641, and Q4 >2641ng/L; and for BNP: Q1 <125, Q2 125-277, Q3 278-570, and >570 ng/L.

		Female	Male	p-value
Clinical data, mean (SD)	**n**	215	163	
Age (years)		77 (9)	75 (9)	0.014
EF (%)		64 (7)	62 (6)	0.005
E/e'		14 (7)	11 (5)	<0.001
SBP (mmHg)		137 (24)	139 (24)	0.45
BMI (kg/m^2)		29 (7)	29 (5)	0.93
NYHA class, n (%)	I	17 (9)	27 (17)	0.009
	II	132 (68)	90 (57)	
	III	45 (23)	34 (22)	
	IV	1 (0.5)	6 (4)	
Biochemistry, median(IQR)				
Haemoglobin (g/L)		125 (110, 132)	129 (110, 139)	0.13
eGFR CKDEPI (mL/min)		74 (66, 80)	72 (65, 83)	0.97
NT-proBNP (ng/L) n=312		1408 (507, 2369)	1480 (611, 2840)	0.17
BNP (ng/L) n=35		301 (229, 476)	108 (98, 570)	0.041
Comorbidities, n (%)				
CAD		58 (27)	62 (38)	0.022
Atrial fibrillation or flutter		132 (61)	107 (67)	0.34
Hypertension		173 (81)	128 (79)	0.64
COPD		29 (14)	24 (15)	0.73
T2DM		55 (26)	45 (28)	0.66
Anaemia		68 (35)	78 (51)	0.004
Treatment, n (%)				
ACEi or ARB		155 (71)	114 (70)	0.98
Potassium sparing diuretic		49 (23)	42 (26)	0.48
Loop diuretic		170 (80)	132 (82)	0.56
Calcium channel blocker		57 (27)	43 (27)	0.99
β-blocker		149 (69)	115 (71)	0.72

SBP, systolic blood pressure; COPD, chronic obstructive pulmonary disease; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Quality of life

Women expressed more difficulties than men related to mobility (53 vs. 41%, p=0.019), usual activities (46 vs. 33%, p=0.013), and anxiety and depression (51 vs. 39%, p=0.013) in EQ-5D-3L. Self-care (22 vs. 17%, p=0.230), and pain/discomfort (60 vs. 53%, p=0.179) were not significantly different. In EQ-VAS, women rated worse global QoL than men [mean (SD), 57 (20) vs. 61 (19), p=0.027]. After adjustment for age and NPs the difference was still significant (p=0.010). When, instead, adjusting for age and NYHA class, there was only a trend towards statistical significance (p=0.056). MLHFQ was similar in women and men, [mean (SD), 31(21) vs. 29 (21), p=0.329], F**igure 13.**

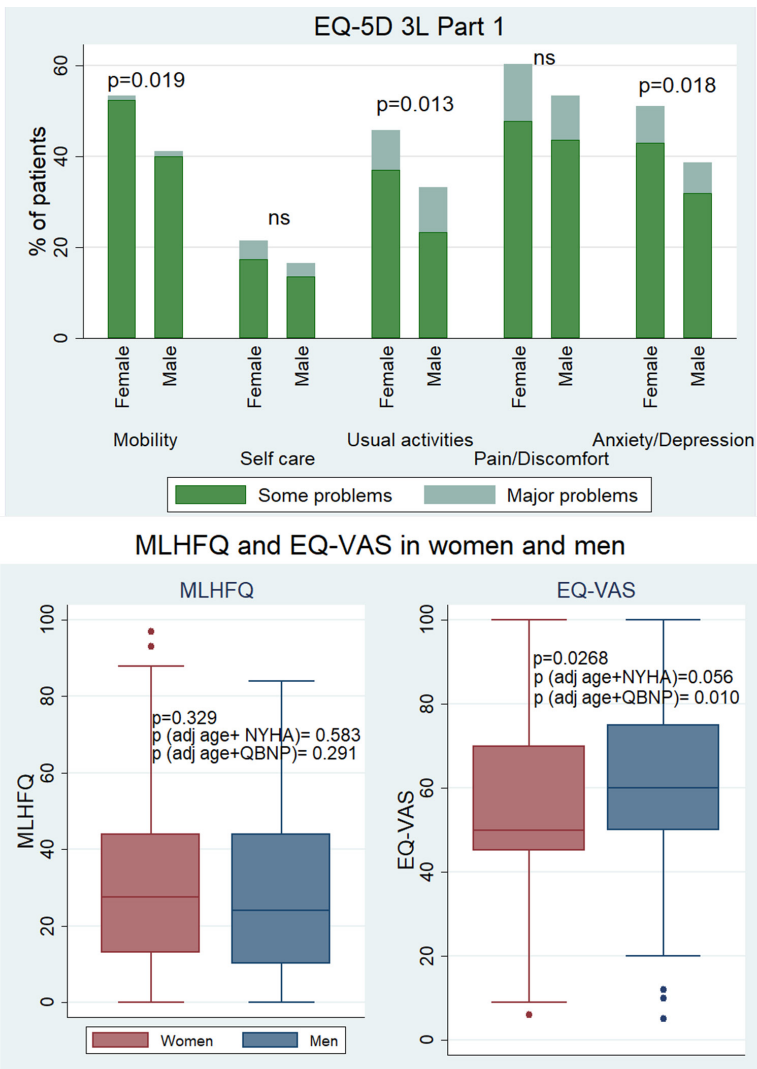

Figure 13. Percentage of patients rating problems in EQ-5D-3L part 1, and rated QoL in EQ-VAS and MLHFQ in women and men.

