Chapter 1

Introduction

Heart failure

Heart failure (HF) occurs when the when the heart is unable to pump blood to the body at a rate commensurate with its needs. Various symptoms may manifest, including shortness of breath at exertion (early stages) or at rest (later stages), decreased exercise intolerance, fatigue, and signs of fluid retention such as swelling peripheral or pulmonary edema [1]. To classify HF, it is categorized into three major groups based on the left ventricular ejection fraction (LVEF), indicating the contractile (systolic) function of the left ventricle. HF with reduced ejection fraction (EF) (HFrEF) is defined by a LVEF of 40% or less, HF with mildly reduced EF is defined by a LVEF of 41% to 49%, and HF with preserved EF (HFpEF) is defined by a LVEF of 50% or greater [2].

Differentiating between HFpEF and HFrEF is not trivial, as it holds significant importance with regards to pathophysiology and management [2]. While numerous treatments for HFrEF have proven effective in reducing mortality risk, attempts to apply these treatments to HFpEF have yielded limited success in reducing the risk of morbidity and mortality [4]. Therefore, it is crucial to recognize the distinctions between these two forms of HF in terms of their underlying mechanisms and pathophysiology.

The typical patients with HFpEF is an elderly female, further characterized by extra-cardiac features such as, obesity, hypertension and type 2 diabetes mellitus (T2DM) (Figure 1) [13]. There is a growing body of evidence suggesting that managing HFpEF should involve addressing these extra-cardiac comorbidities, as they play a substantial role in disease burden and prognosis [16,17].

Cardiac remodeling

In the case of HFpEF, the heart typically exhibits increased myocardial stiffness, leading to impaired diastolic relaxation and reduced diastolic filling capacity [3]. This may result in inadequate cardiac output, despite each contraction successfully ejecting more than half of the blood into the left ventricle (LVEF >50%), and increased left ventricular filling pressure which is transmitted into the pulmonary system resulting in pulmonary venous hypertension and pulmonary edema. In contrast to HFrEF, where contractile dysfunction (systolic dysfunction) leads to reduced ejection fraction (LVEF <50, HFpEF represents a distinct form of heart failure characterized by diastolic dysfunction [2].

Cardiac remodeling is a fundamental process underlying the clinical manifestations of HFpEF, that involves changes in the size, mass, geometry, and function of the heart. The

functional changes (diastolic dysfunction) in HFpEF is characteristically associated with left ventricular concentric remodeling with fibrosis and inflammation and increased levels of cardiac stretch marker -natriuretic peptides [14,15].

Chronic pressure overload on the heart, often caused by conditions such as hypertension, induces myocardial hypertrophy, which is an increase in the size of cardiac muscle cells (cardiomyocytes). This adaptive response leads to the thickening of the walls of the left ventricle, known as concentric hypertrophy, to maintain adequate wall stress (Figure 1). Additionally, there is excessive deposition of collagen and other extracellular matrix components within the myocardium, resulting in fibrosis. Fibrosis reduces myocardial compliance, increases stiffness, and impairs the heart's ability to fill properly during diastole, contributing to diastolic dysfunction (Figure 1). Furthermore, diastolic dysfunction and increased left ventricular filling pressures can cause pressure and volume overload in the left atrium, leading to left atrial enlargement, which is commonly observed in HFpEF.

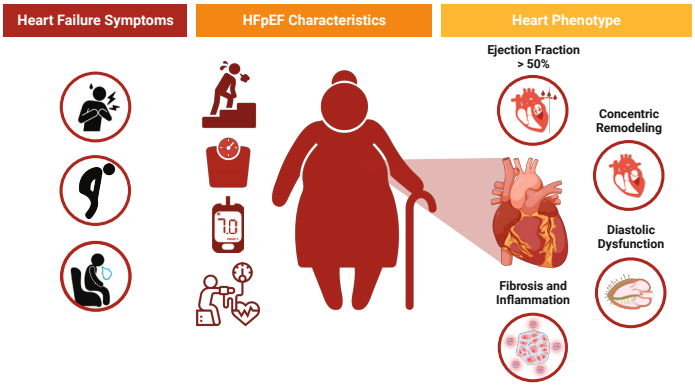

Figure 1. HFpEF is characterized by symptoms of heart failure, preserved ejection fraction, diastolic dysfunction, and a distinct cardiac phenotype

