Chapter 1

General Introduction

and Outline of the Thesis
INTRODUCTION

Background
Heart Failure (HF) is a complex syndrome caused by an abnormality of cardiac structure or function leading to the inability of the heart to deliver oxygen at a rate commensurate with the requirements of the metabolizing tissues, or if the heart is only feasible to achieve this at the expense of increased filling pressures of the left ventricle (LV).\(^1\) The diagnosis of HF requires typical symptoms (such as breathlessness, ankle swelling and fatigue) in combination with signs (such as elevated jugular venous pressure, displaced apex beat and pulmonary crackles).\(^2\) Since these signs and symptoms can have many different causes or are difficult to detect in an elderly or obese population of HF patients, further evidence of cardiac dysfunction is warranted for the diagnosis of HF.

Heart Failure with Reduced and Preserved Ejection Fraction
In the beginning of the 1980s, the importance of LV ejection fraction (LVEF) originated from industry-driven clinical trials and the emphasis on statistics in the light of the novel principle of evidence-based medicine\(^3\). In these trials, only HF patients with a LVEF < 40-45% were included because they were expected to have a grim prognosis and this selection bias was aimed at increasing the statistical power with a reasonable number of patients.\(^3\) This approach led to a tremendous improvement in therapeutic options in patients with HF and a reduced LVEF (HFrEF or systolic heart failure) as described in the current guidelines on the treatment of HF from the Heart Failure Association of the European Society of Cardiology and the American College of Cardiology Foundation/American Heart Association.\(^2,4\) However, during the past decade it became more and more evident that the population of HF patients with a preserved LVEF (HFpEF) increases gradually.\(^5,6\) Since HFpEF is characterized by diastolic LV dysfunction, this type of HF is also referred to as diastolic heart failure. Currently, HFpEF and HFrEF account for roughly equal proportions of HF patients,\(^7\) but in the elderly HFpEF has already become the most common form of HF.\(^8\) Moreover, since modern therapeutic strategies have proven to prevent deterioration of LVEF, it has been suggested that many patients have shifted from a HFrEF to a HFpEF population.\(^7\) Also, mortality in HFpEF patients is only slightly lower than in HFrEF patients,\(^9\) indicative of the burden of this type of HF with its
increasing prevalence. Unfortunately and in stark contrast to HFrEF, no treatment strategy studied to date in large HFP EF trials has proven to improve disease progression and survival, including betablockers,\textsuperscript{10} angiotensin-converting enzyme inhibitors,\textsuperscript{11} angiotensin 2 receptor blockers,\textsuperscript{12,13} and digoxin.\textsuperscript{14} Probably the most important reason for these disappointing trials and the lack of evidence-based treatment options is the absence of a profound knowledge about HFP EF pathophysiology. Indeed, just over a decade ago, knowledge about myocardial structure and function in HFP EF was very poor.\textsuperscript{15} The following years many studies addressed epidemiological, clinical and fundamental aspects in HFP EF, gradually elucidating its pathophysiology.

**Clinical characteristics of HFP EF patients**

HFP EF is characterised by a high incidence of non-cardiac comorbidities. Although waist circumference and overweight patients are often not reported in studies, one third of patients has a body mass index $\geq 30\text{kg/m}^2$.\textsuperscript{6,16} Diabetes mellitus prevalence ranges from 37 to 45% in various registries, while arterial hypertension shows an even higher prevalence, ranging from 76 to 96%.\textsuperscript{6,16–18} Furthermore, around 1/3 of HFP EF patients suffer from chronic obstructive pulmonary disease (COPD) and 26-52% of patients have chronic kidney disease.\textsuperscript{5,18} Moreover, many patients have more than one comorbidity and the number of comorbidities correlates with prognosis.\textsuperscript{19,20} These comorbidities share the capacity to induce a chronic, low-grade inflammatory state and oxidative stress, as will be discussed in detail later.