Associations between quality of life and markers of HF severity

Both instruments were associated with HF severity measured as NYHA class and as levels of NPs. Spearman's correlations between MLHFQ and NYHA class were in women; r_s 0.37 vs. in men 0.41, p for both <0.001. Corresponding data for the associations between MLHFQ and quartiles of NPs were in women; r_s 0.21, p=0.003 vs. in men 0.27, p<0.001. The associations of EQ-VAS with NYHA class were in women r_s -0.28, p<0.001 and in men r_s -0.45, p<0.001, and for EQ-VAS and NPs, r_s -0.17, p=0.018 in women and r_s = -0.27, p<0.001 in men.

Associations between QoL and Outcomes

Associations between MLHFQ/EQ-VAS and outcomes in women and men are reported in **Figure 14.** In women, neither MLHFQ nor EQ-VAS were associated with the composite of HF hospitalization or death. In men, 5 units increase in MLHFQ (worse QoL) was associated with a 6% increase in risk of the composite outcome [HR 1.06, 95% CI 1.01-1.11, p 0.026]. Coherently, 5 units increase in EQ-VAS (better QoL) was associated with a 7% reduction in risk (HR 0.93, 95% CI 0.88-0.98, p=0.010). The association between EQ-VAS and risk of adverse outcome persisted after adjustment for age, kidney function (eGFR) and comorbidities (T2DM, anaemia, chronic obstructive pulmonary disease, and IHD) (HR 0.93, 95% CI 0.088-0.99, p=0.020). The association between MLHFQ and the composite outcome approximated statistical significance (HR 1.05, 95% CI 1.00-1.11, 0.059). The associations were however not independent of HF severity and were lost when NPs were added to the model.

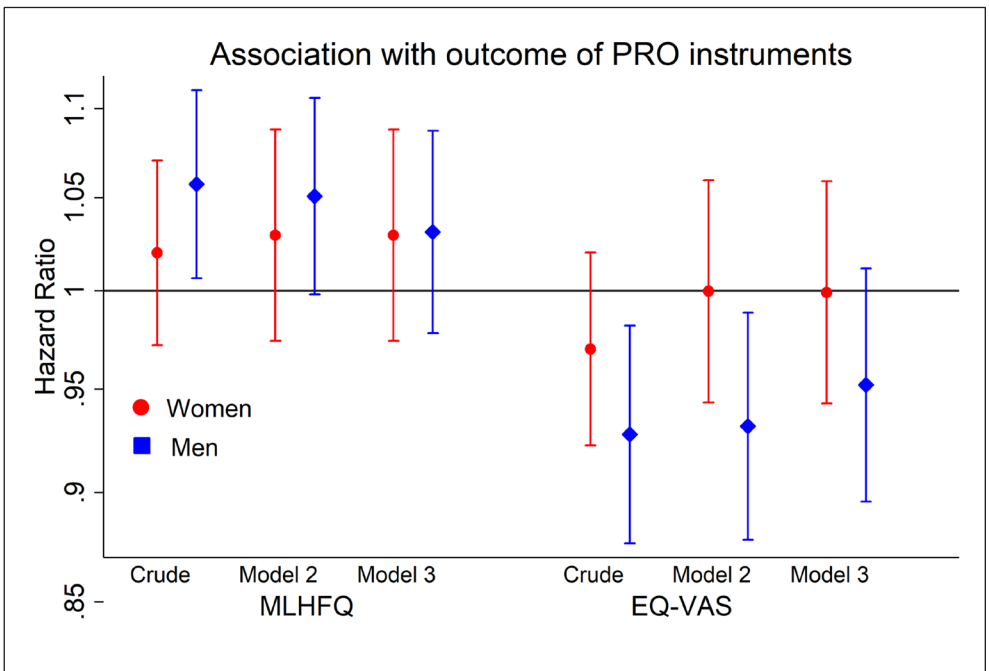

Figure 14. Associations between MLHFQ and EQ-VAS and the composite of HF hospitalization and death. *Model 2* includes adjustment for age, kidney function and relevant comorbidities (T2DM, anaemia, chronic obstructive pulmonary disease, and IHD), and in *Model 3* with addition of quartiles of NPs.

Study IV

Baseline characteristics

Of 9847 patients, 1811 (18%) had HFpEF, 2122 (22%) HFmrEF, and 5914 (60%) HFrEF, **Figure 15**. The proportion of females was higher in HFpEF (49%) vs. HFmrEF (35%) vs. HFrEF (25%). Females of all HF-phenotypes were older, had higher NYHA class, and better renal function as compared with males. The prevalence of T2DM, IHD, and anaemia was lower in females. Atrial fibrillation was less prevalent in females compared with males in HFpEF and HFrEF, whereas in HFmrEF, females were less likely to have hypertension and cancer. Except for more use of diuretics in females with HFpEF and HFmrEF and more use of statins in males regardless of EF, therapy was similar in both sexes.