Patients with HFpEF often present with shortness of breath, fatigue, reduced exercise tolerance, and peripheral and pulmonary edema. Additionally, these individuals may exhibit comorbidities such as hypertension, type 2 diabetes mellitus (T2DM), obesity and hypertension. HFpEF typically affects older individuals, particularly women. The heart phenotype associated with HFpEF involves alterations in cardiac structure and function. A typical intra-cardiac phenotype is observed with increased left ventricular wall thickness

and concentric remodeling, left atrial enlargement, and evidence of diastolic dysfunction and increased stiffness, with fibrosis and inflammation.

The epidemic of heart failure with a preserved ejection fraction (HFpEF)

HFpEF is a global health concern, putting a substantial burden on healthcare systems and societies [5]. HFpEF is common in individuals above 65 years of age, affects more women than men, and significantly impacts overall health, well-being, and quality of life [3]. In the general population, HFpEF is relatively rare among individuals aged 25 to 55 [7,12]. However, its prevalence sharply rises with advancing age, with rates exceeding 8-10% in females, 4-6% in men over the age of 80 [7,12]. Consistent with this the proportion of HFPEF among patients with HF increased with age (70% of cases occurring in individuals aged 65). Over the last 20 years, the incidence and prevalence of HFpEF as a proportion of all patients have been steadily increasing. In 2003, HFpEF accounted for approximately 33% of HF cases, but it now represents approximately 50%, with an annual growth rate of approximately 1% [6]. Patients with HFpEF are hospitalized approximately 1.4 times per year and in 5 years at least half of the patients with HFpEF died (53%-74%) [7] and annual mortality for HFpEF is approximately 15% in observational studies [8]. In contrast to the high mortality observed in observational studies, annualized mortality from large HFpEF trials ranged from 4 -5% per year [9,10]. Given its increasing prevalence resulting from an aging population and the growing incidence of obesity-related diseases, coupled with its substantial morbidity rate (and increased mortality), HFpEF is regarded as one of the most pressing unmet medical needs in the field of cardiovascular medicine [11].

Diagnosing HFpEF

Diagnosing HFpEF can be challenging, given the non-specificity of characteristic symptoms (breathless, fatigue, ankle swelling) and difficulties in assessing diastolic function. In an effort to simplify the process, two clinical scoring methods have been developed. These scoring systems assess HFpEF criteria across various domains and generate a probability score for the presence of HFpEF [18,19]. The European Heart Failure Association score (HFA-PEFF) incorporates pre-test assessment, includes assessment for HF symptoms and signs, typical clinical demographics (obesity, hypertension, diabetes mellitus, elderly, and atrial fibrillation) echocardiography and natriuretic peptides, while the American score (H2FPEF) includes obesity, the use of ≥2 antihypertensive drugs, atrial fibrillation, pulmonary hypertension, age over 60 years, and an elevated E/e' ratio [20,21]. These scoring methods can assist clinicians in diagnosing HFpEF but are not yet incorporated into guidelines.

HFpEF heterogeneity

HFpEF is a heterogeneous condition, and recent advances in understanding the syndrome have led to the identification of HFpEF phenotypes or phenogroups [22]. It is now recognized that HFpEF involves multiple organ systems throughout the body and may not solely be a cardiac disorder. The concept of HFpEF phenotypes categorizes patients into distinct groups based on their clinical characteristics and pathophysiological mechanisms. The number of phenotypes varies across studies, with three to seven phenotypes being reported [23–26]. The most prevalent phenotype in HFpEF is cardiometabolic HFpEF, which is associated with comorbidities of obesity, hypertension, and aging. This phenotype is commonly observed in elderly females and is characterized by lipid accumulation, activation of maladaptive inflammatory pathways, abnormalities in adipose tissue function, oxidative stress, and insulin resistance. These pathological processes contribute chronic systemic inflammation that result in structural and functional impairment of cardiac cells, progressive fibrosis development, and subsequent organ (heart) dysfunction (figure 2) [27,28].