**Cardiac characteristics of HFP EF**

The LV in HFP EF is characterized by concentric remodeling, whereas HFrEF is characterized by eccentric LV remodeling as illustrated in Figure 1.\textsuperscript{21}

Besides morphological differences between HFrEF and HFP EF, pressure-volume (PV) loop analyses reveal two distinct hemodynamical profiles (Figure 2).\textsuperscript{22} The dominant functional abnormalities in HFrEF are decreased LV contractility, as evidenced by a decrease in the slope of the end-systolic PV relationship (systolic elastance) and a global down- and rightward shift of the PV-curve. In contrast, in HFP EF the PV-loop is shifted upward and to the left, indicative of increased LV diastolic stiffness and higher filling pressures in a smaller LV cavity volume.\textsuperscript{2} Many studies thereafter focused on the myocardial mechanisms underlying the increased LV diastolic stiffness observed in HFP EF.
Myocardial characteristics of HFrEF

The myocardium consists of 2 major compartments contributing to LV diastolic stiffness: the extracellular matrix (ECM) and the cardiomyocytes. Studies on LV endomyocardial biopsy samples expanded our knowledge on HFrEF pathophysiology enormously. The first experimental studies showed myocardial fibrosis with an increased collagen volume fraction (CVF) in HFrEF patients compared to controls. Associations of myocardial CVF with parameters related to diastolic dysfunction such as LV end-diastolic pressure or the E:E’ ratio (the ratio of transmitral E velocity to early diastolic mitral annular velocity) have been found in HFrEF patients. However, quantification of total collagen content with CVF seems to have less functional implications than the relative amount of the stiffer collagen type I over the more compliant collagen type III, or the amount of cross-linked collagen by lysyl oxidase (LOX). For example, human HFrEF myocardial biopsy samples contained increased levels of collagen type I, enhanced collagen cross-linking and LOX expression and these findings were associated with parameters of diastolic dysfunction on tissue Doppler echocardiography. Myocardial fibrosis in HFrEF is mainly interstitial, whereas HFrEF is characterized by replacement fibrosis following cardiomyocyte cell death, suggesting different pathophysiological mechanisms in both HF entities.

**Figure 1:** Compared to the normal heart in the left panel, the HFrEF heart is characterized by a normal-sized LV cavity with thickened ventricular walls (concentric LV hypertrophy) and preserved systolic function. In contrast, in HFrEF (right panel), the LV walls are thinner with eccentric remodeling and an overall decrease in systolic function. Reproduced with permission, Copyright Massachusetts Medical Society.
However, besides alterations in the ECM, cardiomyocytes undergo typical changes in HFP EF. For example, irrespective of CVF, cardiomyocyte diameter was larger in biopsy samples from HFP EF patients compared to HFrEF patients. Also, when mounted between a force transducer and piezoelectric motor (Figure 3), HFP EF cardiomyocytes appeared to be stiffer than control or HFrEF cardiomyocytes when stretched over a wide range of sarcomere lengths (SL). Moreover, this increased passive stiffness ($F_{\text{passive}}$) upon stretch correlated with LV end-diastolic pressures and LV diastolic stiffness.

Also, cardiomyocyte stiffness was higher in HFP EF than in HFrEF and highest in HFP EF patients with DM. Upon administration of protein kinase A (PKA), cardiomyocyte stiffness declined more in HFP EF than in HFrEF cardiomyocytes, suggesting that a phosphorylation deficit contributes to myocardial and diastolic stiffness in HFP EF. Finally, in failing hearts, Ca$^{2+}$ reuptake into the sarcoplasmic reticulum by the SERCA (sarcoplasmic/endoplasmic reticulum calcium-ATPase) pump is delayed. This leads to increased cytoplasmic Ca$^{2+}$ concentrations and subsequent increased diastolic stiffness.

It has been hypothesized that when the heart fills and the myocardium is stretched within a physiological range, $F_{\text{passive}}$ is primarily caused by titin in the cardiomyocytes. In pathological settings where the myocardium is acutely...
stretched or in the case of myocardial remodeling, it has been suggested that the ECM plays a more important role, probably to protect the cardiomyocytes from overstretching.\textsuperscript{32}

\textbf{Figure 3:} A myocardial muscle strip mounted between a force transducer and piezoelectric motor above an inverted microscope.