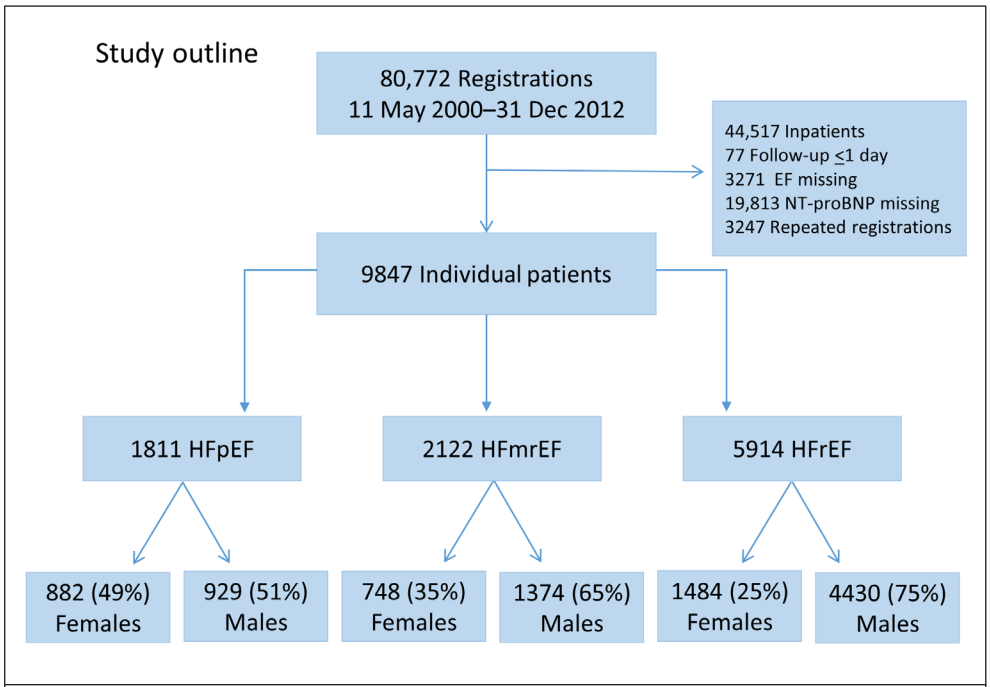

Figure 15. Study population

Concentrations of NT-proBNP

Concentrations of NT-proBNP were higher in females vs. males in all three HF types. Sex-differences in NT-proBNP levels were consistent when rhythm status was considered (atrial fibrillation vs. no atrial fibrillation), except for in patients with HFmrEF where there was no statistically significant difference in females vs. males, **Figure 16 A-C**.

Associations between clinical characteristics and high NT-proBNP

Independent associations between relevant demographics/clinical characteristics/ therapies and NT-proBNP are shown in **Figure 17 A-C**. Factors associated with high NT-proBNP concentrations were similar in both sexes across HF phenotypes with few exceptions. In

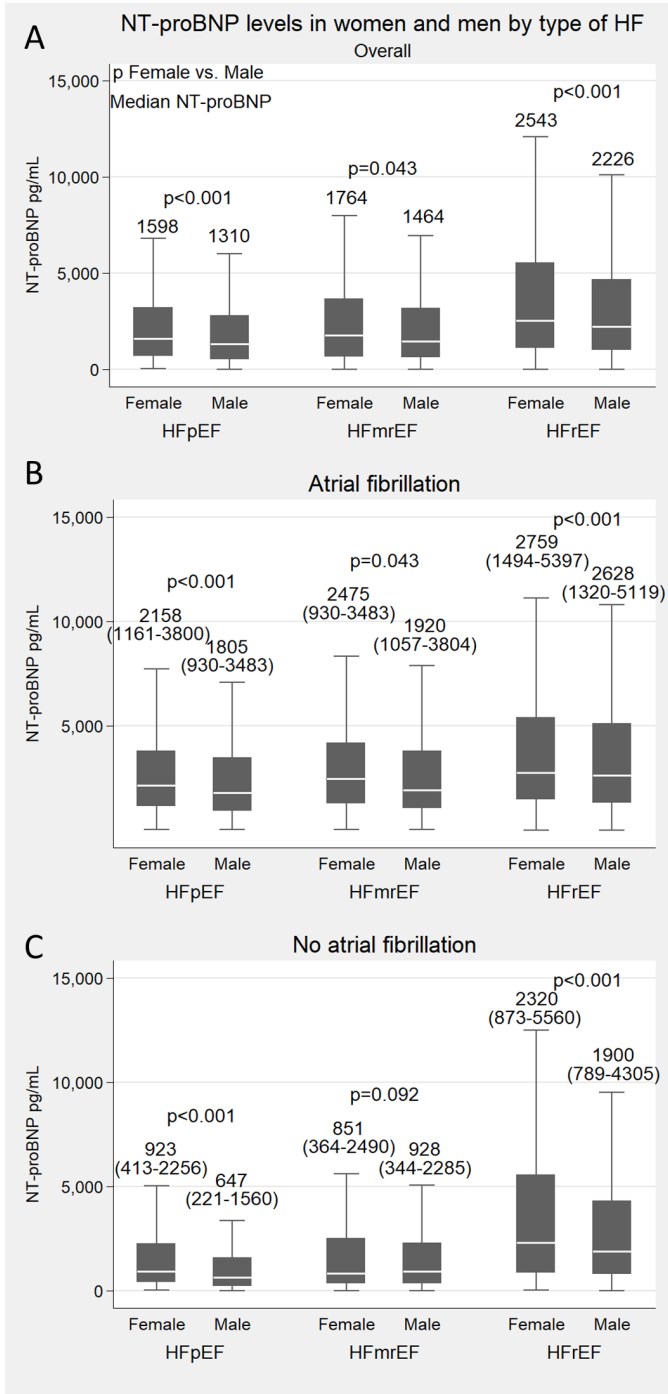

Figure 16 A-C. Concentrations of NT-proBNP in females and males, overall and by rhythm status.