Cardiometabolic HFpEF

In cardiometabolic HFpEF, inflammation plays a crucial role in its pathophysiology. Recent research has revealed that metabolic stress, including increased visceral adipose tissue (VAT), insulin resistance, and hypertension, contribute to metabolic disorders such as obesity, T2DM and exacerbate metabolic inflammation (Figure 2).

Regarding the role of adipose tissue in HFpEF, it is now understood that adipose tissue is not merely an energy storage depot but a highly metabolically active tissue exhibiting a state of low-grade inflammation. Adipokines released from adipose tissue, such as TNF-α and IL-6, contribute to systemic inflammation, endothelial dysfunction, and impaired cardiac relaxation. In the context of obesity, the expansion of adipose tissue triggers the release of fibrosis-promoting factors, including transforming growth factor-beta (TGF-β), leading to collagen deposition, that results in fibrosis in the myocardium [29]. This process contributes to myocardial stiffness and impaired diastolic function. In addition, increased production of reactive oxygen species (ROS) by the endothelium leads to endothelial dysfunction, oxidative stress, and cardiac remodeling in the form of fibrosis or hypertrophy. The inflammatory processes involved in HFpEF are systemic in nature, and comorbidity-driven inflammation is recognized as a critical component of HFpEF pathogenesis (Figure 2).

Obesity-associated HFpEF is particularly prominent in post-menopausal women with increased VAT and epicardial adipose tissue (EAT). While the prevalence of hypertension is lower in pre-menopausal women compared to men of the same age, it significantly increases in women aged 65 and older [30,31]. Female HFpEF patients typically present with a cluster of conditions, including marked visceral adiposity (e.g., BMI >35 kg/m2), insulin resistance with T2DM, and hypertension. All comorbidities are intimately tied to HFpEF development, but visceral adiposity directly contributes to insulin resistance, inflammation, accelerated senescence, and mitochondrial dysfunction [32]. Therefore, excess adipose tissue plays a significant role in the pathophysiology of obesity-associated HFpEF.

In this regard weight loss may promote favorable cardiac remodeling by reducing inflammation, restore the metabolic disbalance with contaminant positive effects on the heart. Drugs that target the cardiometabolic profile such as sodium-glucose co-transporter-2 inhibitors (SGLT2i) or glucose like peptide 1 receptor agonist (GLP-1RAs) are receiving increased attention as potential therapeutic strategies in HFpEF because of their, weight reduction, glucose-lowering, anti-inflammatory, and anti-remodeling effects [33].