\textbf{Molecular characteristics of HFPF EF - Titin}

Since administration of PKA could lower $F_{\text{passive}}$ in HFPF EF cardiomyocytes and this to a larger extent than in HFRF EF cardiomyocytes, molecular changes in HFPF EF cardiomyocytes were to be expected.\textsuperscript{29} The giant protein titin is the main determinant of myocardial stiffness in cardiomyocytes supporting diastolic distensibility. Titin modulates cardiomyocyte stiffness, via either phosphorylation or oxidation.\textsuperscript{32} Titin can, for example, be phosphorylated by protein kinase A (PKA)\textsuperscript{24}, PKG\textsuperscript{33}, by PKC\textsuperscript{34}, calcium/calmodulin-dependent kinase II (CaMKII)\textsuperscript{35} and extracellular signal-regulated kinase (ERK).\textsuperscript{36} PKG is stimulated by cyclic guanosine monophosphate (cGMP), which on its turn is synthesized from guanylyl cyclase.\textsuperscript{37} The latter has 2 isoforms and is either stimulated by nitric oxide (NO) via soluble GC (sGC) or natriuretic peptides (NP) via particulate GC (pGC).\textsuperscript{38} Low PKG activity and cGMP concentration were observed in human HFPF EF LV myocardium and $F_{\text{passive}}$ of isolated cardiomyocytes decreased upon \textit{in vitro} PKG-administration.\textsuperscript{39}

It was recently proposed that the low grade inflammatory state that is induced by metabolic comorbidities, leads to microvascular endothelial inflammation and oxidative stress.\textsuperscript{28} These inflammatory and oxidative processes may decrease NO bioavailability and subsequently NO-sGC-cGMP-PKG signalling, eventually leading to titin hypophosphorylation and increased myocardial stiffness. However, besides titin hypophosphorylation, other mechanisms inside
the cardiomyocytes are expected to increase $F_{\text{passive}}$. Recent studies suggested a role for proteotoxicity in cardiac dysfunction, as previously demonstrated in neurological diseases such as Alzheimer’s and Huntington’s disease. The basis for this proteotoxicity resides in failing quality-control and/or repair mechanisms to correct for aggregated or damaged proteins as a consequence of inflammation and oxidative stress, but it is also part of normal (cardiac) aging and it can affect mitochondrial dysfunction. Knowledge about the role for proteotoxicity and mitochondrial dysfunction in relation to aging, inflammation and oxidative stress in HFP EF is still premature, but might explain the absence of benefit from different treatment strategies so far.

The goal of the current thesis is to investigate the pathophysiological mechanisms leading to increased myocardial stiffness in HFP EF. Moreover, a more thorough knowledge about the pathophysiology might help to improve diagnostic strategies and identify potential therapeutic targets.
1.1 AIM, OBJECTIVES AND OUTLINE

The aim of this thesis was to gain more insight in the pathophysiology (chapter 2, 3, 4), diagnostic (chapter 5, 6) and therapeutic options (chapter 7, 8) of HFrEF. For this, we used a translational approach, ranging from a rat model to human endomyocardial biopsy samples (Figure 4).

In **Chapter 2**, the focus is on myocardial stiffness in HFrEF and its contributors. In this study, a novel, metabolically induced ZSF1-HFrEF rat model was characterized with echocardiography, invasive hemodynamics, metabolic cage studies and on tissue and protein level after sacrifice. Force measurements were performed on small muscle strips and isolated cardiomyocytes to discern the different factors contributing to myocardial stiffness.

**Chapter 3** investigates if systemic, low-grade inflammation of metabolic risk contributes to HFrEF through coronary microvascular endothelial activation. Inflammatory endothelial activation, myocardial oxidative stress, NO bioavailability and cGMP-PKG signalling were investigated in human HFrEF myocardial biopsies and validated in ZSF1-HFrEF rats.

In **Chapter 4**, the role of titin and ECM remodeling in HFrEF are reviewed. Different techniques to assess ECM quality, quantity and its relevance are discussed, followed by changes in cardiomyocytes observed in HFrEF. Also, cardiomyocyte and ECM cross-talk is discussed.

In **Chapter 5**, the focus changes to diagnostic aspects of HFrEF. Weaknesses of the current diagnostic algorithm are discussed, followed by the potential strengths of several biomarkers reflecting myocardial remodelling.

**Chapter 6** highlights the difficulties clinicians are confronted with in patients with pulmonary hypertension (PH), when its aetiology is unclear. Pulmonary capillary wedge pressure (PCWP) aids in this diagnosis. If PCWP is high, PH is likely to be caused by left sided heart failure. However, many patients with HFrEF have a normal PCWP at rest and this chapter focuses on how to cope with this diagnostic trap.

**Chapter 7** focuses on cardiomyocyte based stiffness and a potential therapeutic option (α-B crystallin, a heat shock protein) is examined in vitro. This study uses human endomyocardial biopsy specimens on which force measurements are performed on muscle strips and single cardiomyocytes.
Chapter 8 concludes with a review on the current knowledge on HFrEF, including diagnosis, pathophysiology and current and potential future therapeutic strategies.
REFERENCES


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