HFpEF, hypertension was associated with high NT-proBNP in males but not in females (p-interaction sex*hypertension=0.015). In addition, there was a difference in association between mean arterial pressure ≥90 mmHg and high NT-proBNP in males vs. females (p-interaction 0.040). Diuretic use was also associated with increased odds of high NT-proBNP in males but not in females (p-interaction=0.032). In HFmrEF, in females, whereas not in males, IHD was associated with high NT-proBNP (p-interaction 0.005). In HFrEF there was no significant interaction between the variables explored and sex.

Prognostic associations of NT-ProBNP in females vs. males

In HFpEF, over a median (IQR) follow-up of 2.1 (1.0-3.6) years, 100 deaths per 1000 patient-years occurred in females vs. 107 in males. In HFmrEF rates were 89 vs. 100 per 1000 patient-years over a follow-up of 2.0 (1.0-3.6) years, whereas in HFrEF they were 85 vs. 89 per 1000 patient-years over a follow-up of 2.0 (0.9-3.6) years in females vs. males, respectively.

Rates for the composite endpoint of HF hospitalization or all-cause death were 169 vs. 172 per 1000 person-years in females vs. males in HFpEF, 188 vs. 171 in HFmrEF and 209 vs. 243 in HFrEF.

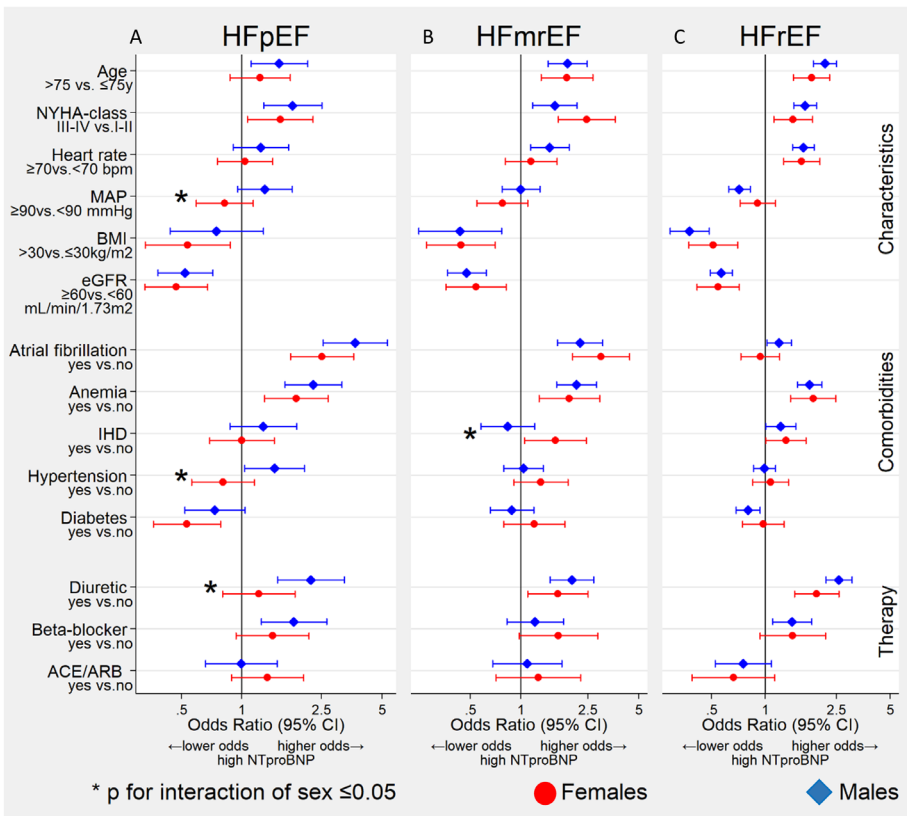

Figure 17 A-C. Associations between patient characteristics and high NT-proBNP in the three HF phenotypes.

Figure 18 show survival free of HF hospitalization together with crude and adjusted HRs for risk of the composite outcome associated with high NT-proBNP in HFpEF, HFmrEF, and HFrEF. NT-proBNP above median was associated with increased unadjusted and adjusted risk regardless of sex and EF. There were no significant interactions between sex and NT-proBNP.