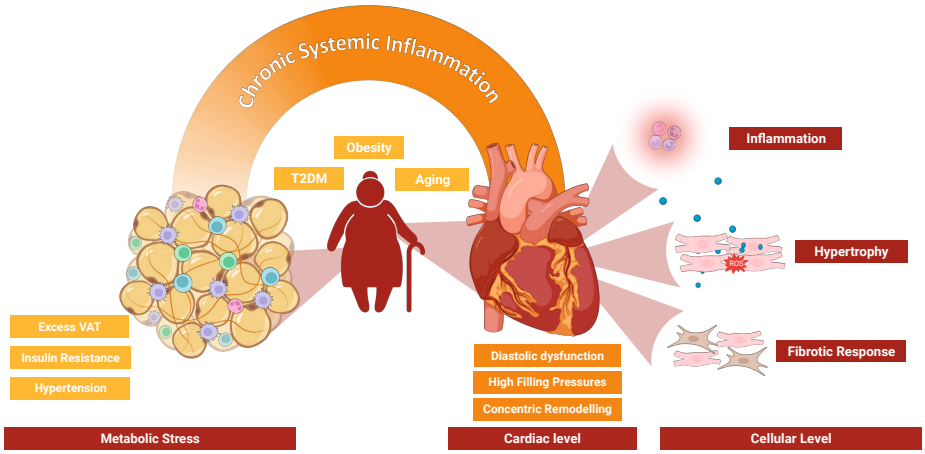

Figure 2. Chronic systemic inflammation plays a crucial role in the development HFpEF
Comorbidity-driven inflammation, often associated with metabolic stress increased visceral adipose tissue, insulin resistance, and hypertension, which leads to obesity and T2DM amplifies the inflammatory response. Metabolic dysregulation exacerbates inflammation and accelerates the aging process, further contributing cardiac inflammation, hypertrophy and the fibrotic response. Ultimately leading to diastolic dysfunction, high filling pressures and concentric remodeling. Together this contributes to the development and progression of HFpEF.

The role of ageing and sex

Comorbidities associated with HFpEF accelerate the normal cardiovascular aging process. With age, blood vessels and heart muscle become stiffer and less elastic, resulting in increased filling pressures with deleterious consequences to the heart (arterial ventricular coupling). Furthermore, chronic low key inflammation, which is more prevalent in older individuals, contributes to long-term pathological remodeling of the heart and interstitial fibrosis, which are characteristic features of HFpEF [34]. Age-related alterations in the extracellular matrix, such as increased collagen deposition, fibrosis, and reduced elastin content, also contribute to the increased cardiac stiffness . In addition to comorbidities and aging, the pathophysiology of HFpEF may also be influenced by sex. This disparity can be partially explained by factors such as longer life expectancy, a higher prevalence of obesity, and post-menopausal changes like hypertension [35]. Interestingly, hypertension can induce distinct patterns of cardiac remodeling in males and females, with females being more prone to develop concentric remodeling and males more likely to exhibit eccentric remodeling. The exact cause of sexual dimorphism in HFpEF is not yet fully understood, and emphasize the need to balance the representation of males and females in clinical trials and pre-clinical research.

The absence of animal models that accurately recapitulate the complexities of human disease

The development of effective drugs for HFpEF is impeded, partly due to an incomplete understanding of the underlying pathophysiological mechanisms. HFpEF is characterized by a multifaceted interplay of impairments throughout the body, involving multiple organs on top of the cardiac dysfunction. The limited success in finding therapeutic options for HFpEF is exemplified by the failure of a large number of clinical trials conducted with common HF (HFrEF) medications. With the growing list of medications without convincing benefits in HFpEF we are faced with the question of how to move forward in identifying novel therapies for HFpEF?

Animal models can play a crucial role in advancing our understanding of disease mechanisms, assessing therapeutic effectiveness and investigating novel interventions. However, the limited and inadequately availability of comprehensive basic and preclinical evidence to substantiate the efficacy of potential treatments poses a challenge. Particularly, the absence of suitable animal models that encompass the entire spectrum of HFpEF pathophysiology is a challenge.

In general, animal models have primarily focused on investigating a single cardiac-specific defect, which has been more applicable to HFrEF where systolic dysfunction is the primary impairment [36]. For instance, animal models inducing myocardial infarction (MI) have significantly contributed to understanding heart remodeling and pathophysiology, leading to the development of effective therapeutics for acute forms of HF. Furthermore, models have mainly utilized comorbidities commonly associated with the HFpEF to induce a single cardiac-specific phenotype that is frequently observed in patients. For example, hypertension has been induced in young male mice through surgical, dietary, or genetic interventions, and subsequent assessments have focused on its impact on cardiac hypertrophy or diastolic dysfunction.