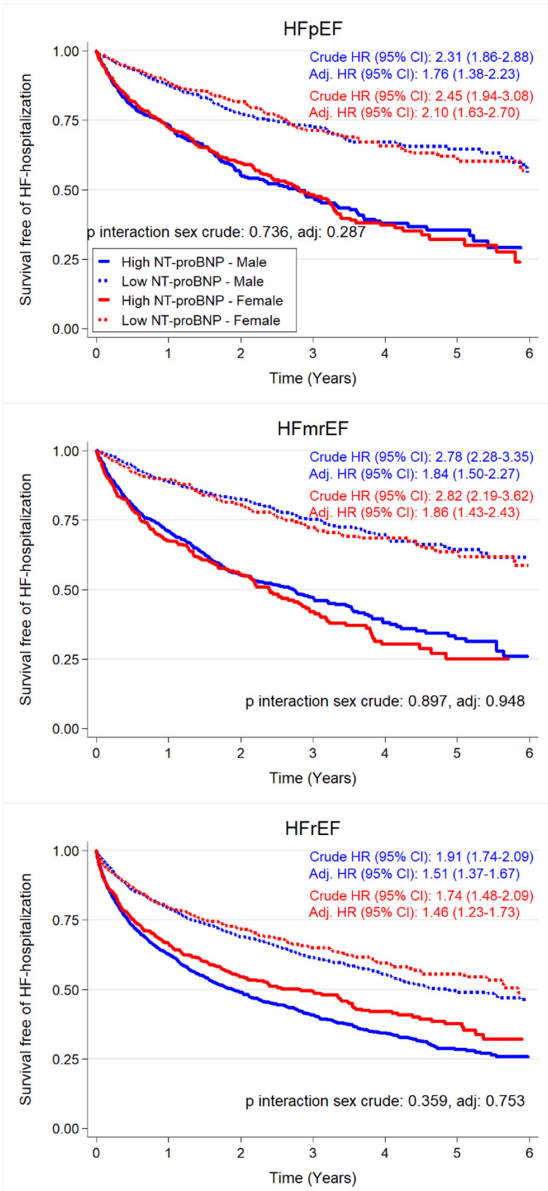

Figure 18. Survival free of HF hospitalization in the three HF phenotypes by sex and high vs. low NT-proBNP and hazard ratios for the composite endpoint in females and males.

GENERAL DISCUSSION

The principal findings of the four studies in this thesis are:

1. The impairments of the IGF-1 axis are more pronounced in HFrEF compared to in HFpEF and are associated with poor prognosis in HFrEF. Still, both EF phenotypes share increased levels of IGFBP-1, indicating inhibition of the IGF-1-axis. IGFBP-1 was also associated with HF severity measured by NT-proBNP. Together with previous data, this suggests a potential mechanistic link between IGFBP-1 and NPs.
2. HFpEF and HFrEF share elevated levels of leptin and adiponectin. The obesity paradox, where higher levels of leptin are associated with better prognosis, was only demonstrated in HFrEF, pointing towards a more conventional metabolic profile in HFpEF.
3. Women with HFpEF express worse global QoL than men. Overall, QoL was only weakly associated with measures of HF severity and the associations were weaker in women. In men only, poor QoL was associated with worse outcome. Overall, the results suggest that to improve QoL in HFpEF patients, in particular in women, other factors than HF, such as comorbidities, must be sought and addressed.
4. Women with chronic HF across the entire EF spectrum have higher NT-proBNP concentrations than men, but associations with patient characteristics and outcomes are largely similar. This supports the current use of NT-proBNP for prognostic purposes but the impact of the relatively large differences between sexes in the lower range warrants further investigation.

The IGF-1 axis

Mounting evidence suggests that HFpEF and HFrEF, despite similar symptoms, are pathophysiologically different[11,15,150]. However, in HFpEF, there is still a vague understanding of the syndrome and many areas, including when and how to treat remain elusive[10]. The aim of **Study I** and **II** was to investigate similarities and differences between HFpEF and HFrEF regarding metabolic biomarkers previously poorly explored in HFpEF.

Anabolic impairment is a feature of HFrEF, but data regarding HFpEF is limited[151]. In **Study I** we confirmed findings in HFrEF of reduced levels of IGF-1 and the association between higher levels and better outcomes[103,104,115]. Depressed IGF-1 may merely be a marker of catabolism and worse HF. Still, the association was independent of HF severity. Considering the presumed inotropic actions of IGF-1 and role in adaptive cardiac remodeling, low IGF-1 activity may directly contribute to HF severity in HFrEF[152,153].

Contrary to in HFrEF, IGF-1 was no different from controls in HFpEF, and there was no association with outcomes. This suggests a more intact somatotropic axis in HFpEF, possibly due to obesity, insulin resistance, or merely absence of catabolism. Our findings were confirmed in a study of the multiple hormone deficiency syndrome, investigating the somatotropic, thyroid, adrenal, and gonadal hormonal axes[154]. Higher concentrations of IGF-1 were reported in HFpEF compared with HFrEF and overall, 46% of patients with HFpEF had no hormonal deficiency vs. 4% in HFrEF[154].

Concentrations of IGFBP-1 were similarly increased in both HFpEF and HFrEF. Since IGFBP-1 regulates IGF-1 activity, this argues for impaired IGF-1 activity in both HF phenotypes, i.e. in contrast to the findings on IGF-1 itself, addressed above. Nevertheless, besides regulating IGF-1, IGFBP-1 has other, independent, actions. Insulin inhibits IGFBP-1 production, and IGFBP-1 is reduced in insulin-resistance where low levels are associated with decreased NO-production, microvascular disease, and cardiovascular risk factors[155,156]. In animal models of insulin resistance, overexpression of IGFBP-1 improves insulin sensitivity, lowers blood pressure, and increases vascular NO-production [157,158]. However, in the development of manifest T2DM, possibly due to hepatic insulin resistance, IGFBP-1 increases[159,160]. High levels of IGFBP-1 are, like NPs, associated with cardiovascular mortality and predict onset HF. In sub-group analysis high IGFBP-1 levels predict HFpEF, whereas not clearly HFrEF[111,161].

While there was no association between IGF-1 and NT-proBNP, the association between IGFBP-1 and NT-proBNP was evident in both HFpEF and HFrEF. Malnutrition, cachexia, inflammatory cytokines, and oxidative stress all lead to increased IGFBP-1 and these factors are also associated with increased BNP[67,162,163]. Indeed, Meirovich et al. demonstrated that rat cardiomyocytes infused with IGFBP-1 secrete BNP[163], findings that we recently have reproduced both in rat and human cardiomyocytes (unpublished).