The current dilemma faced by researchers is twofold: first, a need to precisely define HFpEF in preclinical models, and second, the challenge of developing small animal models that mimic the complexity of this disease in humans. To bridge the translational gap between animal and human HFpEF studies, it is essential to identify animal models that encompass multiple risk factors and comorbidities associated with HFpEF and present a comprehensive cardiac phenotype resembling the pathologies observed in patients. This approach will enable the development of animal models that better recapitulate the multifactorial nature of HFpEF, allowing for a more accurate representation of the disease and facilitating the exploration of extra-cardiac treatments options (Figure 3).

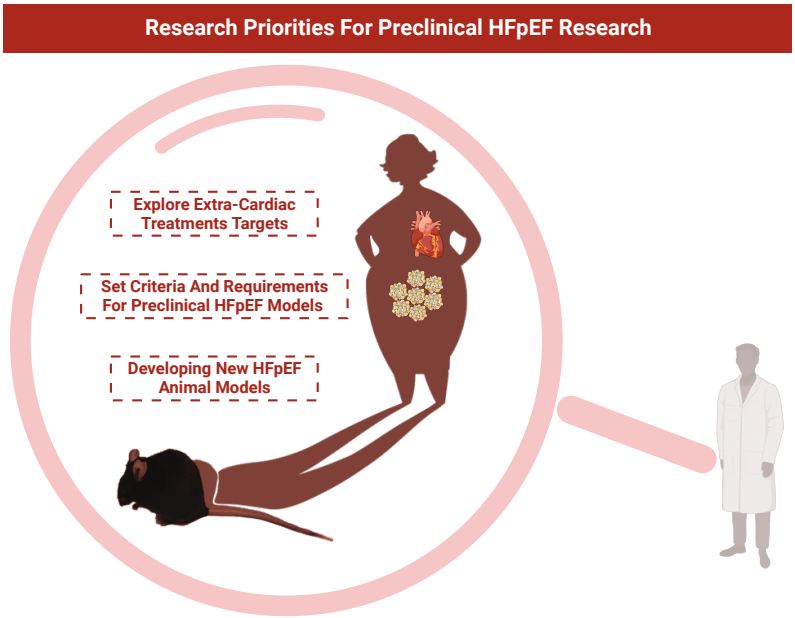

Figure 3. Research priorities for preclinical HFpEF research

Aims and outline of this thesis

The main aims of this thesis are:

1. To establish criteria and requirements for preclinical HFpEF models that take into account the complex human HFpEF pathophysiology to optimize clinical translation.

2. To develop a multi-hit cardiometabolic HFpEF model that resembles the human HFpEF phenotype

3. To evaluate the effect of cardiometabolic interventions in mice with HFpEF

4. To investigate the role of sexual dimorphism in development of murine HF

In **Chapter 2,** we evaluated the translational value of currently available pre-clinical mouse models of HFpEF, and assessed its clinical value by grading them according to the two established HFpEF scores (HFA-PEFF and H2FpEF) [18,19]. Based on these outcomes we propose a novel approach that includes a checklist for small HFpEF animal models to follow when

performing a pre-clinical HFpEF study to optimize bench-to-bed translation. In **Chapter 3,** we recognize the role of ageing in this disease and make a case for the recognition of aging in clinical and preclinical science In **Chapter 4,** we zoom in on the role of obesity in HFpEF and describe the pathophysiological importance of (VAT) in women with HFpEF. In **Chapter 5,** we describe our design and development of a multi-hit mouse model of HFpEF that resembles human HFpEF phenotype. Additionally, we describe in the effects of potential novel cardiometabolic HFpEF therapeutics, such as GLP1-RA, liraglutide and SGLT2i, dapagliflozin in this multi-hit mouse model. In **Chapter 6,** we investigate the effect of weight loss on HFpEF, and compare the actions of drug-induced weight loss with the emerging drug, semaglutide (a GLP1-RA), and studied if they would transcend that of dietary intervention alone. In **Chapter 7,** we investigate the role of sex in HF development in our murine multifactorial model and evaluate potential novel antifibrotic therapeutic targets (Mesalazine). Finally, we discuss main findings, and conclusions of this thesis, as well as current advancements and future perspectives in HFpEF research in **Chapter 8.**

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