Despite the association with NT-proBNP, we found no association between high IGFBP-1 and prognosis in neither HFpEF nor HFrEF. Considering the small sample size, this should be interpreted with caution. and recently, an association between high IGFBP-1 and poor outcome has been shown in HFrEF[164].

High NPs are risk markers in HF but simultaneously protective factors modulating adaptive pathways. Assuming the beneficial endothelial effects of IGFBP-1 and the strong and potentially mechanistic association with BNP, possibly also IGFBP-1 participates in the adaptive neurohormonal activation in HF in concert with NPs. The role of IGFBP-1 in the evolution of and in manifest HF is nonetheless still elusive.

Leptin and adiponectin

Leptin and adiponectin concentrations are elevated in HFrEF and as a part of the obesity paradox, high leptin levels are associated with better outcomes in HFrEF[120,124,137]. In **Study 2,** we show that the levels of leptin and adiponectin are similarly increased in both HFpEF and HFrEF. We also confirmed the obesity paradox of higher levels of leptin being associated with a more favourable prognosis in HFrEF. Still, the association was not independent of HF severity measured as NT-proBNP. This is in contrast to previous findings where leptin was associated with better outcomes in HFrEF independent of NT-proBNP[120]. However, adjustment for HF severity through NPs, considering the interaction between NPs and obesity, may not be optimal when assessing the role of leptin. Furthermore, whether leptin itself has a mechanistic role in the obesity paradox, through beneficial actions in manifest HFrEF, or is merely a bystander related to other factors in obesity, is not clear.

In HFpEF, the obesity paradox regarding leptin could not be confirmed. The obesity paradox seems to be a unique feature of HFrEF, and recent data suggest, in accordance with our findings, that HFpEF displays a more conventional metabolic profile[165]. Possibly, hyperleptinemia in HFpEF is rather associated with the pathogenesis of the syndrome through the negative actions of SNS-stimulation, low-grade inflammation, oxidative stress, and increased aldosterone production mediated by leptin[117,129]. Interestingly, considering the sex distribution in HFpEF, women in the general population have higher concentrations of leptin compared with men, and leptin appears to be more strongly associated with markers of inflammation in women[118].

While low levels of adiponectin are associated with obesity and cardiovascular risk, adiponectin is increased in manifest HFrEF. Adiponectin in HF is furthermore associated with poor prognosis, despite its purported beneficial actions, which is sometimes referred to as the adiponectin paradox[135-137,166]. We report increased adiponectin in both HF phenotypes and confirm the findings of an association with poor prognosis in HFrEF[135-137,167]. There was no association in HFpEF. Again, the absence of association could be explained solely by poor power. However, in the development of HF, the increased risk associated with adiponectin is explained by the risk associated with concomitantly increased NPs[168]. Correspondingly, there was no clear association between concentrations of adiponectin and NT-proBNP in HFpEF.

There are suggestions of adiponectin resistance in HFrEF, both in the myocardium and in skeletal muscle, which would be a possible explanation for the adiponectin paradox[166]. Additionally, NPs stimulate increased adiponectin release from adipose tissue and administration of recombinant NPs increase plasma adiponectin concentrations in HF[138]. Besides natriuresis and reverse remodeling effects, BNP also has favourable effects on lipid metabolism[162] and these actions are possibly mediated through adiponectin[169]. Whether adiponectin is a player in the adaptive NP activation in HF, and whether it represents a potential treatment target remain unknown.

Treatment targets in HF

After decades of therapeutic success in blocking maladaptive neurohormonal activation in HFrEF, the discovery of adaptive pathways in HF is a new arena for therapeutic development. The hitherto most successful example is the combination of neprilysin inhibition and angiotensin receptor blockade with sacubitril-valsartan, reducing NP breakdown[17]. Both recombinant human NPs and designer peptides have been and continue to be investigated despite mixed success in trials[64,170].

Obesity is associated with both incident HFrEF and HFpEF and it is well established that obesity is associated with lower levels of NPs[93,171] . The mechanism is thought to be both increases in neprilysin activity and expression of the NP receptor C, mediating NP degradation[93]. However, besides natriuretic and adaptive remodeling effects, NPs have metabolic effects such as inhibiting the proliferation of adipocytes, inducing lipolysis and increasing the concentrations of the cardiac energy substrate free fatty acids[162,172,173]. As mentioned, possibly, some of these effects may be mediated through adiponectin[169]. Both adiponectin and IGFBP-1 were positively associated with NT-proBNP in our studies and other studies show possible mechanistic links and cardioprotective effects[110,157,158,163,174].

Hence, adiponectin and IGFBP-1 may represent potential players in the adaptive response in HF. An adiponectin receptor agonist, Adiporon is investigated in animal studies in various medical conditions, e.g. diabetic nephropathy[175,176] but any role in the development of or manifest HF, is not yet known.

In HFrEF, neurohormonal antagonists are effective regardless of HF severity. In HFpEF, there is a hypothesis of treatment effect in mild or early stage of HFpEF, in contrast to severe HFpEF with potentially irreversible structural changes, different from in HFrEF. This is based in part on the results from I-PRESERVE and TOP-CAT with treatment effect in patients with less severe HF measured as lower NP-concentrations[72,177]. Considering the role of obesity and T2DM in the evolution of HFpEF through inflammation; adipokines and insulin-related peptides could possibly represent early treatment targets. The impressive cardiovascular effects of the sodium-glucose co-transporter-2 (SGLT-2) inhibitors in the treatment of T2DM, with reductions in HF hospitalizations of 35%, are intriguing [178,179]. While these studies did not assess EF, HFpEF is the most prevalent type of HF in T2DM[180]. Although the early treatment effect of SGLT-2 inhibitors is likely largely related to natriuretic and diuretic effects, the possible roles of direct cardiac, vascular or anti-inflammatory actions are not fully understood. Considering the similar, but opposing actions of leptin, the hypothetical interaction between SGLT-2 inhibition and leptin is interesting[181].

In HFrEF the obesity paradox and the role of the somatotropic axis represent possible treatment targets. Obesity is associated with higher concentrations of IGF-1 and IGF-1 reduces systemic vascular resistance and is inotropic[106]. Possibly, IGF-1 is one of many factors involved in the obesity paradox. GH therapy in HFrEF has been tested in various smaller studies with conflicting results. However, in GH-deficient subjects, GH replacement show promising results, stressing the importance of phenotyping the patients and individualizing treatment[153].

Patient reported outcomes and sex

HF is indeed a deadly and disabling disease with significant impact on QoL affecting not only physical capacity, but also mental health and social life[53,182]. In **Study III** we assessed PROs in patients with HFpEF and potential sex differences. Ideally, HF care and therapies should not only improve morbidity and mortality, but also the patients' well-being or QoL[48,49]. The importance of QoL is stressed by the fact that some patients with HF, in particular patients with higher NP-concentrations, more dyspnoea, and lower general QoL are even willing to trade life longevity for improved QoL[183,184]. However, this issue is complex and rather hypothetical. Furthermore, conflicting data has been reported, where a majority of elderly HF patients are unwilling to trade longevity for improved QoL. Interestingly though, female sex was a predictor of willingness to trade [185]. In HFrEF, women also express worse QoL than men [50].

In **Study III**, women and men with stable HFpEF expressed similar disease specific QoL while women expressed worse general QoL than men, independent of age and HF severity. HF severity was adjusted for through both NYHA class and concentrations of NPs. Nevertheless, the adjustment for HF severity is difficult in comparisons between sexes. The perception of dyspnoea and NYHA class differs in women and men[186], and, as we show in **Study IV**, concentrations of NT-proBNP may be higher in women. Diuretic dose is sometimes used to assess HF severity but considering standard dosing, lower body weight in women, and the fact

that women are more often prescribed diuretics this measure also has inherent difficulties[7]. Despite the severe diagnosis of HF, not even half of patients with HF rate HF as the major determinant of impaired QoL[54]. We found only weak associations of QoL with HF severity, and seemingly weaker in women. One explanation might be that women with HFpEF have more comorbidities than men[8]. Indeed, non-cardiovascular comorbidities, such as T2DM, kidney failure, and chronic obstructive pulmonary disease, may contribute even more to impaired functional status and QoL than HF itself or cardiovascular comorbidities in patients with HF[187]. While QoL is known to be associated with both HF severity and outcomes in both HFpEF and HFrEF[51,188], we surprisingly only found the latter association in men. Since the outcome investigated was HF hospitalization or death, one explanation for the absence of association may be that poor QoL in women is to a larger extent explained by other factors than HF itself. Overall, the results suggest that patients with HFpEF, in particular women, have impaired QoL. To improve QoL, individual factors must be taken into account and a holistic approach to HF therapy and interventions is necessary.

Natriuretic peptides and sex

There seem to be sex differences in the phenotypic expression of HF, prognosis and response to therapy. For multiple reasons women may be more prone to develop HFpEF compared to men[8,189]. Indeed, the biomarkers studied in this thesis exhibit sex-specific patterns[118,141,190]. In **Study I** and **II**, due to the low number of women in HFrEF, sex-specific comparisons between HFpEF and HFrEF were not possible and larger studies are needed to confirm and expand upon our findings.

BNP or NT-proBNP is by far the most widely used biomarker in HF both for confirming and especially for excluding the diagnosis. It may be used as a HF severity or prognostic marker, potentially for guidance of therapeutic decisions, as a therapeutic target, and as a surrogate endpoint in early phase trials[74]. As evidenced by its adaptive cardiovascular effects and the PARADIGM-HF trial, it also represents a therapeutic target[17]. In **Study IV** we show that despite higher concentrations in women across the EF spectrum, the associations between clinical characteristics and high NT-proBNP were largely similar in women and men with HF. There were only few exceptions, mainly in HFpEF. We also show that the prognostic ability of NT-proBNP was comparable in women and men in all EF phenotypes. This is in agreement with studies in acute HF[13], but in contrast to reports in healthy women where associations with relevant comorbidities, like obesity, have a stronger effect on NP levels in women than in men[85]. In healthy individuals, sex is an important predictor of NP-concentrations[191]. Possibly, the role of sex in determining levels of NPs is "diluted" in the higher range of NP-s caused by HF and increased filling pressures.

Indeed, the lower the concentrations of NPs, the more pronounced the differences in NP concentrations between the sexes became. In the present study, in patients with HFpEF without atrial fibrillation, females had 45% higher median NT-proBNP concentrations compared with males. In certain populations, a relatively large proportion, 30%, of patients with HFpEF have low or even normal NPs [192]. In that context, the sexual dimorphism regarding levels of NPs and associations between NP-levels and comorbidities might indeed be relevant. Similarly, the present diagnostic cut-offs and cut-offs for inclusion in trials might yield women with less severe HF than men.

FUTURE PERSPECTIVES

HFrEF, HFpEF and HFmrEF – different challenges

HF is common, deadly, associated with poor QoL, and increasing in prevalence. However, the different HF phenotypes face different challenges. In HFrEF, multiple therapies exist but implementation of evidence based interventions and improving PRO remain a target. The role of the somatotropic axis, the obesity paradox, and potential sex-differences in pathophysiology and response to treatment are still to be elucidated. The obesity paradox and the somatotropic axis are, among other areas, intriguing as potential treatment targets.

In HFpEF, there is still a vague and insufficient understanding of the syndrome, of the relative female predominance, and of how and when to intervene. Considering the presumed role of obesity and T2DM in the evolution of HFpEF and the link between the NP-system, metabolism, and adipose tissue, Adiponectin and IGFBP-1 may represent players in the adaptive response in HF. As such they warrant further investigation as potential treatment targets.

HFmrEF seems to be a heterogeneous phenotype between the two extremes. To what extent the patients with HFmrEF are actually merely a subgroup of HFrEF, a heterogeneous mix of the two phenotypes, or if the pathophysiologically different phenotypes of HFrEF and HFpEF actually can coexist in HFmrEF is still unknown.

Nevertheless, the one-size fits all treatment may no longer be valid for HF. Possibly individualized treatment is needed to improve not only survival, but also PRO. Furthermore, after decades of focus on blocking maladaptive response in HF, the largely unexplored adaptive neurohormonal activation in HF represents a vast field of potential therapeutic targets. And last, since women constitute 50% of the HF population, sex-specific analyses should be imperative and women can no longer be considered merely one subgroup of many in HF.

LIMITATIONS

A limitation in these, and in general in comparisons of HFpEF and HFrEF, is the distinct difference in age, sex distribution, comorbidities, and often HF severity. The three cohorts in **Study I** and **II** were also recruited in different settings, although they were all analysed consistently in the fasting state with the same, validated methods. Due to low sample size and few women in the HFrEF cohort, sex specific comparisons in both sexes were not possible. The outcome definitions in HFrEF and HFpEF furthermore differed. Hospitalization was not included in HFrEF due to a too high event rate, while implantation of LV assist device or heart transplantation were considered as deterioration in HFrEF and included in the composite endpoint. Though data suggests potential differences in HFpEF and HFrEF, adjustment for HF severity was performed, and some of the findings have been confirmed in later studies, we cannot rule out that our findings reflect different severities rather than differences between the syndromes. The interpretations of **Study I** and **II** are also limited by the small sample size, but being the early studies of these biomarkers in HFpEF, they could be considered hypothesis generating.

The three studies in the KaRen cohort (**Studies I-III**) were, albeit pre-specified, retrospective analyses. The KaRen study was also designed before the new diagnostic criteria for HFpEF. Nevertheless, only 2% of the patients in the biomarker sub-study (**Studies I and II**) had EF <50% and in **Study III**, patients with EF <50% were excluded. In **Study III**, data on the different dimensions of MLHFQ were not available why more detailed analyses of HF specific QoL impairment was not possible.

In **Study IV**, a cohort from SwedeHF was investigated. The inclusion criterion in SwedeHF is clinician-judged HF. Hence, it cannot be ruled out that some patients might not have HF and, considering the low awareness of HFpEF in the early study period, that HFpEF is underreported. SwedeHF has furthermore a relatively low coverage in primary care despite many HF patients are being treated there and, thus, selection bias may represent a limitation.

Although extensive adjustments were performed, we cannot exclude potential residual and unmeasured confounding affecting the interpretations. Cause-specific hospitalization but not mortality was considered due to the difficulty in assuring cause of death in registries where there is no adjudication of events. Finally, generalizability of our findings to other settings depends on similarities in population characteristics, health care organization and delivery, and HF management.

CONCLUSIONS

In this analysis of metabolic hormones, NPs, PRO, and sex in HF with different EF we make several observations that may be relevant for understanding how metabolism, neurohormonal activation, and outcomes may differ according to sex and EF phenotype.

Impairment in the IGF-1 axis is more pronounced in HFrEF compared to in HFpEF. Still, both EF phenotypes share increased IGFBP-1 which is associated with, and possibly mechanistically linked to BNP. We also found that both leptin and adiponectin were similarly increased in HFpEF and HFrEF. However, the obesity paradox regarding leptin was only demonstrated in HFrEF, pointing towards a more conventional metabolic profile in HFpEF. The associations between NPs and IGFBP-1 and the adipokines warrant further investigation considering the peptides' potential role in the evolution of and in the adaptive response in HF.

As in HFrEF, women with HFpEF express worse QoL than men and impaired QoL was associated with worse outcome in men only. Overall this suggests that, to improve QoL in HF patients, in particular in women, other factors than HF must be considered and addressed.

Women with chronic, stable HF have higher NT-proBNP concentrations than men, but associations between NT-proBNP concentrations and clinical characteristics, as well as outcomes are largely similar. This supports the current use of NT-proBNP for prognostic purposes. Nevertheless, the impact of the relatively large differences between sexes in the lower range of NPs may require further investigation.

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"In the end, we'll all become stories. Or else we'll become entities. Maybe it's the same."
Margaret Atwood